Biomarkers and Meaningful Endpoints: Perspective from an FDA Research Lab

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Background

- This presentation’s focus will be on liver CIVMs
  a) Most advanced and mature CIVMs
  b) Use in drug metabolism, drug-drug interactions (DDI), and drug-induced liver injury (DILI) assessments
    ▪ **Primary hepatocytes (PH)** are a CIVM described for use in drug metabolism and /DDI studies in the *FDA Guidance for Industry*
  c) CIVM findings from PH are included in *FDA approved drug labels*
    ▪ e.g.: “In in vitro studies using primary human hepatocyte cultures, modafinil was shown to slightly induce CYP1A2, CYP2B6 and CYP3A4 in a concentration-dependent manner...” (Drug@FDA database)
Background continued

• Regardless of the regulatory context of use (COU) of liver CIVMs, biomarkers and endpoints are needed to determine if cells are alive and functioning
  ▪ This is particularly important for CIVMs using PH, as the major challenge of culturing PH is spontaneous time-dependent loss of cell viability and liver functions

• Biomarkers for CIVMs and for the regulatory COU for CIVMs could have similarities and differences
  ▪ CIVMs were developed to address the drawbacks of traditional cell culture, not necessarily for a defined regulatory COU.

➢ This presentation will focus on biomarkers that could potentially be used for both purposes
General considerations

• There are numerous reported liver biomarkers/endpoints that could be used for CIVM, but only a few clinically-used biomarkers are being used by FDA for safety assessment
• Many biomarkers have been proposed for liver CIVMs, but the translation to clinical relevancy remains unclear
• The goal of this project is qualification of select CIVMs for defined COU in regulatory assessments. Therefore, priority levels in biomarker selection:

  FDA Guidance for Industry
  Biomarkers submitted for FDA/EMA qualification
  National and international clinical guidelines for liver disease
  Guideline papers for MPS
  Research articles
General considerations

• Ideally, liver CIVM biomarkers should reflect the dynamics of in vivo biomarkers, but this is not always possible
  ▪ Blood bilirubin: an FDA-accepted DILI biomarker and metabolite of hemoglobin in red blood cells (RBC) that can’t be measured in liver CIVMs, because RBCs are not included in the culture systems

• Liver CIVM biomarkers may **help address the drawbacks** of standard in vivo biomarkers
  ▪ ALT and AST are not specific to liver injury, as muscle injury also leads to ALT/ and AST elevations;
  ▪ CIVMs of liver (and/or muscle) may help differentiate if ALT and AST elevations are mainly from liver or non-liver cells
Candidate biomarkers for liver CIVM

- **Group 1:** Biomarkers described in the FDA DILI Guidance and Draft Guidance for “Noncirrhotic Nonalcoholic Steatohepatitis With Liver Fibrosis”
  
  1) **ALT and AST:** hepatocyte-enriched cytosolic (both) and mitochondrial (AST) enzymes that are released to culture medium when cell membrane integrity is compromised
     - Reflects hepatocyte necrosis (death)
  
  2) **ALP (alkaline phosphatase):** expressed in hepatocytes, bone, and intestine cells; highly induced and then released (excreted) by bile acid
     - Accumulation reflects cholestasis; intrahepatic vs. extrahepatic cholestasis
  
  3) **Vitamin K-dependent clotting factors:** reflected in vivo and clinically by INR (international normalized ratio; prothrombin time)
  
  4) **Bilirubin:** not applicable to CIVM
Candidate biomarkers for liver CIVM

• **Group 2:** Biomarkers submitted for FDA/EMA qualification
  
  1) Glutamate dehydrogenase (GLDH): highly liver-enriched mitochondrial protein released upon cell death
     - Not elevated in response to muscle injury
  
  2) CIVMs using primary liver cells and muscle-derived cells could potentially be used to test the tissue-specific elevations of GLDH after drug treatment
Candidate biomarkers for liver CIVM

- **Group 3:** National and international clinical guidelines for liver diseases

  1) Model for End-Stage Liver Disease: newest version is MELD 3.0;
     - Used to rank patients on the waitlist for liver transplantation by the United Network for Organ Sharing (UNOS) since 2002

  2) Endpoints: serum bilirubin, creatinine, INR, sodium, **albumin**
     - **Creatinine:** an in vivo biomarker for kidney functions, good candidate for kidney CIVM (?) but not liver CIVM
     - **Albumin:** has been widely reported for liver CIVM in the literature
Candidate biomarkers for liver CIVM

- **Group 4:** Guideline papers for MPS
  1) Albumin: >20 or 37 µg/million cells/day; <50% decrease in 14 days
  2) Urea: >56 µg/million cells/day; <50% decrease in 14 days
  3) Drug metabolism enzymes and transporters (DGMTs): stability over time
Candidate biomarkers for liver CIVM

- **Group 5**: Research articles
  1. miRNAs, particularly miRNA-122
  2. Mitochondrial DNAs
  3. Cell death: Lactic dehydrogenase (LDH) release, the most widely used cell death marker for all cell types
  4. Biomarkers reflecting mechanism of toxicity:
     - Redox Homeostasis: Glutathione levels
     - Mitochondrial functions: ATP, oxygen consumption rates, inner membrane potential
     - Oxidative stress: Reactive oxygen/nitrogen species (ROS/RNS)
     - Apoptosis: Caspase activities
  5. Omics-based biomarkers, including expression levels of DMETs
  6. Genetic factors: human leukocyte antigens (HLA) and DMET polymorphism
  7. Biomarkers for nonparenchymal cells (NPC): cytokines, cell-type specific protein markers
# Candidate biomarkers for liver CIVM

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Red fonts: in vivo biomarkers; Blue fonts: CIVM biomarkers
Considerations in measuring biomarkers in CIVM

- The matrix is different from clinically-established biomarkers: medium vs. serum
- The medium will affect biomarker stability

Stability of ALT and AST activity in the lysate of primary human hepatocytes in a liver-chip (n=3). Note: It’s been established ALT/AST activities are stable in human serum.
Considerations in measuring biomarkers in CIVM

- The matrix can affect sensitivity, specificity, and compatibility of detection methods

ALT activity and protein levels in the effluent of primary human hepatocytes cultured in a liver-chip. APAP, acetaminophen.
Considerations in measuring biomarkers in CIVM

- Proposed reference ranges in the liver MPS guideline papers, based on in vivo turnover rates:
  - **Albumin:** >37 or 20 µg/million cells/day
  - **Urea:** >56 µg/million cells/day

- Since the reference ranges of biomarkers in CIVMs may differ from in vivo, these ranges be impractical.

Should these ranges be based on CIVM experience?
- It took decades of effort to establish reference ranges for currently-used clinical biomarkers, such as ALT and AST.
Considerations in measuring biomarkers in CIVM

• The dynamics of CIVM biomarkers also depend on the system design:
  – Perfused vs. non-perfused;
  – Open perfusion vs. closed perfusion

• In open perfusion systems, the perfusing medium was removed at desired intervals to measure biomarkers and then new medium added.
  – Could result in diluting effects
  – May not have enough cells to produce the biomarkers when cell death occurs

➢ This is sharply different from the in vivo condition, wherein only a neglectable fraction of blood is collected to measure the biomarkers
Questions for discussion

- Are ALT/AST good translational biomarkers in CIVM? If so, is there a need to establish the reference range of ALT/AST in CIVM, and how (based on CIVM experience or extrapolations from in vivo data)? Regarding DILI, is the extent of change more important, as suggested in the FDA DILI Guidance (>3-fold elevation vs smaller changes)?

- Albumin is decreased without apparent cell death. What is the in vivo/clinical implications of such changes? Will this be a concern regarding drug-induced liver injury (DILI)?

- The expression/activity of DGMTs will certainly decrease over time in CIVM. What is the threshold for an acceptable model? Will 50% decrease (compared to time 0 when cells are thawed) be considered as acceptable? Will this be COU-dependent, for example, a higher level will be required for drug metabolism?
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Thank you for your attendance!