

# **Drug Metabolizing Enzyme Induction Survey of Current Practices**

A PhRMA Drug Metabolism  
Technical Group Initiative

Gondi Kumar, Celgene Corporation

# Consequences of Enzyme Induction

- Decreased exposure to NME (auto-induction, eg. carbamazepine)
- Decreased exposure to a coadministered medication
- Sub-therapeutic concentrations of NME or co-med
- Altered levels of active or toxic metabolites
- DDI magnitude varies widely for CYP isoforms

CYP	Inducer (substrate)	Clinical effect	Ref
<b>1A2</b>	<b>Omeprazole</b> (caffeine)	39% increase in exhalation of $^{14}\text{C-CO}_2$ in CYP2C19 poor and intermediate metabolizers compared to 12% increase in extensive metabolizers.	Rost et al., 1992
<b>3A4 &amp; 2C8</b>	<b>Rifampicin</b> (repaglinide)	AUC decreased by 57%; Blood glucose decremental AUC(3h) reduced from 0.94 to -0.23 mmol/L	Niemi et al., 2000

# PhRMA PISC Initiative

- One of the ten PhRMA/PISC Working Groups is about *Predictive Models of Safety, Efficacy, and Compound Properties*.
- This initiative is a part of *Predictive Models of Compound Properties*.
- Assess the predictability of various in vitro experimental models currently used across the industry to predict drug-drug interactions due to enzyme induction.

# Enzyme Induction Team

PhRMA Drug Metabolism Technical Group formed an expert team from member companies to steer this effort

Valeria Chu (Sanofi-Aventis)

Heidi Einolf (Novartis)

Raymond Evers (Merck)

Gondi Kumar (Celgene)

David Moore (Roche),

Sharon Ripp (Pfizer),

Jose Silva (Johnson & Johnson),

Vikram Sinha (Lilly),

Michael Sinz (Bristol-Myers Squibb),

Andrej Skerjanec (Novartis)

# Enzyme Induction Initiative - Objectives

The key objectives of this initiative are:

1. identify the current practices employed by PhRMA member companies;
2. collate information on methods and models;
3. assess the success/failure of predictability based on the current methods and models;
4. identify areas with the greatest need for better predictive methods and models;
5. stimulate interest and promote research into the development of better predictive methods; and
6. foster development of general methodologies and frameworks which may help decrease compound attrition during drug development.

# Enzyme Induction Initiative - Goals

- Enzyme Induction Team will conduct a survey on the current practices in this area.
- Information gathered through this anonymous survey will be collated and blinded by the PhRMA office and forwarded to the expert team for analysis.
- The expert team will conduct comprehensive data analysis examining the in vitro-in vivo correlations, and factors leading to successful or unsuccessful predictions.
- The methodological information gathered from this survey and analysis data will be presented as a publication.

# Enzyme Induction Survey

- **Survey Questionnaire** (61 questions)
  - Nuclear Receptor Assays
  - Immortalized Hepatocyte Assays
  - Human Hepatocyte Assays
  - Clinical Induction Studies
  - New Technologies
- **Survey Data Sheet**
  - In Vitro Experimental Conditions and Results
  - Examples of Clinical Studies and Outcome

# Enzyme Induction Survey - Responses

## Information on Survey Responders

Size of Your Company (employees)	10	> 10,000
	4	1,000 – 10,000
	X	< 1000

**Very limited response to Data Sheet request**

**Analysis of published data**



## Perspective

# In Vitro and in Vivo Induction of Cytochrome P450: A Survey of the Current Practices and Recommendations: A Pharmaceutical Research and Manufacturers of America Perspective<sup>S</sup>

Valeria Chu, Heidi J. Einolf, Raymond Evers, Gondi Kumar, David Moore, Sharon Ripp, Jose Silva, Vikram Sinha, Michael Sinz, and Andrej Skerjanec

Survey response details as “Supplemental information”

References cited therein

## Highlights: Nuclear Receptor Assays

- Majority consider PXR very important; CAR and AhR somewhat important
- 14% use *in silico* approaches to assess PXR interactions (some retrospectively)
- Assays: primarily PXR, some AhR, planning for CAR
- Timing: primarily in Discovery (75%), some later
- 64% transactivation assays, 7% binding assays
- Limited efforts for other species (17%)

# Highlights: Transactivation Assays

- Variety of formats employed
  - Stable (29%) vs. Transient (71%) transfection
  - Medium to high throughput assay format
- Rifampicin as positive control (100%) for PXR
- Data expressed in a variety of ways:
  - EC<sub>50</sub> (majority), as % or fold- of PC
  - majority do not assess for partial agonism
  - 50% encounter antagonists, but not clear how to interpret
- Consideration of cytotoxicity, stability and solubility important when interpreting results

## Highlights: Transactivation Assays

- Clinical drug concentrations should be taken into consideration when interpreting results
- Generally used to rank order as low, moderate and high DDI potential
- No false positives in high risk category
- Direct extrapolation for quantitative prediction of DDI magnitude not yet feasible
  
- No regulatory requirement for these assays, but a useful tool at discovery stage and for mechanistic assessments

# Immortalized Hepatocyte Assays

- 63% of responders use immortalized hepatocytes (mostly in discovery, some development and mechanistic use)
- Fa2N-4 cell line (1A2, 3A4), others (HepG2, BC2, HepaRG)
- Issues related to CAR and transporters
- No single cell line affords an exact reproduction of primary hepatocytes
- Not considered adequate replacement of primary hepatocytes for definitive studies

# Primary Hepatocyte Assays

Primary Hepatocyte Related Questions	Survey Response
Percentage of companies routinely employing primary human hepatocyte induction studies	Yes (Discovery): 58% Yes (Development): 83% Use only when requested: 17% Do not use: 17%
Fresh vs. cryopreserved human hepatocytes	Fresh only: 25% Cryopreserved only: 17% Both: 58%
Where do you conduct human hepatocyte induction studies?	In-house only: 46% External only (CRO/academic): 0% Combination of internal and external: 54%
Human hepatocytes are obtained from	Commercial source: 100%; Hospital or university: 14%; In-house: 14%

# Primary Hepatocyte Assays

Primary Hepatocyte Related Questions	Survey Response
What considerations limit your use of human hepatocytes?	Cost: 46%; Availability: 62%; Results not valuable: 8%; Technical difficulty: 8%
Which enzymes are routinely assessed and do you evaluate transporters?	CYP3A4: 100%; CYP2B6: 64% CYP2C9: 50%; CYP1A2: 86% Transporters: 14%
How many donors are routinely used to evaluate induction?	Discovery: 1 donor (73%) 2 or more donors (27%) Development: 3 donors (100%)
What do you routinely measure as an endpoint?	Enzyme activity: 100% mRNA: 77% Protein: 8%
How do you measure enzyme activity?	In situ (single probe): 79% In situ (cassette): 14% Microsomes: 29%

# Primary Hepatocyte Assays

Primary Hepatocyte Related Questions	Survey Response
How do you measure RNA expression?	RT-PCR: 85%; Branched DNA: 15%; Quantitative nuclease protection (HTG): 0%
Do you routinely determine solubility or cytotoxicity	Solubility only: 0%; Cytotoxicity only: 31%; Solubility and cytotoxicity: 46%; Do not routinely assess solubility or cytotoxicity: 23%
How do you interpret human hepatocyte data when assessing enzyme induction potential?	Fold induction above vehicle control: 46% Percent of a positive control: 100% EC <sub>50</sub> : 15%; Other (E <sub>max</sub> /EC <sub>50</sub> , AUC/EC <sub>50</sub> , C <sub>max</sub> /EC <sub>50</sub> ): 15%
Which in vivo drug concentration do you use in interpreting enzyme induction potential?	C <sub>max</sub> total concentration: 71% C <sub>max</sub> unbound concentration: 36% Liver input total concentration: 7% Liver input unbound concentration: 0%



# Hepatocyte Assays - Issues

- **Inverted U-shaped dose response curves:**
  - Solubility/ cytotoxicity may not explain all cases
  - Is it true underlying pharmacology that is not yet understood?
- **Increased RNA expression with little to no increase in activity:** NME likely both an inducer and inhibitor. For reversible inhibitors or potent metabolite inhibitors assays with isolated microsomes, but not for mechanism-based or time-dependent inhibitors.
- **When only one of several donor hepatocyte preparations indicates a positive induction response:** cannot be ignored or discounted as an outlier; follow on studies may be needed.

# Hepatocyte Assays - Activity or mRNA?

- **mRNA data is considered appropriate based on:**
  - induction involves receptor binding and gene transcription; hence mRNA production is a more direct measure of this event than enzyme activity,
  - a better dynamic range than enzyme activity
  - *in situ* activity assays can potentially result in false negatives (mechanism-based or potent inhibitory metabolites).
- A good correlation between CYP3A4 mRNA and activity found when mechanism-based inhibitors or formation of metabolites that are potent reversible inhibitors are excluded.
- Due to the potential for interplay between inhibition and induction, both activity and mRNA endpoints are needed to fully interpret the data.

# Primary Hepatocyte Assays

## Data Analysis and Extrapolation

- Mathematical models:  
Emax model:
  - total or unbound concs,
  - fraction of the substrate metabolized via CYP,
  - fraction unbound in the incubation medium
  - Emax varies widely between donors,  
normalization is needed with a calibrator
- Correlation (calibration curve) approaches
  - Relative Induction Score (RIS)
  - AUC/F2 approach
- Physiologically based models

# Hepatocyte Assays - Recommendations

- Fresh or platable cryopreserved hepatocytes, as either monolayer or sandwich culture, with a 1-2 day recovery period after plating.
- Treatment with NME and positive controls for 2-3 days (changing medium with test compounds every 24 hours) in media containing ITS, dexamethasone, and penicillin-streptomycin as media supplements.
- Test compounds dissolved preferably in DMSO (v/v 0.1%) whenever possible and incubated at 3 or more different concentrations (in triplicate), spanning anticipated or known therapeutic concentration range including a concentration at least an order of magnitude higher. Alternate solvents and NME concentration ranges may be employed, as appropriate.

# Hepatocyte Assays - Recommendations

- The recommended positive controls include omeprazole (25-50  $\mu\text{M}$ ), phenobarbital (1000  $\mu\text{M}$ ) and rifampicin (10  $\mu\text{M}$ ) for CYP1A2, 2B6, and 3A4, respectively, at concentrations known to elicit maximal induction response.
- Assessment of cytotoxicity of the NME under the experimental conditions.
- Knowledge of aqueous solubility characteristics and visual assessment of solubility in the culture medium at the concentrations tested.
- Catalytic activity and mRNA assessments for CYP1A2, CYP2B6 and CYP3A4.
- For a hepatocyte induction experiment to be acceptable, CYP1A2, CYP2B6 and CYP3A4 positive controls should exhibit  $\geq 2$ -fold vehicle control catalytic activity and  $\geq 6$ -fold vehicle control mRNA level.

# Hepatocyte Assays - Recommendations

- At least three donor hepatocytes, with experiment meeting the acceptable criteria for each donor.
- A positive result in at least one of the three donor hepatocytes is considered an indication of induction.
- Interpretation of results is conducted by an empirical approach such as 'percent change compared to the positive control' or a mathematical or correlation based approach, using the therapeutic C<sub>max</sub> drug concentration at steady-state as benchmark.
- The use of free (unbound) or total (bound and unbound) drug concentrations for data interpretation may be based on the available historical data and/or models employed at each laboratory.
- Lack of 3A4 induction can be interpreted as no effect on other PXR-dependent enzymes/transporters.

## Highlights: Clinical Induction Studies

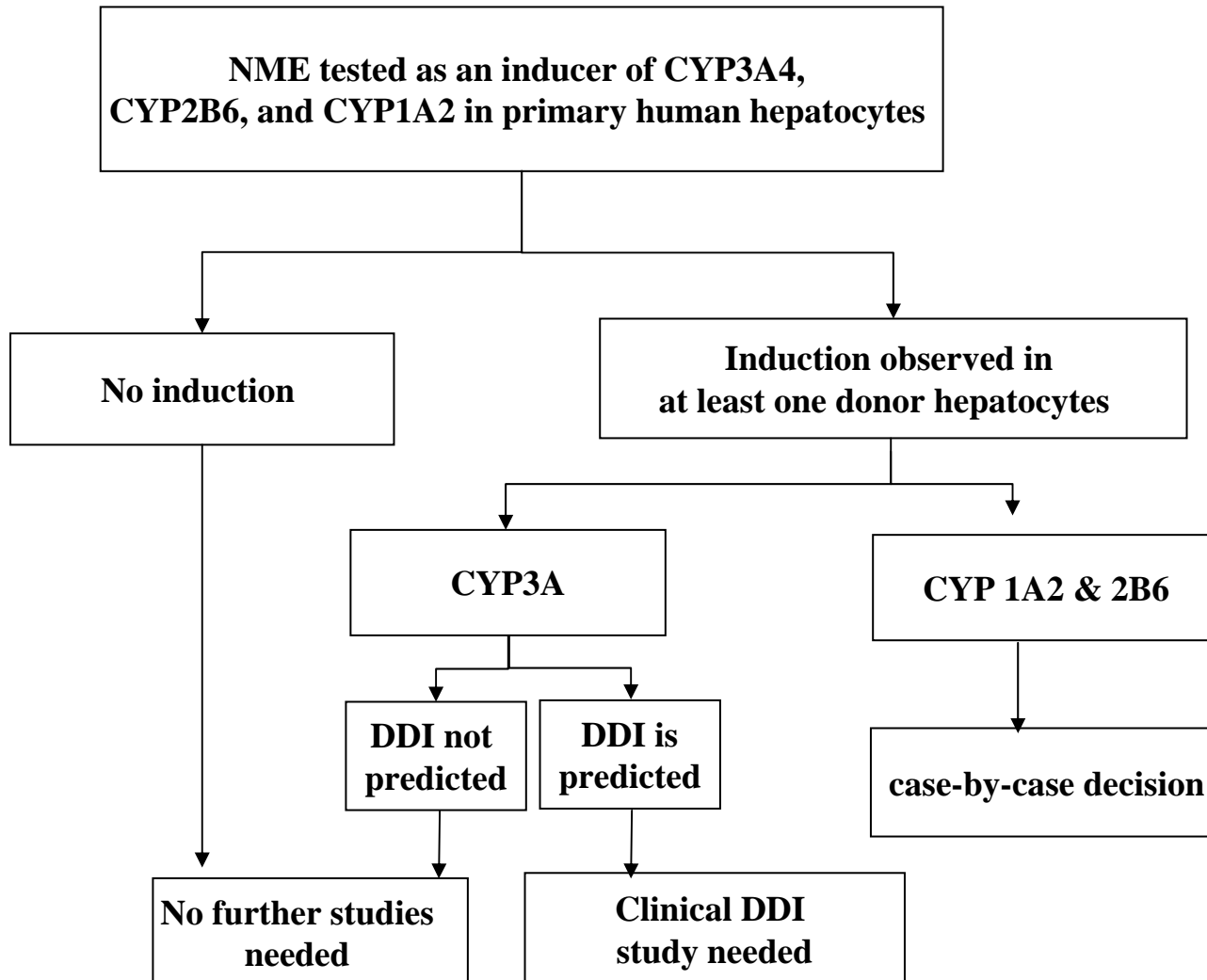
- Well-accepted within companies as a good model for drug metabolizing enzyme induction
- How is the data presented to clinicians?
  - in the context of NME clinical concentrations,
  - comparison to other molecules in the same therapeutic class,
  - clinical relevance & implications for labeling,
  - implications for safety and efficacy in the context of reduced NME exposure (autoinduction)
  - potential for increased exposure to metabolites (active or toxic metabolites).

## Highlights: Clinical Induction Studies

- How do your clinical colleagues use the information?
  - design clinical development program
  - guidance for inclusion/exclusion criteria and contraindications,
  - Go/No decision for further development
- Study population: 77% use healthy volunteers, except for oncologic and cytotoxic NMEs
- Limited use of urinary  $6\beta$ -hydroxycortisol or the erythromycin breath test



# Recommended Decision Tree for Clinical Induction Studies



# Recommendations for Clinical Drug-Drug Interaction Studies

- Choose the most sensitive probe substrate for clinical DDI study.
- NME dosed to steady state or a clinically relevant regimen at the highest dose and shortest dosing interval intended for the marketing approval.
- Assess PK of probe before and after NME treatment, and document NME exposure.
- Data analysis and interpretation should include clinical consequences of induction relative to the therapeutic index of the substrate.

# Alternate or New Technologies

	Not appropriate	Needs more work	Almost ready	Ready for use
Nuclear receptor in silico models	3	7	2	0
In vitro-in vivo modeling techniques, software platforms	3	7	2	0
Humanized mouse (e.g., hPXR mouse)	5	7	1	0
Monkey in vivo models	7	1	1	1

# What is needed for better predictions?

- Better understanding of relationship between concentrations in vitro and in vivo; free vs total; systemic vs. portal
- Expand database for CAR and AhR mediated induction
- Better approaches for IVIVE
- Need for biomarkers without the need for DDI studies

# Conclusions

- In vitro induction assays have gained wide acceptance, leading to avoidance of unnecessary clinical DDI studies
- Notable progress has been made in extrapolating in vitro data for predicting magnitude of DDI.
- It should be clearly stated that a simple experimental approach, for example NME tested at relevant clinical concentrations (with appropriate comparison to PC) would be sufficient for decision making purposes; such data will provide enough information to design an appropriate clinical program.
- If a company feels that a more quantitative prediction is necessary, a more elaborate set of *in vitro* studies may be performed and an *in vitro* – *in vivo* correlation may be attempted, but such strategies are not needed as a routine practice.

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  - Jose Silva (Johnson & Johnson)
  - Vikram Sinha (Lilly)
  - Michael Sinz (Bristol-Myers Squibb)
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