

XENOTECH

A BioIVT Company

CYP Inhibition and Enzyme Induction

Quality Study Designs to Meet the FDA/EMA Guidelines

XenoTech is synonymous with quality. Because of this, we take great pride in making sure the studies you request meet or exceed the FDA and EMA regulations, ensuring that your data are accepted by regulatory agencies worldwide.

XenoTech's CYP Inhibition and Enzyme Induction studies are designed to capture R_1 , R_2 and R_3 data which can be used in the FDA/EMA basic model (shown below). These values can be used to study the combined effects of CYP inhibition and CYP induction, also known as the net effects model, described in the 2012 FDA guidance.

CYP Inhibition

Our efficient 3-curve IC_{50} study design allows us to evaluate CYP enzyme inhibition by drug candidates and their potential metabolites, predicting potential clinical issues in the *in vitro* development phase. XenoTech's CYP inhibition assays are performed with an extensively-characterized dedicated pool of human liver microsomes to assure consistency throughout every study. Automated data acquisition, retrieval and processing capabilities coupled with robotics and Galileo LIMS assures fast turnaround times and publication quality reports. We address many scientific and regulatory concerns when designing our studies in order to provide high content, high quality data.

Our assays are well-equipped to identify direct and metabolism-dependent inhibition in order to calculate the R_1 and R_2 values recommended in the FDA/EMA basic models for inhibition. Our experience has shown that the K_i value needed to calculate R_1 can be predicted from a well designed IC_{50} experiment or it can be experimentally determined. Both methods are accepted by regulatory agencies and XenoTech is well-equipped to predict K_i because our IC_{50} experiment is performed at K_m for each of our marker substrates and utilizes a short incubation time (5 min) and a low concentration of microsomal protein (≤ 0.1 mg/mL). Low protein concentration and a short marker substrate incubation time minimize artifacts caused by protein binding, metabolic instability of the test article and excessive metabolism of the marker substrate. We are also well prepared to

experimentally determine the K_i value that is designed in the same manner as our IC_{50} experiment, but with five multiple concentrations of K_m for each marker substrate.

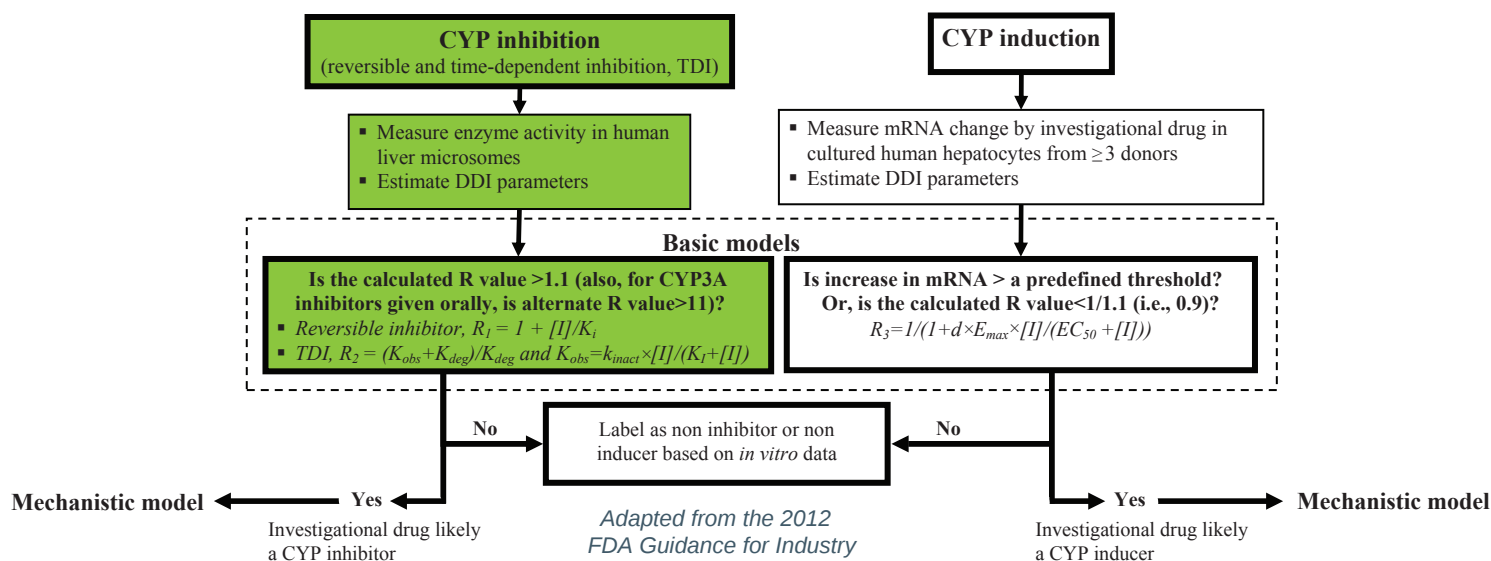
Our follow-up studies support the further characterization of drug candidates that are identified metabolism-dependent inhibitors (for example XTRA: XenoTech's Reversibility Assay and k_{inact}/K_i determinations). This mechanistic information can help you to better understand the potential for clinical issues at this early stage of compound development. The FDA and EMA suggest doing a k_{inact}/K_i study if MDI is detected in order to calculate the recommended R_2 value. XenoTech's automated k_{inact}/K_i design utilizes five test compound concentrations over five preincubation time points to determine how quickly (k_{inact}) and how potently (K_i) the enzyme is inactivated. This information is critical to navigating the R_2 value put forth by regulatory agencies.

XenoTech CYP Inhibition Study Features:

- Robotic incubations
- Efficient 3-curve IC_{50} study design
- Validated methods with FDA-preferred substrates
- Optional follow-up mechanistic studies
- Automated data retrieval and data processing
- CFR 58, Part 11 compliant systems
- Available as GLP or non-GLP

XenoTech CYP Inhibition Study Benefits:

- High-content evaluation of inhibition potential
- Predict clinical outcomes with greater confidence
- Full compliance with all regulatory agencies
- Fast turnaround time
- Submission-quality reports
- Consultative interpretation of results



CYP Inhibition and Enzyme Induction

Quality Study Designs to Meet the FDA/EMA Guidelines

CYP Induction

Enzyme induction studies assess the potential for a drug candidate to induce (up-regulate) the expression of drug-metabolizing enzymes. The metabolic clearance of a drug (victim) can be increased and its efficacy reduced, if it is co-administered with a second drug (perpetrator) that induces the enzyme responsible for the victim drug's metabolism. The increased clearance may compromise the therapeutic effectiveness or safety of the victim drug. As such, the FDA and other regulatory agencies recognize enzyme induction as a potential cause of drug-drug interactions. XenoTech offers well-designed Cytochrome P450 (CYP) and UDP-glucuronosyltransferase (UGT) *in vitro* studies that meet or exceed the FDA and EMA's latest guidance for enzyme induction. XenoTech addresses many scientific and regulatory concerns when designing studies in order to provide high content, high quality data.

Our studies are designed to obtain induction data for a drug candidate that is necessary to calculate R_3 values as recommended in the FDA / EMA basic models for induction. Using a dynamic drug candidate concentration range with cryopreserved hepatocytes (pre-characterized with nine mild to strong CYP3A4 clinical inducers), the estimated EC_{50} and E_{max} can be obtained for a compound's induction potential using a variety of methods. These include the correlation method (i.e., relative induction score or RIS), the basic model and the net effect / mechanistic model. In our induction reports, data interpretation is discussed several ways in order to meet or exceed the FDA or EMA guidelines. If there is induction with the drug candidate, trends within the drug candidate treatment groups and trends among the three donors are discussed individually, along with the relationship of the drug candidate CYP positive control (or % of the prototypical inducer).

Our submission-quality induction study design allows us to evaluate CYP enzyme induction by drug candidates and their potential metabolites to better predict clinical DDIs during the preclinical *in vitro* development phase. This saves you valuable time and money down the road as you have quality induction data that will be accepted by regulatory agencies worldwide.

Recommended Study Design:

- Cryopreserved human hepatocytes, RIS characterized
- CYP1A2, 2B6, 3A4 mRNA expression by qRT-PCR
- 6-8 concentrations of test article
- EC_{50} / E_{max} data
- Vehicle control (test article solvent)
- Negative control for induction (clinical & *in vitro* non-inducer)
- Multiple positive controls (prototypical inducers)
- Pre-study solubility testing
- Spent media analysis with multiple time point collections
- Comprehensive, full submission-quality reports

Enhanced Study Design (to Exceed FDA / EMA):

- CYP1A2, 2B6 and 3A4 *in situ* based activity using two-step cocktail incubation
- Microsomal based activity by single substrate incubation
- Pre-induction study toxicity assessment
- Full GLP Dose Solution Analysis on-site
- CYP2C8, 2C9 and 2C19 activity and/or mRNA expression endpoints
- Additional commercially available probes for mRNA expression endpoints (e.g., MDR1)

Automation and Analysis:

- Validated qRT-PCR methods
- Tecan liquid handling systems
- Validated LC/MS/MS methods
- Deuterated internal standards (all CYPs, most UGTs)
- Automated data retrieval and processing
- High throughput Shimadzu autosamplers
- Available as GLP or non-GLP

