Novel Genomic, Cell, and Biomarker-based Approaches for Accelerating Drug Development

Bob Young
MilliporeSigma, BioReliance® Services
Rockville, Maryland

The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the U.S. and Canada.
Our aim at MilliporeSigma* is to:
accelerate access to health for people everywhere

20,000
employees

>1.6M
customers
globally

60
manufacturing
sites worldwide

>300,000
products & services
including
- Genomics
- Bioinformatics
- Gene editing
- Toxicology
- Custom cells
- Chemicals and reagents
- ...

*The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the U.S. and Canada.
Addressing FDA’s Predictive Toxicology Roadmap

**CHALLENGE:** Can we improve and accelerate drug discovery and development through Innovative Predictive Toxicology?

**FDA Roadmap Needs**
- Predict drug failure early
- Predict drug-drug interactions
- Data sharing for failed drugs
- Improved carcinogenicity predictions

**MilliporeSigma Proposed Solutions**
- New Predictive *In Silico* methods
- New *In Vitro* Cell Models for Predictive Toxicology
- New *In Vitro* and *In Vivo* Biomarkers Predictive of Human Risk

Deliver a complete tool box to identify, address, and predict drug toxicity early
**In Silico Approaches: Bioinformatics Supports Predictive Toxicology**

- Data aggregation & sorting
- High content data analysis
- Deep learning & trend prediction

**Application:**
**Prediction of Kinase Selectivity**

Thousands of compounds screened in hundreds of kinase assays
Different applications of deep learning to develop algorithms for predictive toxicology

In Silico Applications: Deep Learning Using Convolutional Neural Network

Cytometry Cell Classification

Genomics Variants

Literature Text Mining
Predictive Toxicology: MilliporeSigma Novel In Vitro Cell Models

2D-cell cultures

Cell lines for Improved Identification of Drug/Transporter Interactions

- Transporter KO/KI cell lines: Caco-2, MDCKII; HepaRG KO cell lines; Reporter-tagged cells
- Immortalized cell lines: LX-2 hepatic stellate, hCMEC/D3 blood brain barrier, ReNcell human NSCs, AC16 & HL-1 cardiomyocytes

Primary cells, iPSCs

Primary cells from multiple tissues for ADME/Tox applications

- Human hepatocytes, non-parenchymal cells, proximal tubule cells
- ~800 normal, diseased & CRISPR edited hiPSCs.
- >100 primary cells sourced from human & rodents with media

3D-cell cultures

Liver spheroids, Organoids

- Hydrogels, scaffolds, ECM proteins, spheroid plates, media & protocols for improved 3D culture

Complex 3D cultures

Organ-on-Chip, Microphysiological Systems (MPS)

- Collaborations with multiple organ-on-chip/MPS platform providers
- Cellasic® ONIX microfluidics system
Gene Editing: Tools for Better ADME Tox Models

**Tools & Reagents:**
- Zinc Finger Nucleases
- CRISPR pooled and arrayed libraries
- Sanger whole genome lentiviral libraries
- SAM activator libraries
- Cas9 proteins
- shRNA
- si/esiRNA
- sygRNA

**CRISPR & ZFN licenses for Research use CRISPR/Cas9 IP portfolio**

**Cell Design Studio:**
- Cell model development services
- Discovery models
- Immunotherapy models

**In Vitro Safety Systems**
- Primary human hepatocytes
- Gene edited models
  - Intestine
  - Liver
  - Kidney
Biomarkers: Improving Nonclinical Biomarkers of Human Cancer Risk
Error-Corrected Next Generation Sequencing

Mutant frequency

Trinucleotide Mutant Spectra

MilliporeSigma FDA Roadmap for Predictive Toxicology 12Sep2018
# Biomarkers: Multiplexed DNA Damage Assay Using Machine Learning

## CAN Flow Assay

### Global Evaluation Factors

<table>
<thead>
<tr>
<th></th>
<th>Clastogenic Signature?</th>
<th>Aneugenic Signature?</th>
<th>Evidence of Genotoxicity?</th>
<th>Mode of Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 FU 1000</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Non-Genotoxicant</td>
</tr>
<tr>
<td>AMG 900 0*1</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Aneugen</td>
</tr>
<tr>
<td>Brefeldin A 10</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Non-Genotoxicant</td>
</tr>
<tr>
<td>Car bendazim 1000</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Aneugen</td>
</tr>
</tbody>
</table>

### Diagrams

- **5 FU 1000**
  - 24Hr p53: 8
  - 4Hr H2AX: 6
  - 24Hr ph-H3: 0

- **AMG 900 0*1**
  - 24Hr p53: 353
  - 4Hr H2AX: 250
  - 24Hr ph-H3: 177
  - 4Hr H3: 125
  - 4Hr p53: 88

- **Brefeldin A 10**
  - 24Hr p53: 24Hr
  - 4Hr H2AX: 24Hr
  - 24Hr ph-H3: 24Hr
  - 4Hr p53: 4Hr

- **Car bendazim 1000**
  - 24Hr p53: 24Hr
  - 4Hr H2AX: 4Hr
  - 24Hr ph-H3: 24Hr
  - 4Hr p53: 4Hr

---

**MilliporeSigma FDA Roadmap for Predictive Toxicology 12Sep2018**
Lessons learned on managing change

Case Study 1: *In vivo* carcinogenicity assays
- Validated early 1990s leading to ICH S1B and HESI consortium
- Lead to early adoption by pharma and submission for NDAs

Case Study 2: *In vivo* TGR mutation assays
- Validated early 1990s but no guidelines, so little use for over 20 years
- OECD TG 488 in 2011 immediately led to strong interest use by industry

Adoption of new technologies is driven by scientific, business, and most importantly, regulatory reasons
Innovation in Predictive Toxicology

**In Silico**

**Technique**
- Leverage advanced statistical methods and latest machine learning approaches to develop novel models for a variety of toxicity assays based on public and proprietary data sets curated and annotated by internal experts
- Leverage big data technologies and cloud computing power to manipulate massive data sets

**Application**
- A unique combination of experts in bioinformatics, chemoinformatics, and data science within the organization specifically working on toxicity model development with lab scientists
- Through manual curation and annotation, building integrated, high quality training data sets/database on different assays including compound properties, toxicity, and omics data sets (genomics, proteomics, metabolomics, pathways, etc.)
- Improved many specific existing toxicity models using machine learning/deep learning algorithms and better curated data
- Developing novel predictive toxicity models like cell engineering & gene toxicity based on our lab proprietary and unique assay data sets
- Digitalizing web lab processes including automation, LIMS, ELN, compliance, etc.

**References**
- Fernandez, et al, Toxic colors. JCIM, July 31, 2018
- Yang, et al, Frontiers in Chemistry, 6:30, 2018
- Mayr et al., DeepTox: Toxicity prediction using deep learning, Front Environ Sci. 3:80, 2016
Innovation in Predictive Toxicology
Engineered 3D models

**Technique**

- Use genetic engineering tools such as Zinc Finger Nucleases (ZFNs) or CRISPR/Cas9 to modify human cell models (primary cells, immortalized cells, patient specific iPS derived cells)
- Create more complex 3D human models (eg. organoids, spheroids) containing immune & endothelial cell compartments

**Application**

- Market leader in ZFN and CRISPR gene editing tools
- Expertise in human material that are more predictive, can be passaged & genetically manipulated to generate knock-out and knock-in models
- Knock-in technology may include target genes of interest, correction of single nucleotide polymorphisms (SNPs), generation of isogenic cell lines targeting known mutations in human diseases, and expression of fluorescent reporter molecules
- CRISPR technology can also be used to activate or silence endogenous genes of interest
- Establishing reproducible toxicity end point assays on organ on a chip platforms

**References**

- Sampson et al. (2015) Drug Metab Dispos. 43, 199-207 [PMID:25388687]
- Chen F et al. (2017) Nat Commun. 8, 14958 [PMID:28387220]
- Forsythe et al. (2018) Front Public Health. 6, 103-112 [PMID: 29755963]
Innovation in Predictive Toxicology

Improved Biomarkers of Human Risk

**Technique**

- **In Vivo:** Enhanced biomarker of cancer risk - Error-Corrected Next Generation Sequencing (EC NGS)
- **In Vitro:** Cell based *in vitro* assays with multiplexed biomarkers for DNA damage

**Application**

- **In Vivo:**
  - EC NGS detects rare mutants following drug exposure
  - Integrate into early repeat dose preclinical toxicology studies
  - Alternative to 2 year cancer bioassays – FDA/PhRMA NEGCARC Program

- **In Vitro:**
  - Predictive Early Screening for Genotoxicity.
  - To determine genetox mode of action and to design GLP assays in a smarter way to avoid late failures

**References**

- Genotoxic mode of action predictions from a multiplexed flow cytometric assay and a machine learning approach, Bryce SM *et al*., Environ Mol Mutagen. 2016 Apr;57(3):171-89
For Follow-up Information:

In Silico/Bioinformatics
Yang Liu, Ph.D
MilliporeSigma
Digital & Information Technology
St Louis, MO
yang.liu@milliporesigma

Cell Models/Gene Editing
Patrick Sullivan, MS
MilliporeSigma, Sigma-Aldrich
Research and Development
St Louis, MO
patrick.sullivan@sial.com

Biomarkers of Cancer Risk
Bob Young, MS
MilliporeSigma
BioReliance® Testing Services
Rockville, MD
bob.young@sial.com