Enzyme Induction Studies:
Services Overview

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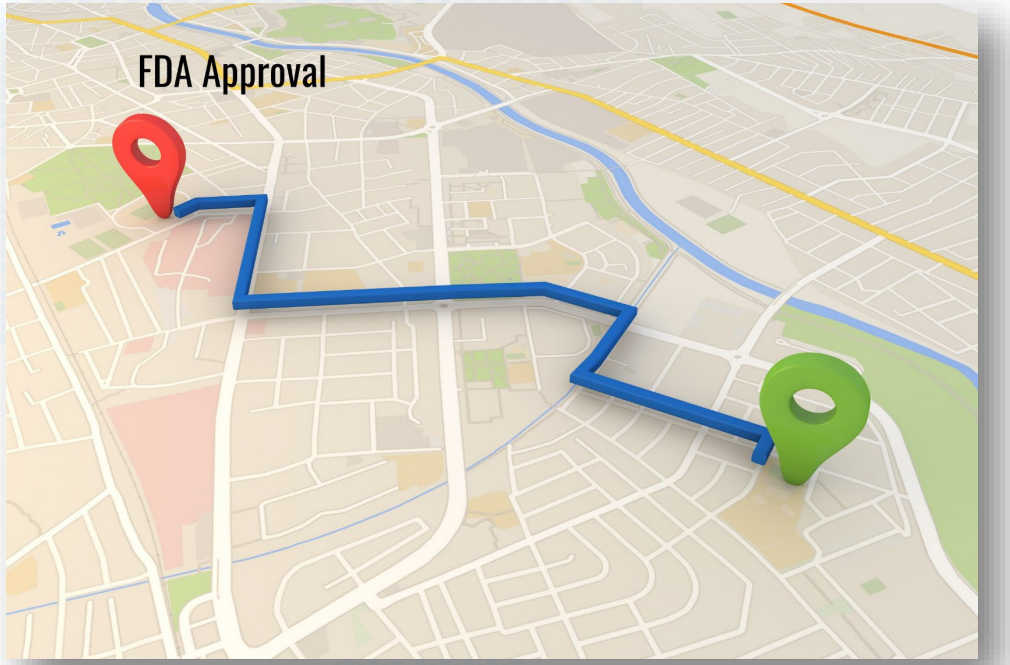
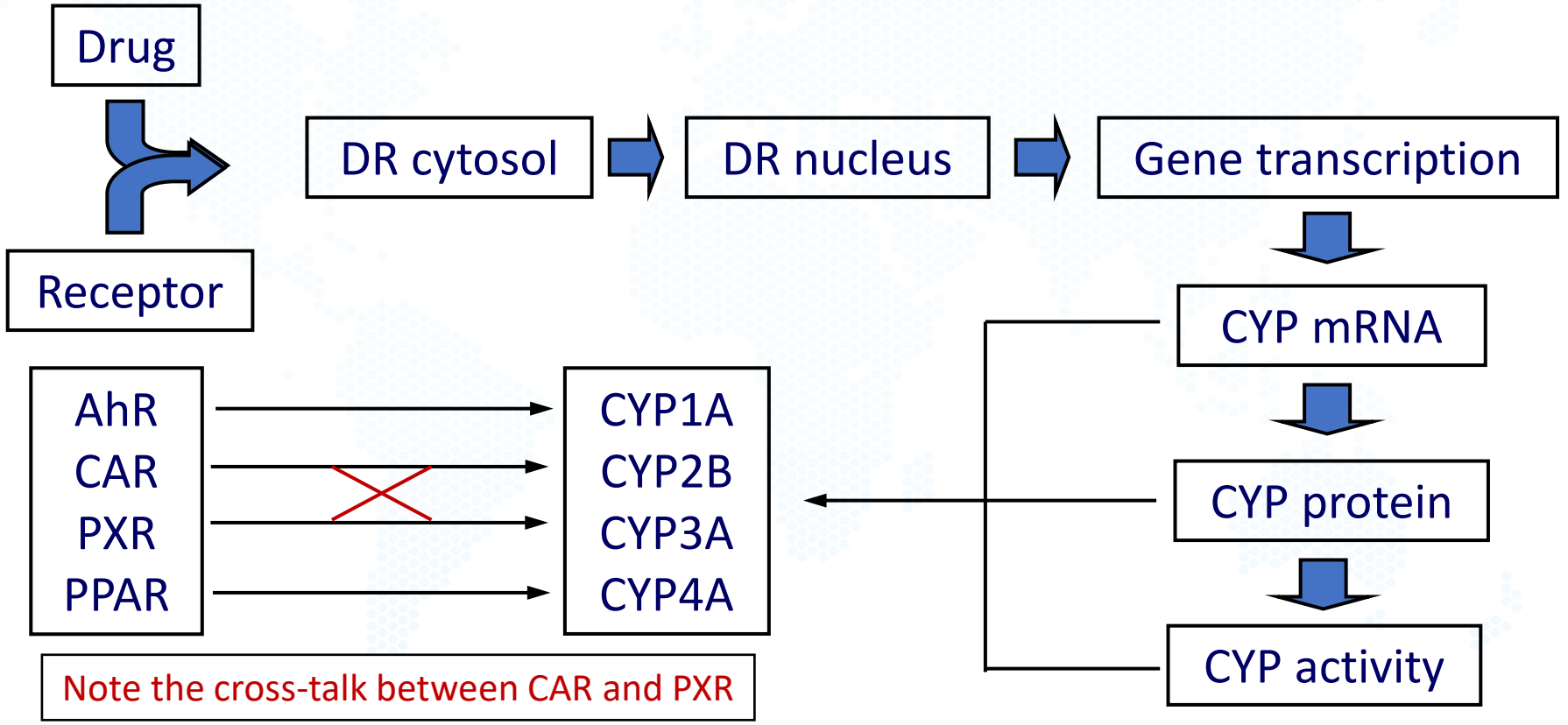
Overview

- What is enzyme induction?
- Why is measuring CYP (or other enzyme) induction important?
- Basic terminology
- Regulatory guidance & expectations
- Types of induction studies
- Typical study design (definitive vs. screening)
- Example results
- Considerations & questions we ask clients up front
- XenoTech products for induction

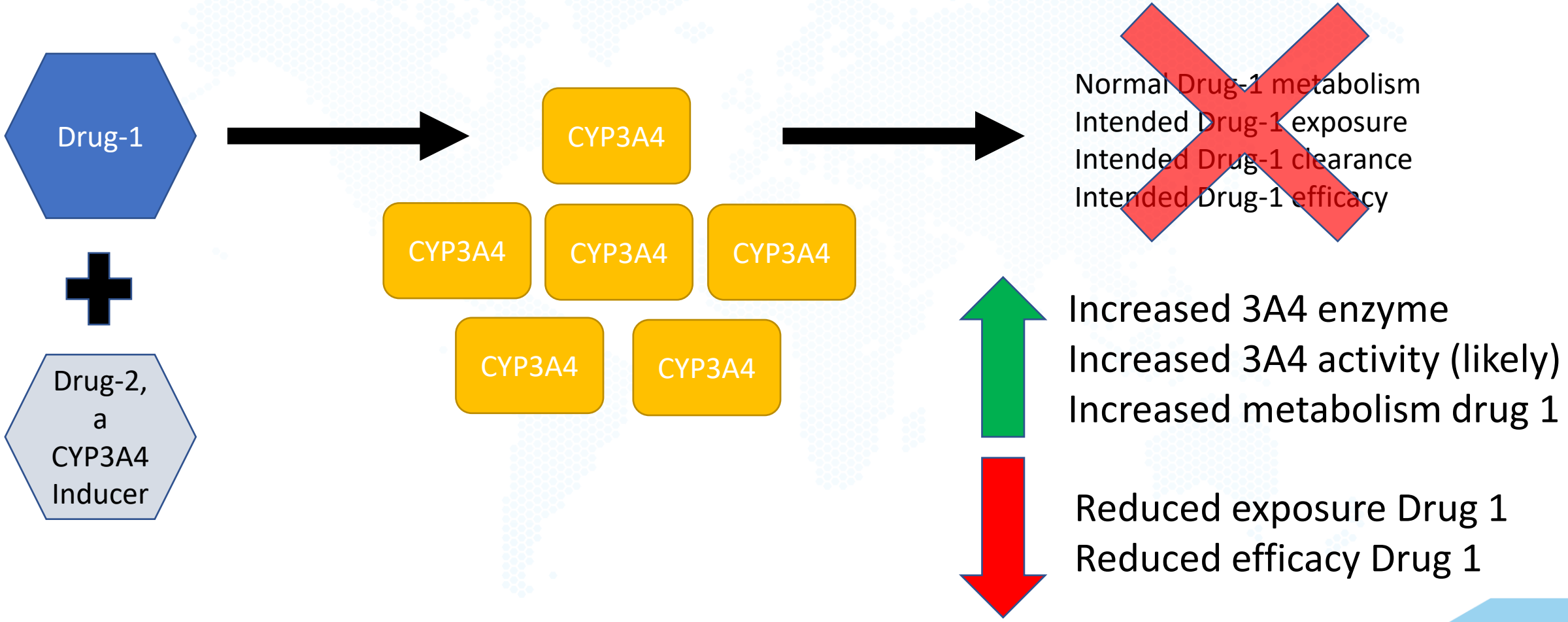


General mechanism of enzyme induction

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



Induction DDI General Mechanism



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)

Meeting Regulatory Expectations

Requirement	FDA (2020)	EMA (2013)	XenoTech
Test system (number of donors)	Cryopreserved or fresh human hepatocytes (*other systems considered complimentary*) n ≥ 3		Cryopreserved human hepatocytes (*pre-characterized with mild to strong clinical CYP3A4 inducers*) or fresh human hepatocytes n ≥ 3
TA concentrations	Sufficient to reach Emax not based on Cmax-ss total unbound	50x Cmax-ss unbound or if orally dosed 1/10th dose in 250 mL for CYP3A4	1/10th dose in 250 mL or 50x Cmax-ss total unbound, limit of in vitro solubility or in toxicity, includes Cmax-ss (and ideally reaches Emax) (*recommend 8 concentrations*)
CYP emphasis	1A2, 2B6, 3A4		1A2, 2B6, 3A4
Controls	Negative: Not specified CYP specific Positive: Omeprazole (25-100 µM) **Phenobarbital** (500-1000 µM) Rifampin (10-50 µM)	Negative: Not required CYP specific Positive: Omeprazole (50 µM) **CITCO** (100 nM) Rifampin (20 µM	Vehicle: Test article specific Negative: Flumazenil (25 µM) CYP specific Positive: Omeprazole (50 µM) **Phenobarbital** (750 µM) Rifampin (20 µM)

Meeting Regulatory Expectations

Requirement	FDA (2020)	EMA (2013)	XenoTech
End-point measurement	mRNA	mRNA (activity if decreases observed)	mRNA and activity
Positive induction response	Unspecified	6-fold increase in mRNA	6-fold increase in mRNA (in pre-characterized cryopreserved hepatocytes)
Concentration of TA in medium	Yes, on last day of treatment to obtain C_{avg} ***OR*** change medium at intervals to reduce TA loss		Spent media collection on last day of treatment over 4 time points (analyzed by test article specific LC/MS/MS method or stored for future analysis)

Meeting Regulatory Expectations

Requirement	FDA (2020)	EMA (2013)	XenoTech
Toxicity assays	Not specified	Yes, at highest TA concentration before and after incubation period	Recommended Lactate Dehydrogenase Release (a measure of membrane integrity) (daily evaluation) Reduction of Resazurin (a measure of mitochondrial respiration) (day of harvest)
Individuals vs. Average	Individual cultures, worst-case scenario		
Methods for data interpretation	• Basic models • Mechanistic-static model (net effect) • Dynamic models (PBPK)	• Basic models • Correlation methods (RIS) • Mechanistic-static model (net effect)	• Correlation methods (RIS, etc.) • Basic models • Dynamic models • Mechanistic static models
GLP-compliance	Not required (spirit of GLP)	Not required	Available on request (Requires DSA)

Study Types

Ex vivo

- Investigates induction in laboratory animals (rat, mouse, dog, monkey)
- Typically done following toxicology studies by the Sponsor as **GLP multi-site study**
- Animals usually dosed by Sponsor and liver samples sent to XenoTech
- Animals dosed with large amounts of test article
- Microsomes or RNA from individual animals isolated from liver tissue
- Tecan assays for activity or qPCR for mRNA expression levels performed

In vitro

- Enzyme induction examined in **cryopreserved, characterized** hepatocytes
- Cultured hepatocytes treated with test article over 3 days **in 60 mm dishes only**
- Small amounts of test article required
- Hepatocytes from rat, dog, mouse, monkey and human have been used
- **Microsomal** activity (**or In situ** activity)
- Microsomes or RNA from treated hepatocytes
- Tecan assays for activity or qPCR for mRNA expression levels performed

Definitive vs MTS EI Study Design

Definitive EI Study

- mRNA fold change for CYP1A2, 2B6 and 3A4 using qRT-PCR
- 3 lot hepatocytes, 72 hr treatment period (n =3 biological replicates)
- 6-8 TA concentrations
- EC_{50}/E_{max} data
- Vehicle control, Negative control for induction, Multiple positive controls (1 concentration)
- Spent media analysis at multiple time points
- Full submission report

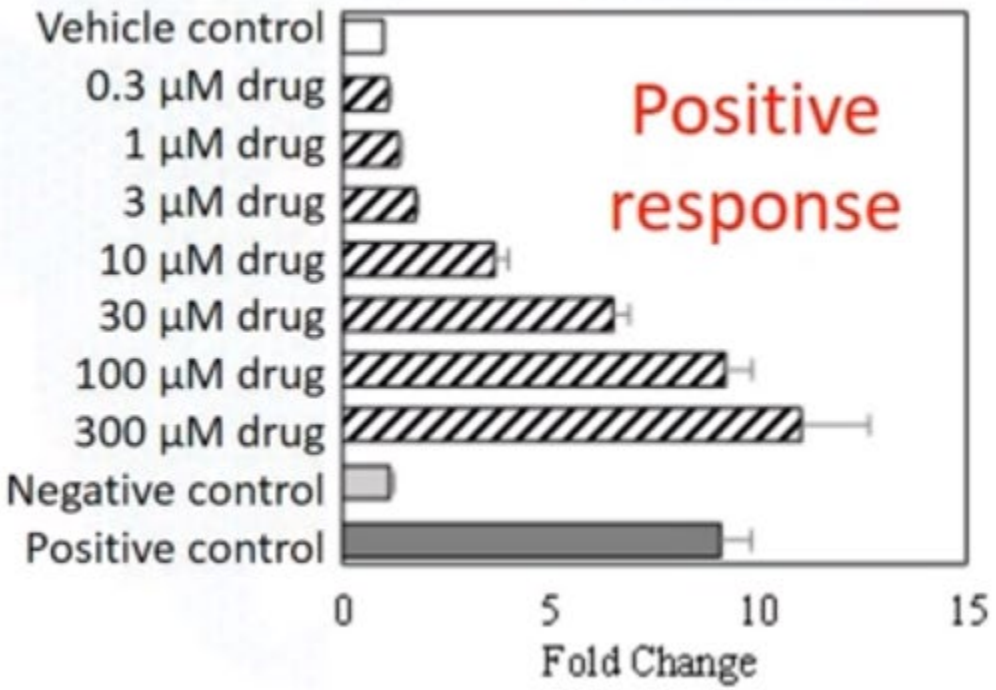
Additional options:

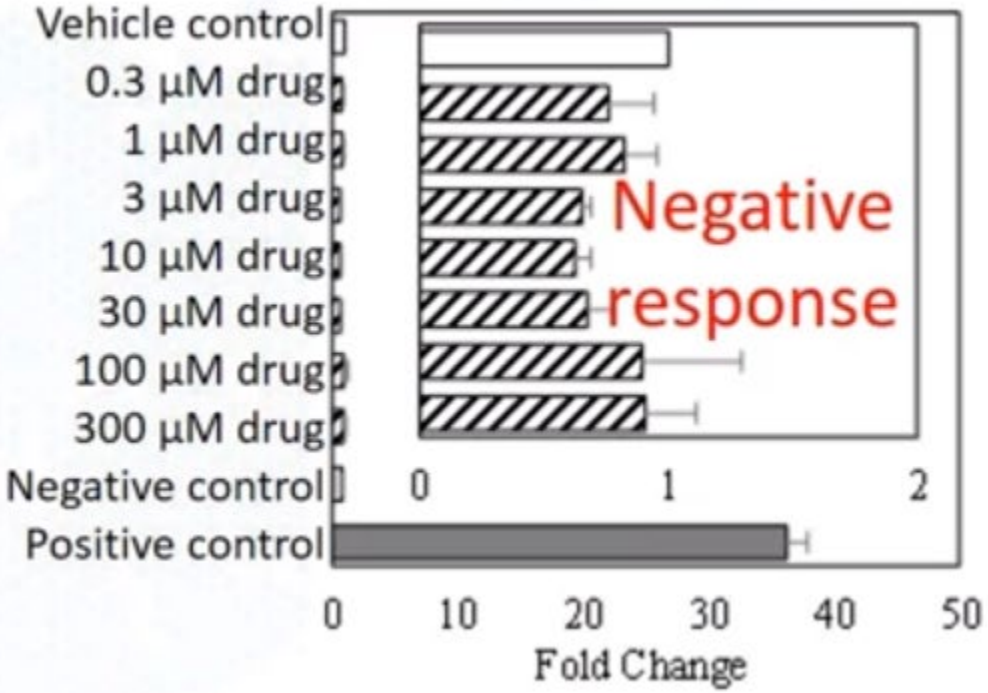
- 2C8, 2C9 and 2C19 follow up (activity and/or mRNA)
- GLP dose solution analysis
- Pre induction study toxicity assessment

Medium-Throughput Screening EI Study

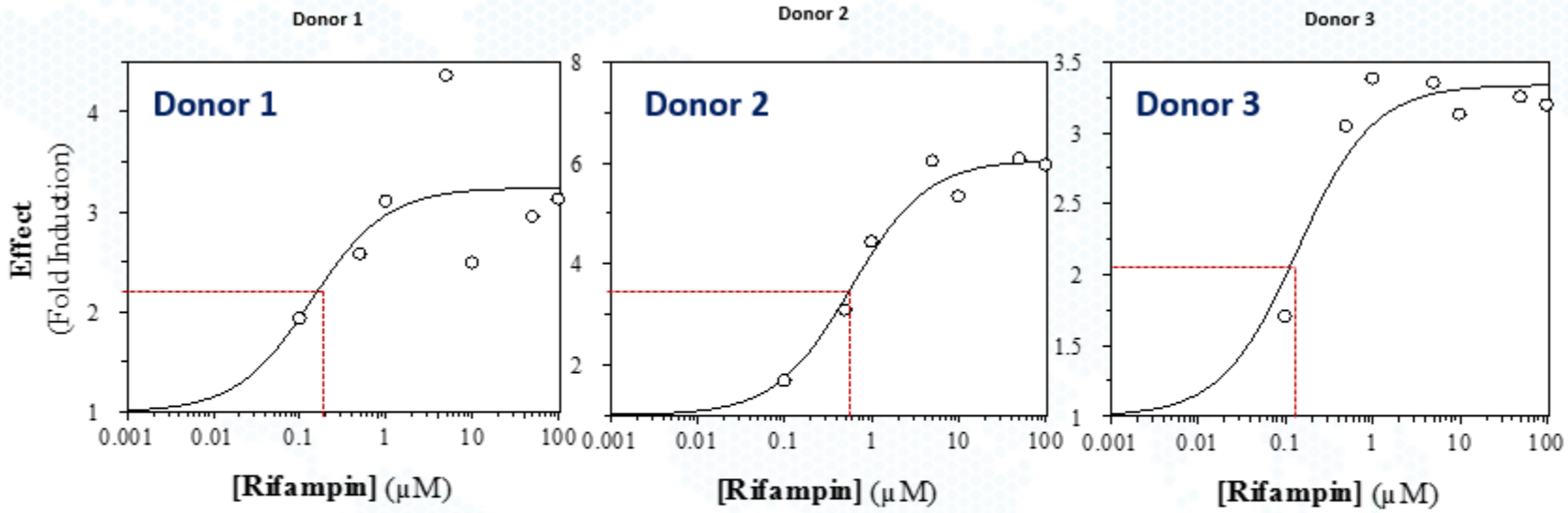
- mRNA fold change for CYP1A2, 2B6 and 3A4 using qRT-PCR
- 1 lot hepatocytes, 24 hr treatment period
- 3 TA concentrations (n = 3, pooled measured)
- Positive control included
- TA shipped in solution or as pre-weighed aliquots
- LDH
- Tabular data summary

Induction Example Data





Induction EC_{50} and E_{max} Example Data



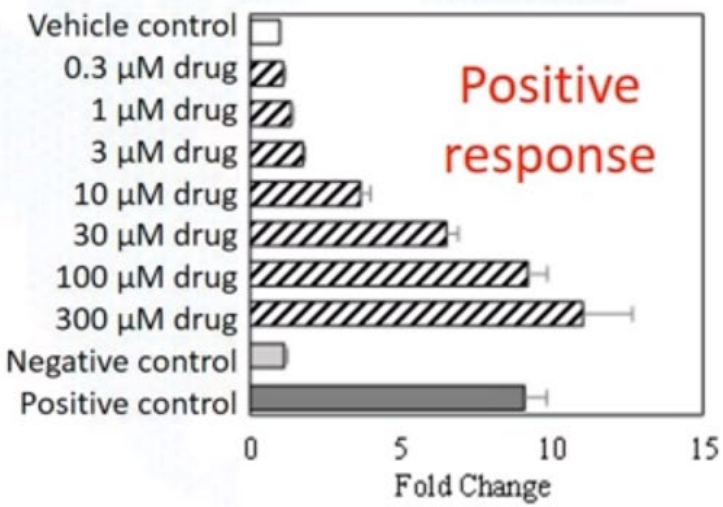
Parameter	Value	Std. Error
Emax	3.2417	0.3163
EC50	0.1371	0.1427

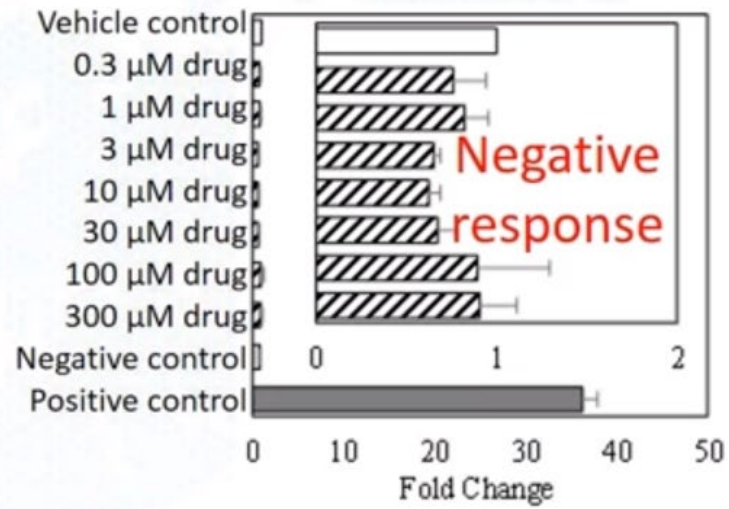
Parameter	Value	Std. Error
Emax	6.0649	0.1981
EC50	0.5795	0.1244

Parameter	Value	Std. Error
Emax	3.3378	0.1200
EC50	0.1362	0.0528

Induction Data Interpretation

- Determine fold change compared to vehicle control
  - Did the test article cause a fold change in mRNA or activity levels of greater than 2-fold?
  - Did the test article cause a response in mRNA or activity levels greater than 20% of the positive control?
  - Were the increases observed concentration dependent?
- If increases are observed, determine EC_{50} and E_{max} values for R_3 calculations (FDA 2020) – (requires I_{max-u})
- Alternatively, compare test article response to range of weak to strong inducers (Relative Induction Score – CYP3A4 only)





Additional Considerations:

- Cytotoxicity observed?
- Does the mRNA response match the activity response?
- Should the mechanistic prediction model be used (e.g., AUCR values)?

Considerations and Questions for the Sponsor: In Vitro Studies

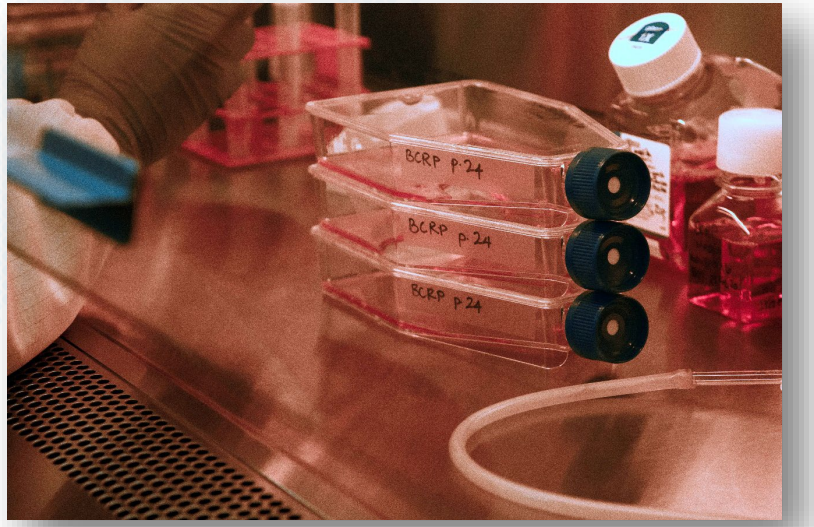
- TA specific considerations (solubility, binding, stability, molecular weight, molecule type, etc.)
- What question are you trying to answer? Just checking FDA boxes (human study) or anything other than human always good to know.
- FDA, EMA or both?
  - What do you plan to do with the data, plan on additional modeling, going to consultant, depend on us for interpretation? If using additional modeling (SimCYP for EMA) may suggest additional PC for induction. I.e., some clients may also want EC_{50} of prototypical inducers in experimental design.
- C_{max-u}, physiological relevant concentrations, Plasma Protein Binding

Considerations and Questions for the Sponsor: Ex Vivo Studies

- What species
- Number of animals per sex
  - Pooled or individual
- Number of treatment groups
- GLP vs Non-GLP
- Multi-site work plan or protocol
- CYPs/UGT to be analyzed
- What question are they trying to answer
- Endpoints
  - mRNA, activity, Western Immunoblotting, ELISA—for activity WI and ELISA need 4-g liver tissue, mRNA only need ~150 mg
- CYP specific probe substrates preferred
- Do we have a preferred method of preparing the livers we do have a protocol to share? Yes!

XenoTech Products (CYP/UGT Induction)

- **Cryopreserved Attaching Primary Hepatocytes**
  - Individual donors
    - >6-7 fold mRNA induction (CYP1A2, CYP2B6, and CYP3A4)
    - >2 fold activity induction
  - Pooled primary human donors (screening only)
  - Multiple small animal species
- **Support Reagents**
  - Hepatocyte media
    - Thaw, Plate, Incubate, and Culture
- **Immortalized Hepatocytes (Fa2N-4)**
  - CYP induction screening (not so popular anymore)
- **Support Reagents**
  - MFE Hepatocyte mediums



XenoTech Products (CYP/UGT Induction)

Treated Animal Liver Subcellular Fractions

- **Male Sprague-Dawley Rat**
  - β-Naphthoflavone — CYP1A
  - β-Naphthoflavone and Phenobarbital — CYP1A & 2B
  - Phenobarbital — CYP2B
  - Isoniazid — CYP2E
  - Dexamethasone — CYP3A
  - Clofibric acid — CYP4A
  - Saline — Control
  - Corn oil — Control
- **Male Beagle Dog**
  - β-Naphthoflavone — CYP1A
  - Phenobarbital — CYP2B
  - Rifampin — CYP3A
  - Clofibric acid — CYP4A
  - Saline — Control
  - Corn oil — Control
- **Male and Female Cynomolgus**
  - β-Naphthoflavone — CYP1A
  - Omeprazole — CYP4A
  - Phenobarbital — CYP2A & 2B
  - Pyrazole — CYP2E
  - Rifampin — CYP3A
  - Saline — Control

Thank you for watching!

For questions get in touch through the Contact Us tab on our website or use our Products pages to find Research Biobank tissue preparations & microarrays currently available

Ex vivo versus *in vitro* enzyme induction

Animals treated *in vivo*
*Mice, **rats**, dogs and monkeys*
↓
Livers shipped frozen to SXT
(-80C, air tight)
↓
Liver microsomes and RNA lysates prepared
↓
CYP and UGT activities determined *in vitro*
↓
Western immunoblotting
Spectral binding
mRNA analysis

Hepatocytes plated in collagen sandwich configuration
***Human** (Mice, rats, dogs, monkeys)*
↓
Hepatocytes treated *in vitro* and harvested
↓
Microsomes from hepatocytes or RNA from hepatocytes prepared
↓
CYP and UGT activities determined *in vitro*
↓
Western immunoblotting
mRNA analysis