Report of a Workshop on Dose-Response Approaches for Nuclear Receptor-Mediated Modes of Action

The 2010 Society of Risk Analysis Meeting
Salt Lake City, Utah

Robert Budinsky
The Dow Chemical Company
Outline

• Nuclear Receptor Background
  – CAR/PXR, PPARα and AHR Rodent Liver Tumors

• Nuclear Receptor Workshop
  – Government, Academic, Consulting and Industry Scientists
  – NIEHS facility
  – Case studies guided by charge questions, human relevance framework and mode-of-action key events assessment

• Dose-Response Modeling
Summary of signaling pathways for CAR and PXR, and the nuclear steroid receptors ER and GR.

Timsit and Negishi, 2007
Nuclear Receptor-Promoted Liver Tumors

- **Rodent Liver Tumors**
  - Phenobarbital: CAR/PXR
  - Cholesterol-lowering drugs: PPARα
  - TCDD: AHR

- **Human Liver Cancer Evidence**
  - Negative for phenobarbital
  - Negative for statins
  - Negative to equivocal for TCDD (lung and all cancer mortality in slight excess)
Conference Co-Chairs:  J. Preston and M. Andersen

Steering Committee Members:  M. Cunningham, M. Dourson
R. Becker, M. Honeycutt, R. Budinsky, C. Elcombe, J. Klaunig

CAR/PXR
D. Wolf/C. Elcombe
  R. Barrs
  D. Bell
  R. Cattley
  R. Conolly
  K. Crump
  S. Ferguson
  D. Geter
  A. Goetz
  J. Goodman
  S. Hester
  A. Jacobs
  B. Lake
  C. Omniecinski
  R. Peffer
  J. Ross
  R. Schoeny
  A. Vardy
  W. Xie

PPARα
C. Corton/J. Klaunig
  P. Bentley
  M. Cunningham
  Y. Dragan
  T. Hummer
  B. Meek
  J. Peters
  J. Popp
  L. Rhomberg
  J. Seed

AHR
D. Schrenk/B. Budinsky
  N. Walker
  A. Brix
  T. Simon
  A. Aylward
  B. Allen
  T. Starr
  G. Perdew
  T. Gasiewicz
  M. van den Berg
  N. Kaminski
  M. Andersen
  R. Thomas
  C. Rowlands
  A. Maier
Structure of Workshop

• Day 1
  – Morning Plenary Sessions
  – Begin Afternoon Case Study Discussion (Background review of key events)

• Day 2
  – Continuation of Case Study Discussions (Charge Questions)

• Day 3
  – Report of Case Study Discussion to the Workshop Attendees
Major Goals of the Workshop

Establish a mode of action (MOA) for NR-mediated rodent liver tumors
• Apply the IPCS Framework for Human Relevance and the modified Hill Criteria applied to MOA

General Charge Questions
1. Is a minimum threshold of receptor ligand required for
   • gene transcription?
   • biochemical, cellular and tissue responses?
2. Is linear low-dose modeling of receptor ligands appropriate, based on the underlying science of nuclear receptor signaling biology, and if not, provide insights into more appropriate low-dose modeling approaches?
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Draft Human Relevance Framework (ILSI-HESI risk 21)

1.) Is the Weight of Evidence Sufficient to Establish a MoA in Animals?
   - Not Sufficient
   - Sufficient

2.) Is the Animal MoA Plausible in Humans?
   - No
   - Yes

3.) Taking Into Account Kinetic and Dynamic Factors, Is the Animal MoA Plausible in Humans?
   - No
   - Yes

- Relevant to human health
- Not relevant to human health

Use MoE approach on most sensitive apical endpoint

What is the dose response for each key event?

What are the modulating factors for key events of the human DR (e.g., repair, polymorphisms)?

How do the key events and their modulating factors vary within the human population?

Establish acceptable exposure limit, using MoE approach on most appropriate key event

Clearly communicate all steps in assessment

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Using Hill Considerations to Determine Key Events for Rodent Liver Tumor MOA

Evaluate Possible Key Events

- Strength
- Consistency
- Specificity
- Temporality
- Biological Gradient
- Biological Plausibility
- Coherence

- Causal Key Event
- Associated Event (marker)
- Modulating Factor
- None of the above
AHR-Key Events: Rodent Liver Tumorigenicity

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CAR MOA-Key Events: Rodent Liver Tumorigenicity

CAR Over Activation

- Altered gene expression specific to CAR activation
- Altered epigenetic changes specific to CAR activation
- Cyp2b Induction
- Hypertrophy
- Increased cell proliferation
- Decreased apoptosis
- Clonal expansion leading to altered foci
- Gap Junction Communication Inhibition
- Liver Adenomas/Carcinomas
**PPARα-Key Events: Rodent Liver Tumorigenicity**

*Key Events: 2010 version*

1. Metabolic activation – *if necessary*
2. Activation of the PPARα
3. Increased hepatocellular proliferation with or without decrease apoptosis
4. Selective expansion of preneoplastic hepatocytes
5. Neoplasm formation
## AHR Species Concordance

<table>
<thead>
<tr>
<th>Key Event</th>
<th>Rats</th>
<th>Humans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sustained AHR Activation</td>
<td>Yes (in vitro and in vivo based on associative events – XME gene expression)</td>
<td>Yes (in vitro and in vivo based on associative events – XME gene expression)</td>
</tr>
<tr>
<td>Inhibition of Apoptosis</td>
<td>Yes (in vitro and in vivo data) – mechanism not yet clear</td>
<td>Yes (based on in vitro data in human cells); no in vivo data</td>
</tr>
<tr>
<td>Altered Hepatic Foci</td>
<td>Yes (observed in rat bioassays)</td>
<td>Inadequate data</td>
</tr>
<tr>
<td>Liver tumors</td>
<td>Yes</td>
<td>Negative to equivocal for liver and bile duct tumors</td>
</tr>
</tbody>
</table>

Note: There are data for early key events that suggest quantitative differences – but magnitude is likely to be endpoint specific.
## CAR Species Concordance

### Species Concordance Table – CAR Activation MOA with Phenobarbital as One Example

<table>
<thead>
<tr>
<th>Key Event or Marker</th>
<th>Mouse</th>
<th>Rat</th>
<th>Hamsters</th>
<th>Primates</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAR activation</td>
<td>Yes (1, 2) (in vitro and in vivo)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes (157) acknowledge differences (in vitro)</td>
</tr>
<tr>
<td>Altered gene expression</td>
<td>Yes (16)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes (in vitro)</td>
</tr>
<tr>
<td>Altered DNA methylation/epigenetic changes</td>
<td>DNA methylation altered (35-37)</td>
<td>DNA methylation altered(^{24})</td>
<td>No data</td>
<td>No data</td>
<td>Possible but no data</td>
</tr>
<tr>
<td>Cyp 2B induction</td>
<td>Yes (16)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes (154) (in vitro)</td>
</tr>
<tr>
<td>Hypertrophy</td>
<td>Yes (16)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes (121, 168, 169) (in vivo)</td>
</tr>
<tr>
<td>Increased cell proliferation</td>
<td>Yes (16)</td>
<td>Yes(^{28})</td>
<td>No</td>
<td>No? (check for refs)</td>
<td>No (171) (in vitro) (and hCAR/ hPXR mice in vivo)</td>
</tr>
<tr>
<td>Decreased apoptosis</td>
<td>Yes – but mixed results (44, 47, 145, 146)</td>
<td>Yes</td>
<td>No</td>
<td>?</td>
<td>No (137) (in vitro)</td>
</tr>
<tr>
<td>Gap Junction Intracellular Communication inhibition</td>
<td>Yes</td>
<td>Yes</td>
<td>No (Klaunig)</td>
<td>No (Klaunig)</td>
<td>No (Baker, 1995) (in vitro)</td>
</tr>
<tr>
<td>Clonal expansion (Foci)</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No data</td>
<td>Possible but No data either way</td>
</tr>
<tr>
<td>Tumors</td>
<td>Yes – most strains (9, 10, 15)</td>
<td>Yes – certain strains (9, 10)</td>
<td>No (131)</td>
<td>No data</td>
<td>No (9, 10) in vivo</td>
</tr>
</tbody>
</table>
## PPARα Species Concordance

<table>
<thead>
<tr>
<th>STEP</th>
<th>Qualitative</th>
<th>Quantitative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolism</td>
<td>plausible</td>
<td>Same for rodents and humans</td>
</tr>
<tr>
<td>Activation</td>
<td>plausible</td>
<td>Higher MEHP concentrations needed to activate human receptor (~3-10 fold)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Target genes not responding in human cells compared to rat cell line</td>
</tr>
<tr>
<td>Proliferation</td>
<td>plausible</td>
<td>Non-human primates don’t respond (cell proliferation)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Liver size not changed in humans (Based on MRI)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Humanized mice – no effect at tumorigenic doses</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Uniformly negative for DNA replication in human (&amp; non-human primate) hepatocytes</td>
</tr>
<tr>
<td>Foci</td>
<td>plausible</td>
<td>No evidence</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fairly rare observation in human liver</td>
</tr>
<tr>
<td>Tumors</td>
<td>plausible</td>
<td>Epi data – no evidence (decades of exposures) – albeit @ lower doses than tumor production in rodents (gemfib and clofib)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Usually in humans requires chronic injury, infection (Hep B,C, etc), chirrosis (alcohol)</td>
</tr>
</tbody>
</table>
Desired MOA Data Sets

• **Exclude other MOAs** (cytotox, mutagenicity, if not already available).

• **Data to confirm the rodent MOA**
  – Appropriate rodent studies to examine endpoints including:
    • early, observable key events (e.g. Cell proliferation, CYP induction, apoptosis suppression, hypertrophy, liver wt)
    • Evaluate in a dose-response design.
  – Knockout models
  – Genomics, Proteomics

• **Confirmation of lack of human relevance via NR MOA**
  – e.g. use of primary human hepatocytes and when appropriate humanized models
Quantitative Dose-Response Modeling

Nuclear Receptor Molecular Interactions

Ligand Binding
Partner Protein(s)
Co-Regulatory Proteins

Transcription and Translation

mRNA (RT-PCR, genomics)
Protein Formation

Change in Enzyme Activity or Protein Function

CYPs
ROS

Foci, BrdU, Apoptosis
Histopathology
Clinical Changes

Change in Cell, Tissue, or Organ Function

Timing? AUC

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Dose Response Example for AHR: Hepatocellular Cancer Key Event

Multinucleated Hepatocyte RfD: 2 – 70 pg/kg/day (UFs: 1.0 – 30)
Hepatocellular Cancer RfD: 20 to 600 pg/kg/day (UFs: 1.0 – 30)

[Graph showing dose-response relationship with TCDD in blood vs. frequency of effect]

based on Simon et al., 2009
Summary

The AHR expert panel, for the first time in an expert panel format, rigorously applied the MOA framework and agreed on a mode of action.

The CAR expert panel identified the relevant data and rigorously applied the MOA and HRF with emphasis on the qualitative and quantitative aspects of human relevance.

The PPARα expert panel built upon previous applications of the framework using significant new data that allowed for refinement of the key event descriptions and updated considerations related to human relevance.

Each panel identified key data needs and suggested improvements for application of the MOA/HRF.

A series of manuscripts will be forthcoming on the results of this workshop.