Solving Challenges Faced in Early Clinical Development

J. Fred Pritchard, Ph.D.
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The Pressure is On for Proof-of-Concept!

- Increase in novel new drugs from discovery research
- More difficult disease states to study and treat
- Greater regulatory expectations on clinical trials
- Increased cost of clinical research
- Need for more informed decisions at clinical proof-of-concept
- Regulatory acceptance of adaptive-like study designs

Innovation

- Expanding universe of new technology applications
New Technology Drives Innovation
So Many New Tools in So Little Time
Understanding Relevance of Preclinical Signals Early in Clinical Development

Focus of Presentation

- Examples of common and uncommon problems faced by drug developers early in clinical development.
- How to leverage new technologies to provide early answers to tough questions
Troublesome Problems Encountered in Early Clinical Research

- **Safety**
  - Positive or equivocal signals in preclinical safety assessment
    - Common – hERG signals
    - Less Common – liver injury signs
    - Uncommon – testicular toxicity

- **PK / Metabolism**
  - Drugs with potentially poor absorption or unknown hepatic first-pass metabolism
  - Active metabolites, species-unique metabolites, or disproportionate human vs. tox species metabolite(s).

- **PD**
  - Establishing if drug gets to site of action and actually works as designed

**High definition digital ECG collection and analysis**

**Assessment of drug induced liver injury (DILI)**

**Testicular biomarkers - semen collection/analysis**

**Use of microtracers with Accelerator Mass Spectrometry**

**Efficacy/Mechanism biomarkers**
QT Prolongation
QT Safety Biomarkers

- Nonclinical risk assessment:
  - In vitro IKr
  - In vivo QT
  
  *Ref: ICH S7B*

- Clinical risk assessment:
  - ECGs in Phase I - II
  - Thorough QT/QTc study
  
  *Ref: ICH E14*
# Comparing ECG Acquisition Modalities

<table>
<thead>
<tr>
<th></th>
<th>Stand alone 12 Lead</th>
<th>Standard Holter</th>
<th>Telemetry System</th>
<th>Blue-tooth Holter</th>
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<tbody>
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<td>Continuous ECG Collection</td>
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<td>Retrospective data collection</td>
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<td>View Safety ECG</td>
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<td>Data capture out of range</td>
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<td>Transportable</td>
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</table>
Digital ECG Reading Enables Overlay of Lead Signals for Better Accuracy in Measurement of Intervals
ECG Extraction: Find a Period of Stable Heart Rate and Reduced Noise

Accurate QTc require a stable preceding heart rate
Antares Optimal ECG Extraction: Decreases Variability

Searching for best extraction, noise and HR stability criteria…

Second extraction
Nominal Timepoint
Third extraction
Best ECG! First extraction

F Badilini, Vaglio, Sarapa, A.N.E 2009;14(Supp1):22-29

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Better data, faster, cheaper
Drug Induced Liver Injury
## Regulatory Action on Marketed Drugs due to DILI (1995-2010)**

**Partial List**

<table>
<thead>
<tr>
<th>Withdrawals (US* &amp;/or other countries+)</th>
<th>Boxed Warnings (US)</th>
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<tbody>
<tr>
<td>troglitazone*</td>
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<tr>
<td>lumaricoxib+</td>
<td>cytarabine</td>
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## Restricted (US)

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<th>trovofloxacin</th>
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<tbody>
<tr>
<td>felbamate</td>
<td>acetaminophen (Rx)</td>
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<tr>
<td>pemoline</td>
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*FDA/C-Path/PhRMA HepTox Steering Committee, 15 March 2012*
Drug-Induced Liver Injury Biomarkers

- Classic serum chemistry tests include total bilirubin (TBL) and enzyme activities of ALP, AST and ALT.
  - “Validated” by more than 60 years of clinical use
  - Serum enzyme activity are NOT tests of liver function
  - TBL and prothrombin time ARE tests of liver function
  - ALT high sensitivity; TBL high specificity

- Problems:
  - Don’t discriminate between drug and non-drug etiologies
  - Are not early predictors of DILI outcomes
    - Resolvable versus serious acceleration of injury
  - Normal ranges not agreed upon
Drug-Induced Liver Injury Biomarkers

- There is a need for DILI biomarkers to predict
  - Clinical resolution vs progression at an early stage of mild liver injury
  - Which drugs can cause idiosyncratic DILI
  - Which patients are susceptible to develop DILI

From Mark Avigan, CDER, FDA, presentation at FDA/C-Path/PhRMA HepTox Steering Committee Meeting, 15 March 2012
Liver-enriched microRNAs were shown to be promising serum biomarkers of acetaminophen-induced acute liver injury in mice\(^1\) and humans\(^2\).

- miR-122 and miR-192 were detected earlier than ALT and at lower doses.
- miR-122 had improved liver tissue specificity vs ALT.
- There is a high degree of cross-species conservation of microRNA sequences.

\(^1\)Wang K et al. Proc Natl Acad Sci USA 106: 4402-4407 (2009)
Other Exploratory Biomarkers of DILI Currently under Investigation

- Albumin mRNA
- α-glutathione S-transferase
- High-mobility group box 1
- Cytokeratin 18
- Glutamate dehydrogenase
- Sorbitol dehydrogenase
- F-protein
Assessing Potential for Testicular Toxicity in Men
Male Reproductive Safety – Preclinical Evaluation

- Preclinical toxicology studies may demonstrate testicular histopathological abnormalities in one species or in multiple species at high doses.
  - Histopathological changes – degeneration of spermatocytes, dilation of seminiferous tubules
  - Decrease in fertility, spermatagonia, increase in abnormal sperm in rodent reproductive toxicology studies
- There is no single species that is best for prediction of human risk.
- Abnormalities in any species may be a cause for regulatory concern leading to clinical holds.
Testis is a Dual Organ in Function and Structure

**Interstitial Compartment**
- Endocrine Function
- Leydig cell
- Low metabolic rate
- Fibroblast stem cells
- Resistant to toxicity
- Functional serum biomarkers are testosterone and LH

**Seminiferous Compartment**
- Exocrine Function
- Sertoli and germ cells
- Active turnover rate
- Spermatogonial stem cells
- Exquisitely sensitive to toxicity
- Functional serum biomarker is FSH (highly variable) and Inhibin B (needs further clinical validation)

Semen analysis still best choice to evaluate effects on sperm production despite challenges in collecting good samples
Strategy for Assessing Testicular Safety in Men

- Conduct Phase I multiple dose safety/PK study in a healthy volunteer population of vasectomized males and postmenopausal women.
  - Data used to select dose for testicular safety study, ensuring adequate safety margins.
- Conduct study in healthy men after single dose or short term treatment where a “responder” is defined as someone who has >50% reduction in sperm concentration from baseline.
  - Needs to cover at least 90 days after dosing – full spermatogenesis cycle (e.g. semen collections at baseline - predose, 65 days, 95 days, 125 days after dosing)
  - Power study to test non-inferiority (no increase in number of responders in treated group compared to placebo-treated group) – usually 100-150 participants
Strategy for Assessing Testicular Safety in Men

- Primary Endpoint
  - Concentration of viable sperm in semen

- Secondary Endpoints
  - Sperm motility in semen
  - LH, FSH, testosterone and Inhibin B in serum at timepoints shortly after drug dosing and at periods of semen sampling

- Controlling variability is critical to obtain statistical valid results
  - Limit number of sites (ideal one or two)
  - Limit number of laboratories doing sperm testing (ideal just one with same team that sets up at clinical site during sampling periods)
  - Multiple samples per time period (ideal is 3 over 24 hours after 48 hour abstinence)
Use of Accelerator Mass Spectrometry
Accelerator Mass Spectrometry

- Measures isotope ratios – can detect ultra low levels of $^{14}$C radioactivity
- Technology used in carbon dating of antiquities
- First biological application in 1989

Applications in Pharmaceutical Research (since 1998)

**Preclinical:** Special bioanalysis (proteins, monoclonal antibodies, interfering RNA); Phase 0 (subtherapeutic dose) clinical studies

**Early Clinical:** MIST (Metabolism in Safety Testing) solution, metabolic profiling, absolute bioavailability

**Clinical:** Bioanalysis of high potency drugs
Isotopic Tracers: Determination of Absolute Bioavailability (F)

- Oral dose
- IV dose (labelled)
- Blood samples over time
- LC-MS measures total drug
  - Providing the $^{14}$C-drug concentration is very small this = oral dose
- HPLC-AMS measures $^{14}$C-drug
  - This = IV dose

$$F = \frac{AUC_{po}}{AUC_{iv}} \times \frac{Dose_{iv}}{Dose_{po}}$$
When AMS Provides Enriched Data?

- Poor or variable bioavailability
  - Is absolute bioavailability too low?
  - Is it influenced by formulation?
  - Role of gut absorption/metabolism vs. hepatic metabolism and efflux
- Different metabolic profiles between species used in toxicology
  - Which species reflect human metabolic profile qualitatively and quantitatively?
- Exposure in tissues
  - Cerebral spinal fluid (CSF) exposure for CNS-acting drugs?
  - Systemic exposure for dermal, inhaled, optical, etc. drug delivery
- High potency drugs
  - Ultra-low concentration measurements

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Use of Mechanism/Efficacy Biomarkers in Early Clinical Development
Plethora of Biomarkers for Diabetes

- Glucose
- Fatty Acids
- C-Peptide
- TGs
- Glucagon
- Insulin
- HbA1c
- Adiponectin
- TCF7L2
- PPARγ (glitazones)
- KCNJ11
- Gastric Emptying
- CRP
- TNF-α
- IL-6
- IGF
- IGFBP
- GIP
- GLP-1
- OCT1 (metformin)
- OCT2 (metformin)
- DPP4
- Adiponectin
- TNF-α
- IL-6
# SAD Study of a Novel DPP-4 Inhibitor in Mild Diabetic Patients

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<th>Patients</th>
<th>Treatment Periods</th>
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Results of SAD Study in Mild Diabetic Patients: Early Evidence of Efficacy

**Drug Plasma Concentration**

F=4-8%

**Percent DPP-IV Inhibition**

**GLP-1 Concentration**

Good Activity

**Glucose Concentration**
Takeaways

- Solutions to Troublesome Problems in Early Clinical Development
  - New technologies, properly applied
  - Creative study designs – answer more questions in each study
  - Focus on reducing variability in key indicators of safety and efficacy

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