2C or Not 2C: CYP2C Induction Studies for Successful Preclinical Risk Assessment

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Presentation Outline

- Why is measuring CYP induction important?
- What is the mechanism of enzyme induction leading to DDI?
- Real world induction DDI examples
- Basic terminology for enzyme induction
- General mechanism of enzyme induction
- Induction study guidance recommendations
- Common questions on CYP2C induction

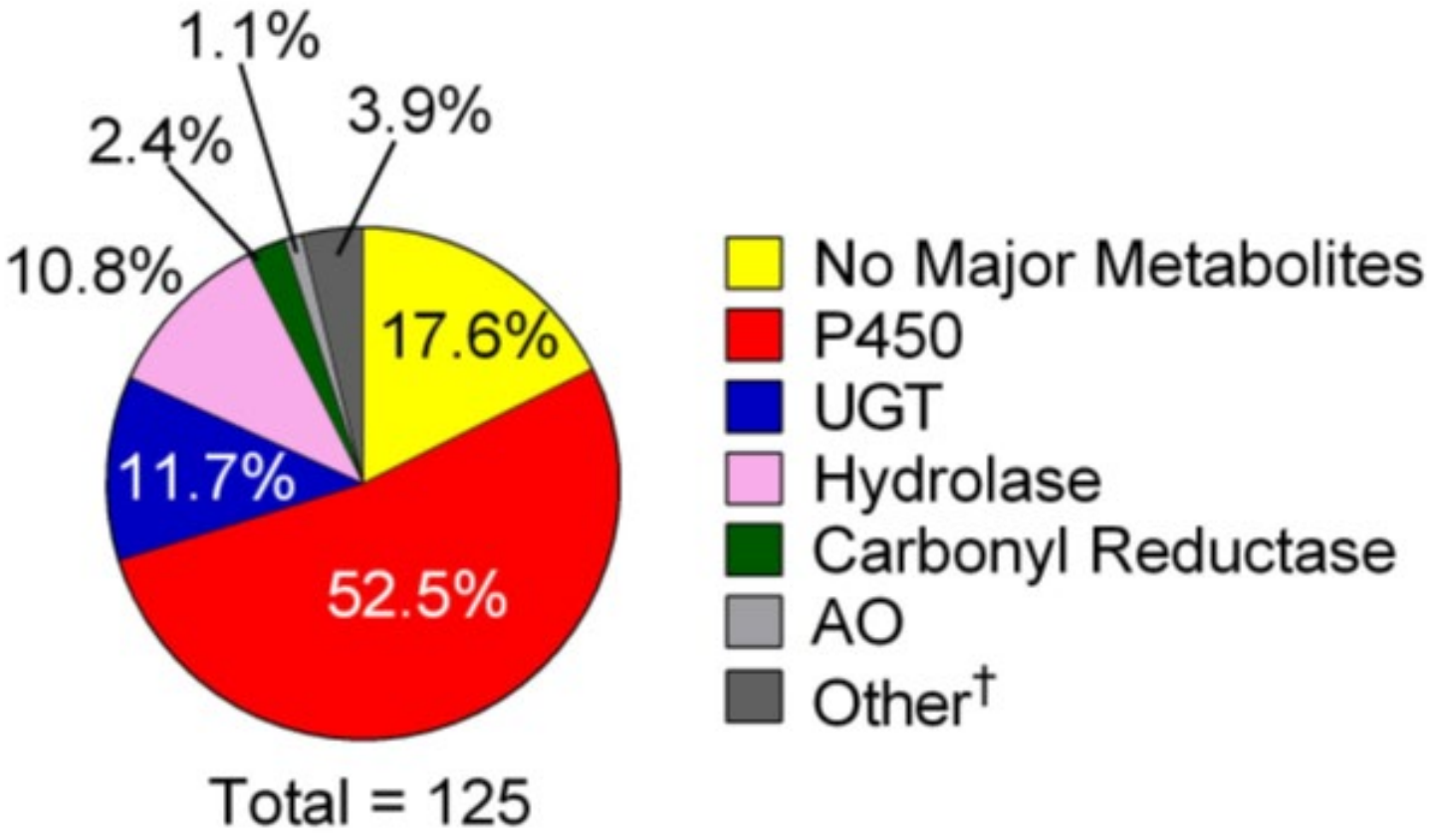
Interactive Webinar Questions for Audience

Who has ever needed to incorporate 2C induction into panel?

If so, were your induction studies done initially (pre-IND or pre-NDA), or as a follow up based on clinical findings or regulatory comments?

Why is Measuring CYP Induction Important?

Fractional assignment of approved drugs (2006-2015)



† - Other represents: sulfotransferases (0.8%), cytidine deaminase (0.8%), dehydropeptidase-I (0.8%), nucleotidases (0.8%), alcohol/aldehyde dehydrogenase (0.4%), flavin-containing monooxygenases (0.3%), glutathione conjugation (0.3%), gut microbes (0.3%), undefined/unknown (0.3%)

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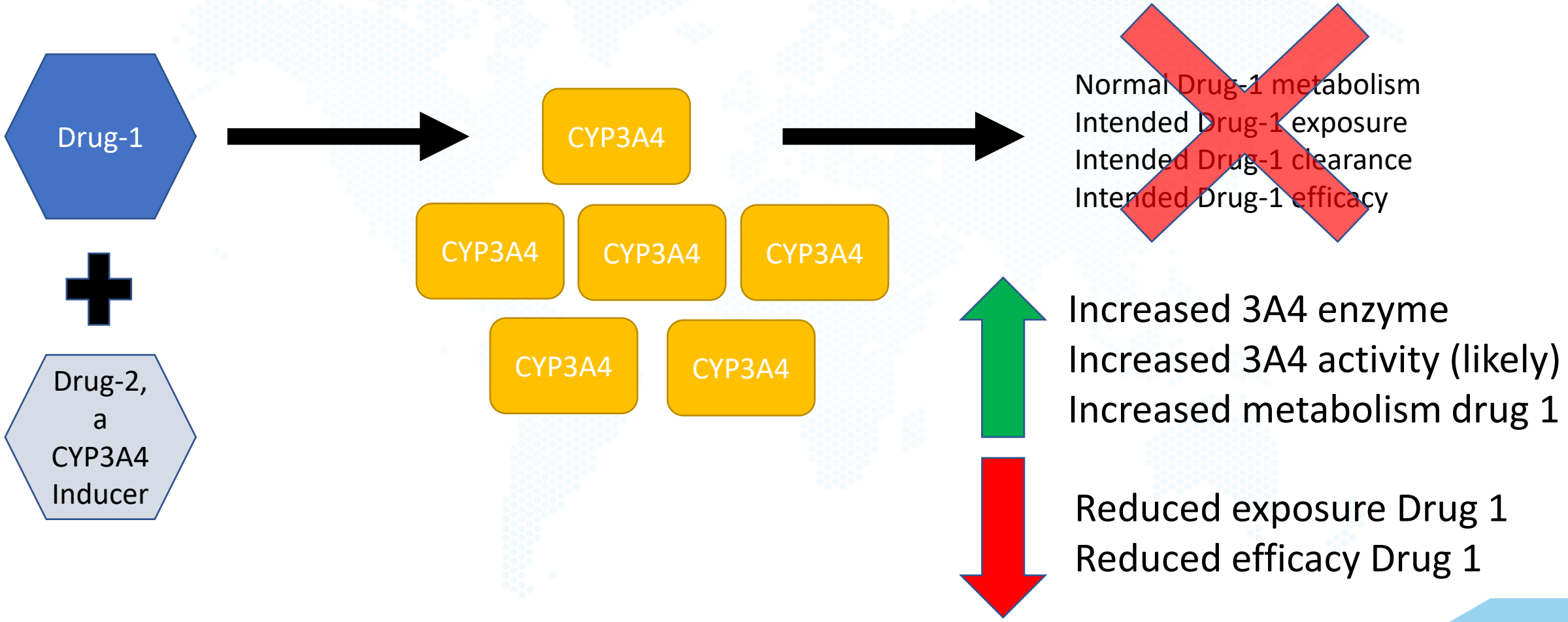
Induction DDI General Mechanism



Induction DDI Real World Examples

Oral contraceptives and rifampin (birth control and antibiotics)

Oral contraceptives increase plasma estrogens/progestins

Estrogens/progestins are metabolized by CYP3A4

Rifampin is a strong CYP3A4 inducer

Oral contraceptive + rifampin → increased CYP3A4 → decreased plasma estrogens→ decreased contraceptive efficacy

St. John's wort

St. John's wort is a CYP3A4 inducer

Can reduce plasma concentrations of other drugs that are CYP3A4 substrates

Basic Terminology for Enzyme Induction

EC_{50}

- Concentration of the drug that gives the half-maximal response
- Conceptually similar to $IC_{50,}$ but looking at response instead of inhibition
- It is a concentration (e.g., µM or mg/mL)

E_{max}

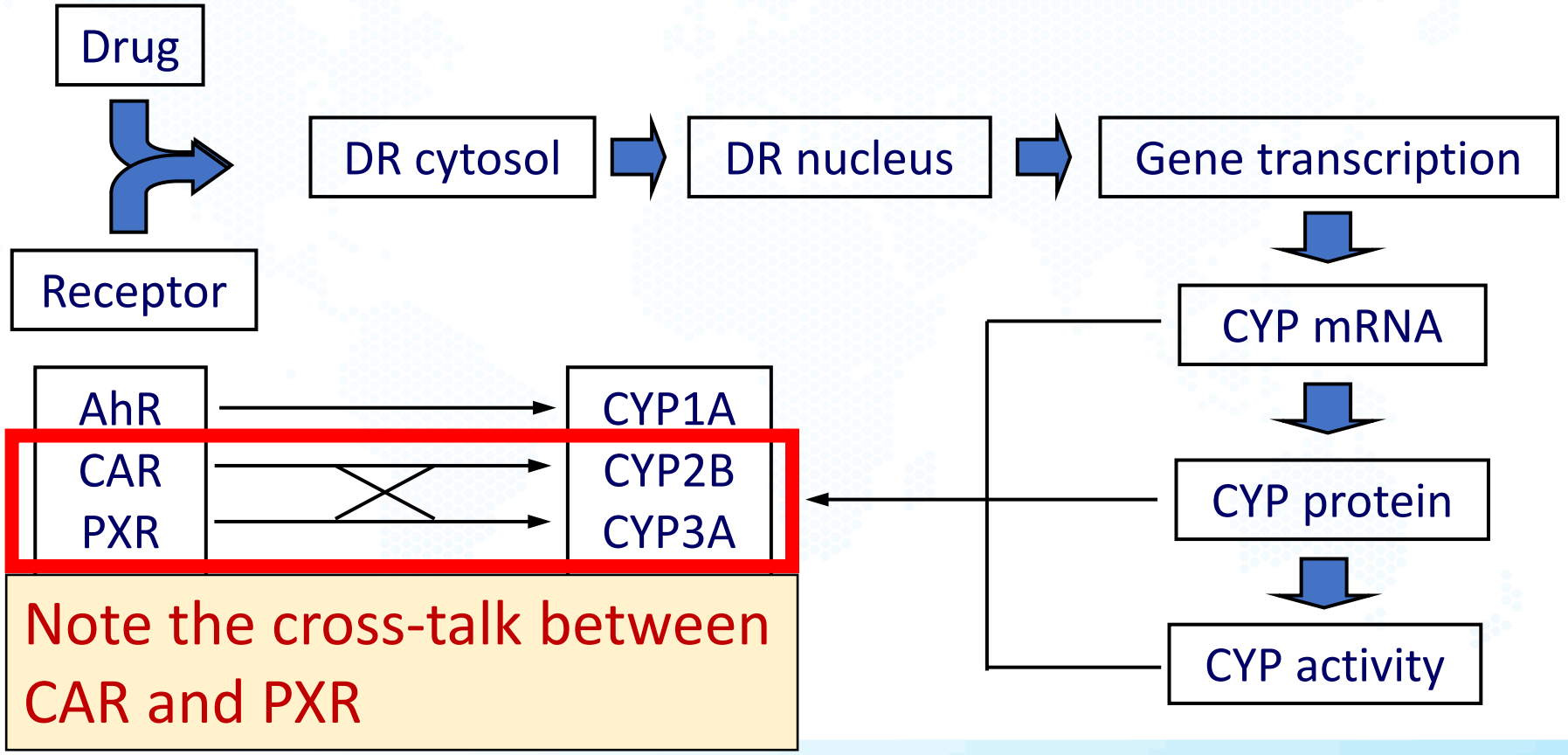
- "E" is the effect at drug concentration C
- E_{max} is the maximal effect at high drug concentrations when all receptors are occupied by the drug
- It is fold change (compared to vehicle control)

Prototypical Inducer

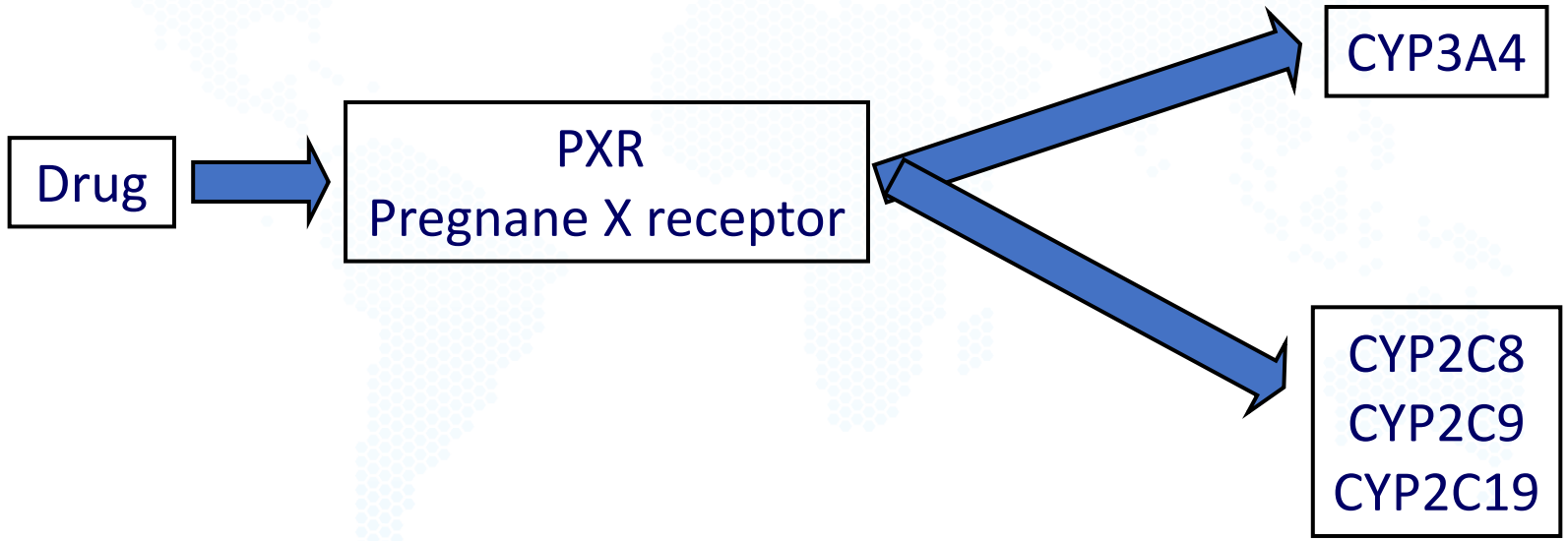
Compound known to induce a particular enzyme, a positive control (E.g., rifampin or phenobarbital → CYP3A4)

General mechanism of enzyme induction

A receptor-mediated response to xenobiotics (**xenosensors**)



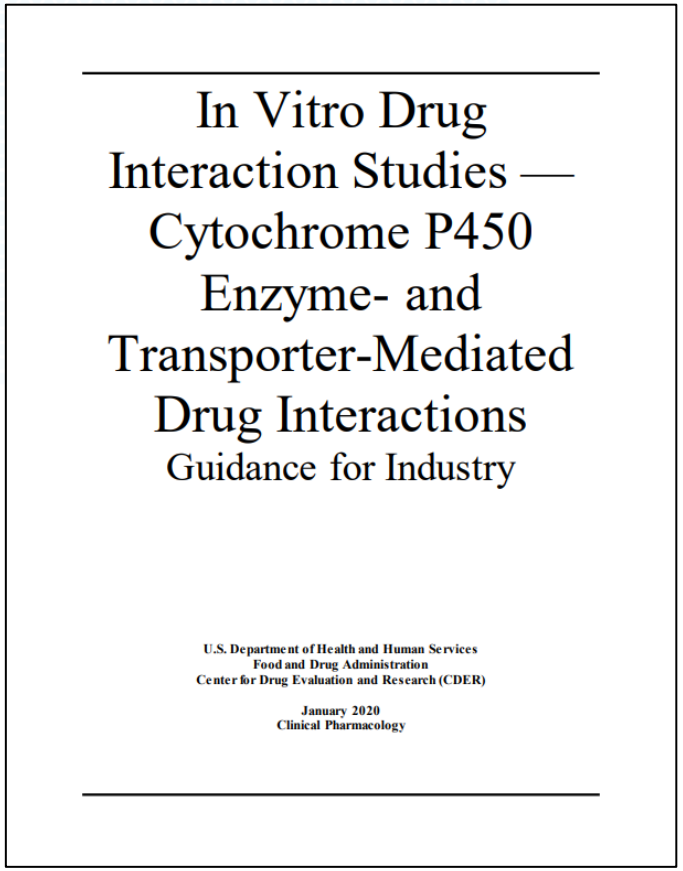
General mechanism of CYP3A4 and CYP2C enzyme induction



When Do I Need to Assess CYP2C Induction?

"If no induction of CYP3A4 enzymes is observed, evaluating the induction potential of CYP2C enzymes is not necessary because both CYP3A4 and CYP2C enzymes are induced via activation of the pregnane X receptor (PXR). **If the investigational drug induces CYP3A4 and the results suggest that a clinical study is warranted, the sponsor should evaluate the potential of the investigational drug to induce CYP2C.**"

--FDA In Vitro Drug Interaction Studies – Cytochrome P450 Enzyme- and Transporter Mediated Drug Interactions Guidance for Industry



In Vitro Drug Interaction Studies — Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions

Guidance for Industry

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

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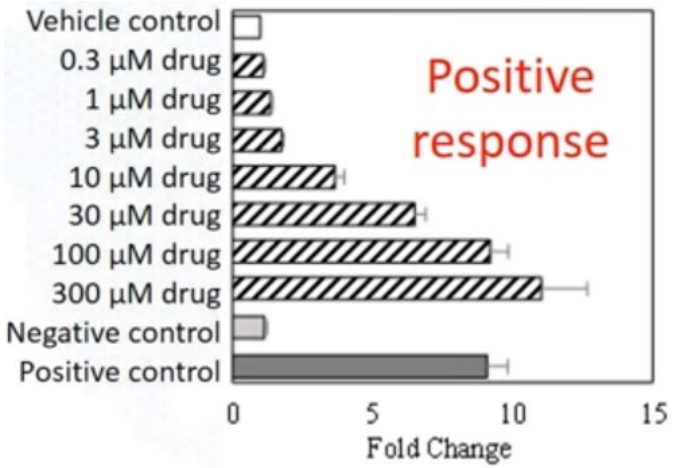
Clinical Pharmacology

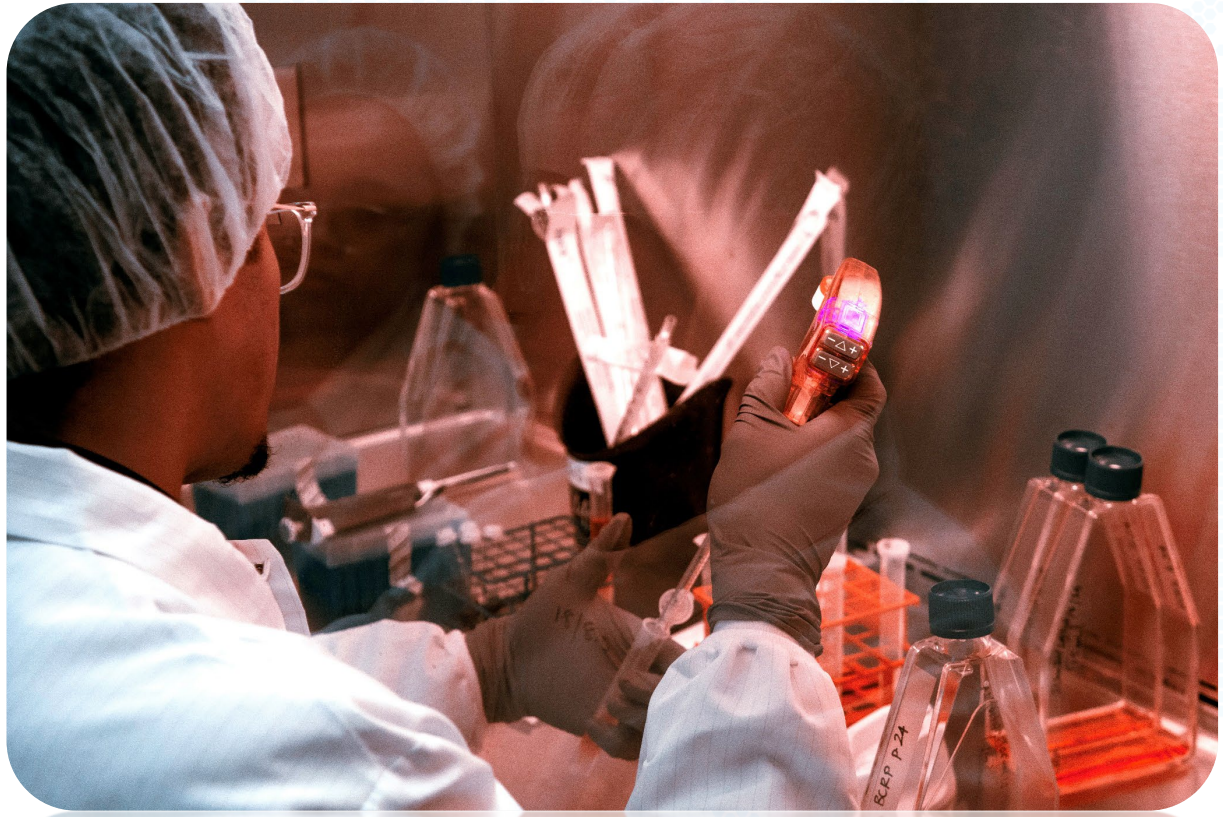
When Do I Need to Assess CYP2C Induction?

CYP3A4 Induction? ✓

→ Evaluate CYPC8, 2C9 & 2C19 Induction ✓



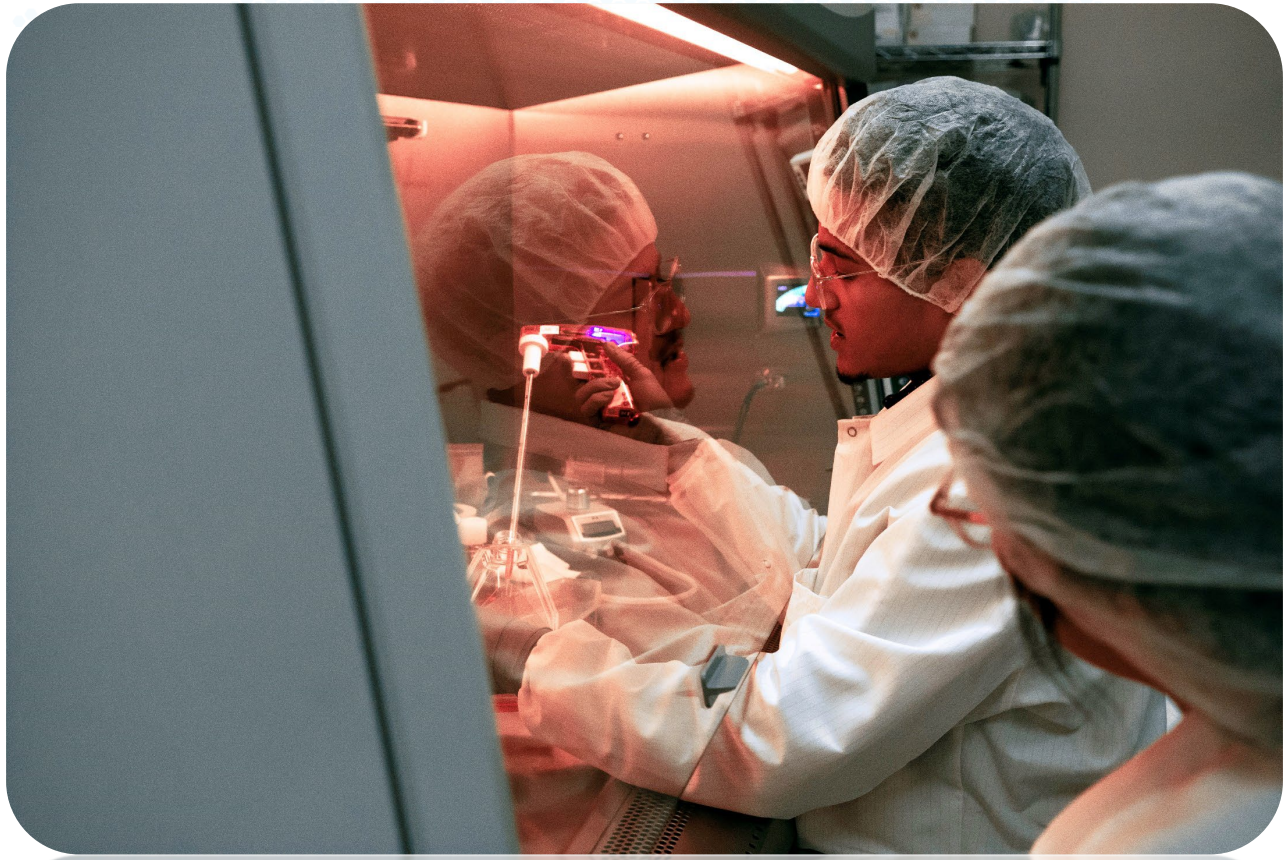
CYP2C Study Design



- CYP2C induction study design is (mostly) the same as for standard design
- Conducted in plated hepatocytes
- 3 donors
- 72 hr incubation with test article treated every 24 hr
- Includes SMA and cytotox
- Endpoint for the 2Cs we recommend to look at activity instead of mRNA (more on this coming up)

End points – for 2Cs

- mRNA is the preferred endpoint for 1A2, 2B6 and 3A4
- Potential inhibition from test article or metabolites may mask induction when activity is used as the endpoint
- Due to industry wide known phenomenon of poor 2C19 induction determination via mRNA, activity is actually preferred endpoint for 2C19
- XT recommends activity for all 2C induction experiments



Interactive Webinar Questions for Audience

If you had 3A4 induction but didn't do 2C in vitro, did the agency require it at a later date?

Common Questions on CYP2C Induction

Common Questions on CYP2C Induction

"Why do you recommend looking at activity instead of mRNA for CYP2C induction?"

ANSWER:

- CYP2C19 mRNA is known to not be a reliable indicator of 2C19 induction

Common Questions on CYP2C Induction

"Could you comment more on how 2C induction may result in data looking like downregulation? Is this only when using activity as the readout, or mRNA as well?

ANSWER:

- When looking at activity, there is the possibility that the test article, or its metabolites, may inhibit the enzyme under investigation. This would lead to a decrease in activity and could appear as downregulation.
- This could also lead to a false negative on induction.

Common Questions on CYP2C Induction

"Do you typically save the cell lysate for the potential need to analysis CYP2C in case the CYP3A4 readout is positive, so that no separate hepatocyte incubation is needed?"

ANSWER:

- It's a two step process. We can save the samples for 2C8/2C9 mRNA; however we don't save the lysates for the 2C19 activity because we are not sure of stability.

Common Questions on CYP2C Induction

"Can you use correlation method for 2C enzymes to understand the induction potential?"

ANSWER:

- In theory, yes. However, the correlation method requires the sponsor/CRO to look at several clinical and in vitro inducers for the specific CYP in question.
- The ceiling for induction is great for 3A4 than the 2Cs, so you can't get as nice as correlation curve as you get for something like 3A4.

Common Questions on CYP2C Induction

"What would be the effect of a less than 2x 2C induction?"

ANSWER:

- Case by case basis, based on the data set. You need to look at all three cultures and look at the big picture. Was induction concentration dependent? How many concentrations was it increasing for? How did the prototypical inducer function?

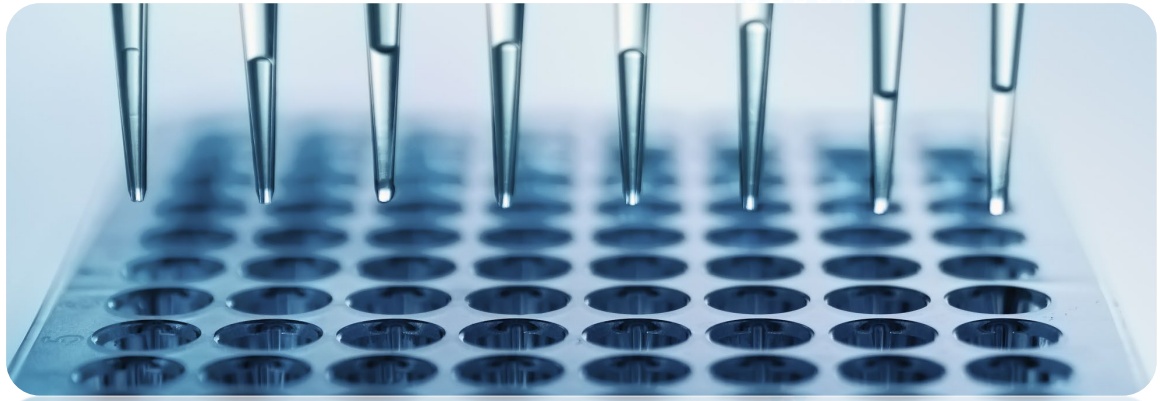
Interactive Webinar Questions for Audience

If you only did 2C activity in the last 5 years was there any push back from agency?

If 2C19 mRNA didn't show induction by prototypical inducer was there pushback?

In Conclusion

- If CYP3A4 induction is observed guidance requires to follow up with CYP2C induction (in vitro or in the clinic)
- If 3A4 induction is suspected ahead of time it is best to include 2C induction in the initial study to save time and cost



- mRNA is the preferred endpoint for induction studies…
- …except for CYP2C's where activity is the preferred endpoint

Interactive Webinar Questions for Audience

Based on what you have learned, if evaluation of CYP2C activity was offered as a cocktail add-on to CYP1A2, 2B6 and 3A4 mRNA induction assessment, would you be more inclined to include it and run it up front?

For More In-Depth Information on CYP Induction Studies…

https://www.xenotech.com/preclinical-drug-development/in-vitro-studies/enzyme-induction/

- Videos
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- Blogs
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Thank you for your attention!
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Consulting...

Cellular Products
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- Non-Parenchymal Cells (Kupffer Cells)

Subcellular Fractions
- Liver Microsomes
- S9 Fractions
- Cytosol
- Homogenate
- Lysosomes & Tritosomes
- Mitochondria
- Extrahepatic Fractions

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Research Biobank
- Normal & Diseased Tissue Samples

Recombinant Enzymes

Substrates & Metabolites

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