Argutus Medical Ltd

Develops and commercializes novel biomarkers for the early detection of organ damage in liver and kidney

Applications:

1. Safety in drug development
   - *In Vitro* – cell culture
   - *In Vivo* – rat/mouse and primate/human
Recent Name Change

- Argutus Medical is a spin-out of Biotrin International
- BD Biosciences distributor

Partners in the search for new drugs through innovative ADMET solutions
BD Biosciences – Webinar Series
Optimal Safety Biomarker Criteria

- **Specific**
  - Organ/tissue/site/cell selective
- **Sensitive**
  - Significant changes can be measured and correlated with injury
- **Easy to measure**
  - Simple assays, no special equipment
- **Dynamic**
  - Short half life and rapid return to baseline
- **Bridging**
  - Transportable data interpretation between cell culture, preclinical species, and humans
- **Histological correlation**
  - Assays correlate with the “Gold standard”
- **Scientific community support**
  - Cited in significant number of scientific journal articles
- **Pharma consortium validation**
  - Reproducible data that meets the above criteria from multiple labs
  - ILSI/HESI
Safety Biomarker Kit Overview

- Liver and kidney safety markers
  - Serum and urine biomarkers
    - Get the “result” without the need for histology
- ELISA kits for GSTs and RPA-1
  - All reagents for 1 x 96-well plate of stripwells
  - Glutathione S-transferases (GSTs) are released into blood, urine, or culture media when particular tissues are damaged
  - Rapidly cleared from tissues after insult is removed
  - Cell- and organ-specific distribution
  - Strong validation support in scientific literature
  - ILSI/HESI has evaluated GSTs and RPA-1 as a nephrotox (kidney tox) marker in rats
  - Rat and human kits
  - Urine stabilizing buffers
### Argutus Medical Tox Biomarker Products

<table>
<thead>
<tr>
<th>Product</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEPKIT® Alpha and High Sensitivity GST EIA (1994)</td>
<td>- Serum GST – biomarker for hepatocyte injury and recovery</td>
</tr>
<tr>
<td>Urinary GST EIA (NEPHKIT®)</td>
<td>- Urinary GST – biomarker for renal proximal tubular injury</td>
</tr>
<tr>
<td>GST EIA</td>
<td>- Human urinary GST – biomarker for renal distal tubular injury</td>
</tr>
<tr>
<td></td>
<td>- Human serum GST – biomarker of liver biliary injury</td>
</tr>
<tr>
<td>Rat GST EIA</td>
<td>- Urinary and serum GST – biomarkers for renal proximal tubular and hepatocyte injury, respectively</td>
</tr>
<tr>
<td>GST Yb1 EIA</td>
<td>- Rat urinary GST Yb1 – a unique biomarker of injury to the renal distal tubules</td>
</tr>
<tr>
<td>Rat RPA-1 EIA</td>
<td>- Unique biomarker of injury to the renal collecting ducts</td>
</tr>
</tbody>
</table>
Argutus Medical Tox Biomarker Products

• Urine stabilizing buffers
  – For GST sample prep
  – Available for Rat and Man
  – Patented
Enzyme Immunoassay (EIA) Kits

Partners in the search for new drugs through innovative ADMET solutions

BD Biosciences – Webinar Series
Partners in the search for new drugs through innovative ADMET solutions

BD Biosciences – Webinar Series
Partners in the search for new drugs through innovative ADMET solutions

BD Biosciences – Webinar Series

- > 100 publications using these products
- Summaries available for key articles
Partners in the search for new drugs through innovative ADMET solutions

BD Biosciences – Webinar Series

http://wwwbdbiosciences.com/admet/toxicity.php
Argutus Medical Tox Biomarker Kits

Distributed by BD Biosciences

Hepatotoxicity Markers
Immunohistochemical Localization of GST Isoforms in the Liver

GST in Hepatocytes

GST in Bile Duct Epithelium
In Vitro Hepatocyte Assays

- Comparison of LDH vs. GST leakage using human cryopreserved hepatocytes

Miller, V. and Xiang, Y., BD Biosciences (2006)
In this study, the cells were not killed (no release of GST), but the number of cells was reduced indicating reduced growth. Measurement of MTT and ATP indicate inhibition of mitochondrial activity. Measuring individual biomarkers could give conflicting results (GST - no effect), cell numbers (reduced growth or cell death), and mitochondrial biomarkers (mitochondrial effects or cell death). However, combining them showed that Rotenone under these conditions was cytostatic, not cytolytic and this was due to inhibition of mitochondrial function. This correlates with the known mechanism of Rotenone toxicity. Data courtesy of CeeTox® Inc.

When rat H4IIE cells are exposed to increasing levels of a compound, eventually toxicity biomarkers start to show a change. Using a panel of specific biomarkers, this concentration is a good indicator of the likely in vivo toxic concentration. Data courtesy of CeeTox Inc.
Hepatotoxicity in the Rat

Samples taken 16 hours after administration
Clarke, et al. (1997)

* p<0.05
** p<0.01

Compared with zero dose

Partners in the search for new drugs through innovative ADMET solutions
BD Biosciences – Webinar Series
Methotrexate Hepatotoxicity

Human Clinical Study

Partners in the search for new drugs through innovative ADMET solutions
BD Biosciences – Webinar Series
Liver Injury in Man

Declaration of No Rejection (NO REJN) and GI Rejection (GI REJN)

- **BILI/17**
- **ALP/135**
- **ALT/40**
- **AST/37**
- **GST/10**

Multiple Upper Limit of Normal Range

Days Post-LTx

Partners in the search for new drugs through innovative ADMET solutions

BD Biosciences – Webinar Series
Liver Injury in Man

- HEPKIT  GST is used widely in phase I safety assessment globally
  - Accurate indicator of early hepatocellular injury
  - Correlated well with liver biopsies in clinical studies (transplant patients)
Argutus Medical Tox Biomarker Kits

Distributed by BD Biosciences

Nephrotoxicity Markers
Basic Renal Structure

- **Glomerulus**
  - Collagen IV
- **Loop of Henle**
  - RPA-2 / LOH
- **Distal tubules**
  - GST Yb1 / GST
- **Collecting ducts**
  - RPA-1 / HCD
- **Proximal tubule**
  - αGST / αGST

Red = Rat Markers. Blue = Human Markers
Immunohistochemical Localization in the Kidney

GST in Proximal Tubules

GST in Distal Tubules

RPA-1 in the papilla
Proximal Tubular Injury: Compound A Nephrotoxicity in Rats

After Kharasch, et al. (1998)
Data on file at Biotrin International

% Necrotic Tubules

GST Excretion, µg/24 hrs

Partners in the search for new drugs through innovative ADMET solutions
BD Biosciences – Webinar Series
Biomarker Changes and Histological Scoring – Preclinical

Shaw, et al. 2007

Partners in the search for new drugs through innovative ADMET solutions
BD Biosciences – Webinar Series
Proximal Tubular Injury: Aminoglycoside Therapy

After Sundberg, et al 1994

Partners in the search for new drugs through innovative ADMET solutions
BD Biosciences – Webinar Series
Proximal Tubular Injury: Foscarnet Therapy

Days After Initiation of Therapy

GST (ng/mL) vs. Serum Creatinine (µmol/L)

Sundberg, et al 1994
Therapy between days 0 and 13

Partners in the search for new drugs through innovative ADMET solutions
BD Biosciences – Webinar Series
Proximal Tubular Injury: Cardio Pulmonary Bypass Early Prediction of AKI Following Surgery

Predict Development of AKI

GST ng/mL

Pre CPB  Post CPB  ICU  6 hr  Day 1  Day 2  Day 3

No AKI (n=40)
All AKI by AKIN (n=36)
Proximal Tubular Injury: Cardio Pulmonary Bypass Prediction of AKI Severity Following Surgery

Predict Severity of AKI

\[ \pi \text{GST (ng/mL)} \]

- No AKI (n=40)
- Stage 1 AKI by AKIN (n=28)
- Stage 3 AKI by AKIN (n=8)

Timeline:
- Pre CPB
- Post CPB
- ICU
- 6 hr
- Day 1
- Day 2
- Day 3

Partners in the search for new drugs through innovative ADMET solutions
BD Biosciences – Webinar Series
HESI Nephrotoxicity Working Group

Allergan
AstraZeneca
Bayer – Schering
Biotrin International
Bristol Myers Squibb
Biogen Idec
FDA
GlaxoSmithKline

Johnson & Johnson
Lilly
Merck
NIEHS
Pfizer
Roche
Sanofi-Aventis
Wyeth

Test Compounds
Gentamycin > Proximal tubules
Cis-platin > Proximal tubules
NPAA > Renal papilla & collecting ducts
Biomarkers for Monitoring Proximal Tubular Injury

Harpur, et al. 2008
Biomarkers for Monitoring Collecting Duct Injury

Harpur, et al. 2008

Partners in the search for new drugs through innovative ADMET solutions
BD Biosciences – Webinar Series
Nephrotoxicity Biomarker Validation

**2004**

1. ILSI HESI Nephrotoxicity Consortium starts in 2004
2. Major Pharma collaborate in study to identify novel preclinical kidney biomarkers
3. Multicenter study with 3 toxins and 100s of animals comparing histology and traditional markers
4. Study concluded *Rat alpha GST* to be the optimal tool for identifying early proximal injury
5. Also concluded that RPA-1 was the only useful tool for identifying early collecting duct injury
6. Preliminary data presented in 4 back to back presentations at 2007 SOT.
7. Further data presented at 2008 SOT meeting

**2008**

- May 2nd ILSI HESI Nephrotoxicity Consortium submits proposal to FDA VxDs
- Full study with statistical analysis and conclusions devised by members of the group
- July 2nd face to face meeting with FDA and EMEA to clarify submission
- September meeting proposed to conclude the content and questions
- Decision expected beginning of October
- It is the expectation of the ILSI HESI group that rat alpha GST, Clusterin and RPA-1 will be qualified rat urinary biomarkers as a result of this process

Nephrotoxicity working group active participants

- GSK
- Schering
- Bristol-Myers Squibb
- Sanofi Aventis
- AstraZeneca

BD
<table>
<thead>
<tr>
<th>Product Name</th>
<th>Cat. No.</th>
<th>Organ-Specific Biomarker</th>
<th>Biomarker Analyte</th>
<th>Detection Limit (µg/l) and CV%</th>
<th>Sample Matrix</th>
<th>Dynamic Range (µg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEPKIT® Alpha</td>
<td>458601</td>
<td>Human Liver</td>
<td>GST</td>
<td>0.05 µg/l, &lt;12.5</td>
<td>Serum, plasma, tissue culture media</td>
<td>0.05-40</td>
</tr>
<tr>
<td>High Sensitivity GST EIA</td>
<td>458602</td>
<td>Human Liver</td>
<td>GST</td>
<td>9.0 ng/l, &lt;11</td>
<td>Serum, plasma, tissue culture media</td>
<td>9-2000 ng/L</td>
</tr>
<tr>
<td>GST EIA</td>
<td>458603</td>
<td>Human Liver</td>
<td>π GST</td>
<td>3 µg/l, &lt;7</td>
<td>Serum or tissue culture Media</td>
<td>3-100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Human Kidney</td>
<td></td>
<td></td>
<td>Urine or tissue culture media</td>
<td></td>
</tr>
<tr>
<td>Rat GST EIA</td>
<td>458604</td>
<td>Rat Liver</td>
<td>GST</td>
<td>0.2 µg/l, &lt;8</td>
<td>Serum or tissue culture Media</td>
<td>0.02-100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rat Kidney</td>
<td></td>
<td></td>
<td>Urine or tissue culture media</td>
<td></td>
</tr>
<tr>
<td>Urinary GST EIA (NEPHKIT® Alpha)</td>
<td>458600</td>
<td>Human Kidney</td>
<td>GST</td>
<td>0.05 µg/l, &lt;10</td>
<td>Urine or tissue culture media</td>
<td>0.05-40</td>
</tr>
<tr>
<td>GST Yb1 EIA</td>
<td>458605</td>
<td>Rat Kidney</td>
<td>GST Yb1 (uGST)</td>
<td>0.2 µg/l, &lt;10%</td>
<td>Urine or tissue culture media</td>
<td>0.2-100</td>
</tr>
<tr>
<td>Rat RPA-1</td>
<td>458609</td>
<td>Rat Kidney</td>
<td>RPA-1</td>
<td></td>
<td>urine</td>
<td>3.1-100U/L</td>
</tr>
<tr>
<td>Human Urine Stabilizing Buffer</td>
<td>458606</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>For use with human urine</td>
<td>n/a</td>
</tr>
<tr>
<td>Rat Urine Stabilizing Buffer</td>
<td>458607</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>For use with rat urine</td>
<td>n/a</td>
</tr>
<tr>
<td>OxyDNA Kit</td>
<td>458608</td>
<td>Oxidized DNA</td>
<td>8-oxoguanine DNA adducts</td>
<td></td>
<td>Oxidized DNA in animal or human cell cultures</td>
<td>n/a</td>
</tr>
</tbody>
</table>
### Features and Benefits

<table>
<thead>
<tr>
<th>Feature</th>
<th>Benefit</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA technology</td>
<td>• Unaffected by drugs and metabolites</td>
</tr>
<tr>
<td></td>
<td>• Commonly used assay, no special equipment required</td>
</tr>
<tr>
<td>Serum and urine biomarkers</td>
<td>• Non-invasive sample collection and easy to measure.</td>
</tr>
<tr>
<td></td>
<td>• Allows determination of tissue damaged without histology</td>
</tr>
<tr>
<td>Measure proteins released only upon loss of cell membrane integrity</td>
<td>• Low background, high sensitivity, and earlier biomarker compared to common biochemical markers</td>
</tr>
<tr>
<td></td>
<td>• Animal/human subject serves as its own control</td>
</tr>
<tr>
<td>Many biomarkers are present in tissue culture, rat, and human tissues</td>
<td>Transportable biomarker</td>
</tr>
<tr>
<td>GSTs are not stable <em>in vivo</em></td>
<td>Biomarker shows kinetics of damage and recovery</td>
</tr>
</tbody>
</table>
### In Vitro Assays

<table>
<thead>
<tr>
<th>Feature</th>
<th>Benefit</th>
</tr>
</thead>
<tbody>
<tr>
<td>GST is not found in culture media</td>
<td>Low Background</td>
</tr>
<tr>
<td>Very little release from healthy cells</td>
<td>Sensitive. Allows users to detect low levels of injury.</td>
</tr>
<tr>
<td>Cell specific</td>
<td>Ability to monitor different cell types simultaneously</td>
</tr>
<tr>
<td>Rapid release from injured cells</td>
<td>Sensitive. Allows user to notice as soon as trauma is experienced.</td>
</tr>
<tr>
<td>Correlation with rat and human results</td>
<td>Allows easier model comparison. Acts as transportable biomarker.</td>
</tr>
<tr>
<td>GST is stable in medium</td>
<td>Samples can be stored longer, as they are, without disturbing study timeline.</td>
</tr>
</tbody>
</table>
Toxicity Biomarker Criteria

- **Specific**
  - Organ/tissue selective
- **Sensitive**
  - Significant changes can be measured and correlated with toxicity
- **Easy to measure**
  - ELISA kits
- **Dynamic**
  - Short half life and rapid return to baseline
- **Bridging**
  - Transportable data interpretation between cell culture, preclinical species, and humans.
- **Histological confirmation**
  - “Gold standard”
- **Scientific community support**
  - Biotrin/Argutus and GSTs are cited in > 100 scientific journal articles
- **Pharma consortium validation**
  - ILSI/HESI has favorably evaluated GST and RPA-1 as nephrotox biomarkers
Questions?

Contact information:
Chris Patten
e-mail: chris_patten@bd.com

Technical Support:
tel: 877.232.8995
e-mail: admetox@bd.com
bdbiosciences.com/webinars

For research use only. Not intended for use in diagnostic or therapeutic procedures.
Argutus, HEPKIT, and NEPHKIT are registered trademarks of Argutus Medical.
CeeTox is a registered trademark of CeeTox Inc.
BD, BD Logo, and all other trademarks are the property of Becton, Dickinson and Company. ©2009 BD