Webinar at November, 2012

A Comparison of USFDA and EMA Guidance in the Conduct of Enzyme Induction Studies In Vitro.

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Gentest Contract Research Services

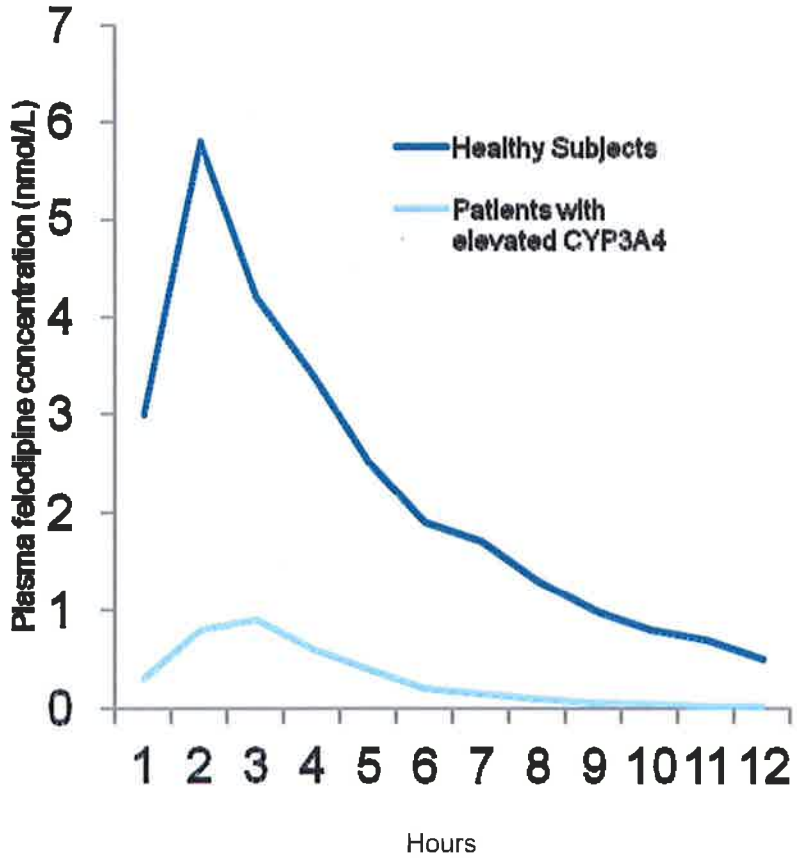
Notice: Gentest products and services are transferred from BD to Corning as of November 1, 2012. So the following presentations are provided with Corning style format.

Presentation Overview

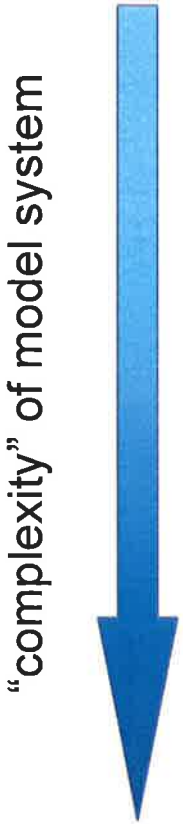
- Background and introduction
- Overview of recent drug-drug interaction guidance from the FDA and EMA
- Focus solely on enzyme induction in vitro
- Comparison of selected parameters

Importance of enzyme induction

- Therapeutic failure and safety issues
  - Higher rate of drug inactivation, so *less* of the (oral) parent drug reaches target
    - Autoinduction (self)
    - Drug-interaction (co-medication)
  - More potentially toxic metabolite
  - May point to other adverse situations
- Inducing drugs are less likely to be commercially competitive
- May be clinically manageable
  - Unmet medical need
  - Therapeutic indication



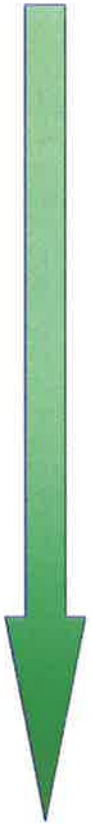
Models for induction testing



- Binding assays
  - SPA
- Cell-based hPXR, hAhR reporter gene assays
- Human "hepatocyte-like" cell lines
  - HepaRG, HepTRU-1, Fa2N-4
  - Stem cell-derived (?)
- Human hepatocytes
  - Freshly isolated
  - Cryopreserved
  - "Quasi" human hepatocytes

Recommended in Guidance documents

- Whole animal models
  - Poor predictors of human response
  - Exception: Transgenic "Humanized" mouse (human PXR)
- Others
  - Slices, chimeric mice



Discovery

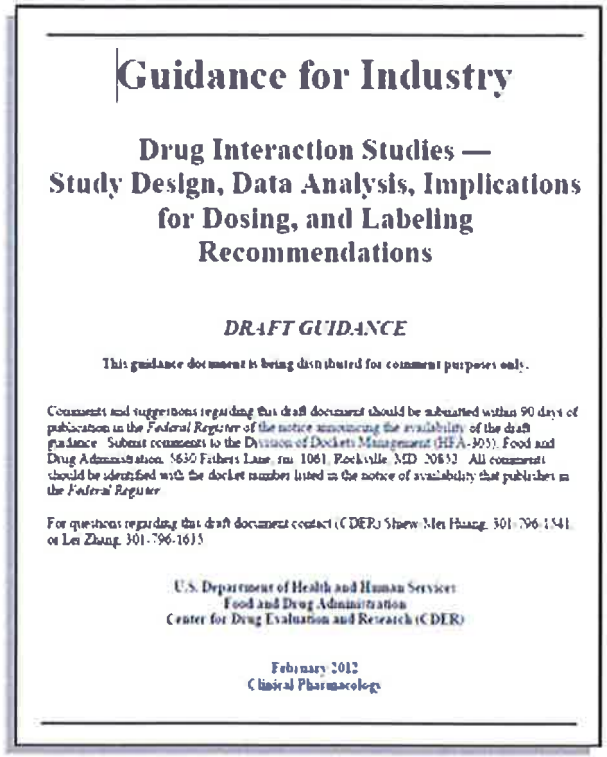
Development

Guidance Documents

- Represent the Agencies' current thinking
- Do not operate to bind Agency or the public
- If in doubt, contact the originating office (e.g. CDER, CHMP)
- Serve to reduce uncertainty among practitioners tasked with providing information to these agencies for review

Guidance documents for drug interactions

- FDA "Guidance for Industry: Drug Interaction Studies"
- Draft Guidance – Issued Feb, 2012



Guidance for Industry

Drug Interaction Studies — Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations

DRAFT GUIDANCE

This guidance document is being distributed for comment purposes only.

Comments and suggestions regarding this draft document should be submitted within 90 days of publication in the *Federal Register* of the notice announcing the availability of the draft guidance. Submit comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852. All comments should be identified with the docket number listed in the notice of availability that publishes in the *Federal Register*.

For questions regarding this draft document contact (CDER) Shiew-Mei Huang, 301-796-1541, or Lei Zhang, 301-796-1615.

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

February 2012
Clinical Pharmacology

http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM292362.pdf

- European Medicines Agency "Guideline on the Investigation of Drug Interactions"
- Final, effective January 1, 2013

EUROPEAN MEDICINES AGENCY

Guideline on the Investigation of Drug Interactions

Final

http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/07/WC500129606.pdf

High level summary

- Provide guidance for conducting *in vivo* and *in vitro* investigations of drug interaction potential
- Covers effects of the medicinal product on other drugs as well as the effect of other drugs on the medicinal product
- Most interactions are based on metabolism or transport
  - Inhibition
  - Induction
  - Phenotyping

Observations – relative to previous iterations

- More emphasis on
  - Mechanisms
  - Models
- Expansion into newer areas
  - Transporters
  - PBPK modeling and simulation
- Additional data is being requested

Drill down for today: Enzyme induction *in vitro*

- Provide a comparison of recommendations from the EMA and FDA guidance concerning the conduct of enzyme induction studies *in vitro*
- Categories and parameters selected to be of interest to the practitioner
- Focus on:
  - Experimental
  - Data Interpretation
- While there are over-arching similarities, there are multiple key differences that can impact the experimental approach

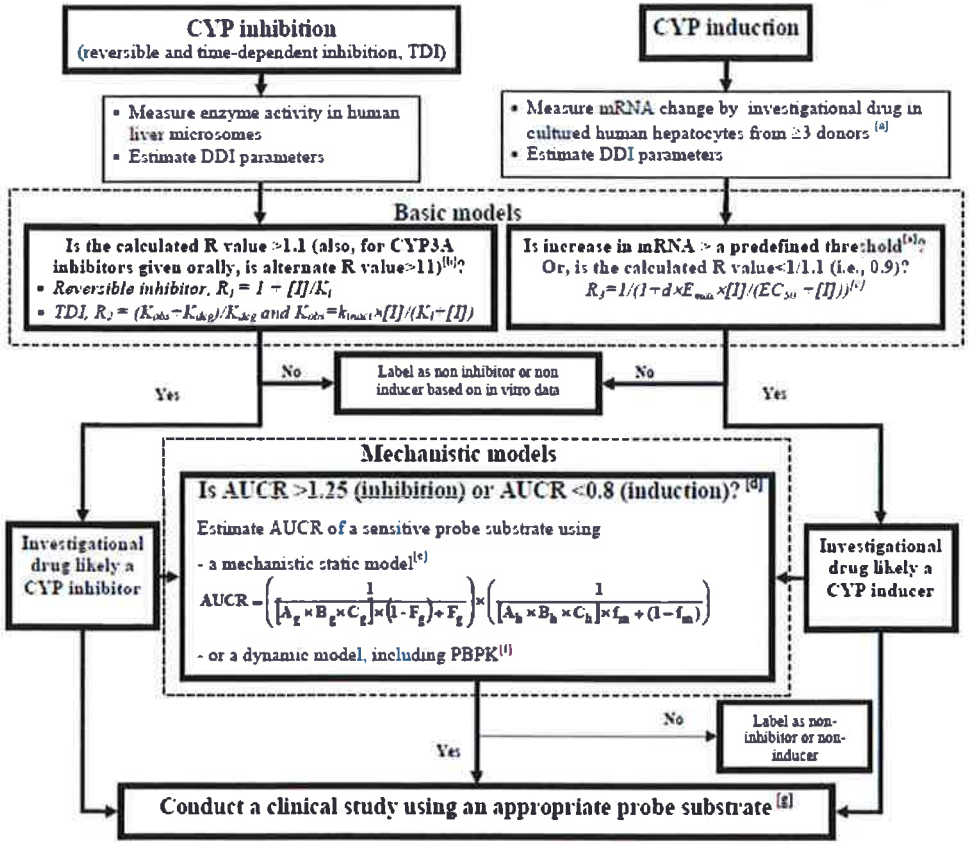
Decision trees

FDA

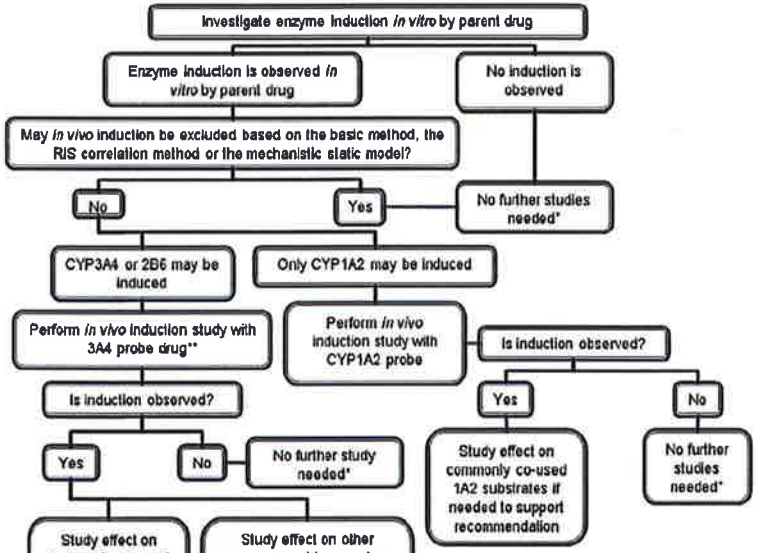
Contains Nonbinding Recommendations

Draft – Not for Implementation

Figure 4. General Scheme of Model-Based Prediction: The Investigational Drug (and Metabolite Present at ≥25% of Parent Drug AUC) as an Interacting Drug of CYP Enzymes



EMA



*A DDI study with an oral contraceptive is always needed if the drug is a potential human teratogen.
** If CYP3A4 is inhibited as well, study the effect on an additional PXR/CAR regulated enzyme in vivo. If CYP2B6 is markedly induced or induced to a greater extent than CYP3A4, in vivo studies with probe drugs of CAR regulated enzymes are recommended

Experimental

Item	FDA Guidance (Draft)	EMA Guidance (Final)	Degree of alignment
Test articles	Applies to small molecules only	Applies to small molecules only	+
Test system	Human Hepatocytes. Data generated from other in vitro systems are considered complementary.	Human hepatocytes. Minimally derived hepatocyte lines (e.g. HepaRG), nuclear receptor binding assays, or reporter gene assays are considered as supportive data only.	+
Fresh or cryopreserved	Cryopreserved is strongly implied	Either are acceptable for the Basic method evaluation; Cryo for RIS correlation method or Mechanistic Static Model	+/-
Number of Donors	3 or more	3 or more for Basic method; "one well performing batch" for follow up RIS correlation method or Mechanistic Static Model	+/-
Prequalification of hepatocytes	Yes, with a sufficient number of clinical inducers and noninducers	Yes, for non-Basic methods, and with 8 or more covering the full in vivo induction potency range	+/-

Experimental

Item	FDA Guidance (Draft)	EMA Guidance (Final)	Degree of alignment
Enzymes required	CYP1A2, 2B6, 3A; CYP2C required in vitro or in vivo if CYP3A positive	CYP1A2, 2B6, 3A4	+/-
Other enzymes	Should be considered if important for the drug	"A number of enzymes could be investigated"	+
Transporters	There is no validated in vitro system and in vitro studies are of limited use; any definitive study must be in vivo	If induction or down-regulation observed in vitro, "the effect on...transporters should preferably be quantified in vivo".	+
Number of test article concentrations to test in the basic model	3 or more	3 or more	+
Concentration of test article	Unspecified	Range should cover the worst case concentrations expected in hepatocytes in vivo (50X mean unbound;Cmax obtained at SS during treatment with maximum therapeutic dose for liver drug metabolizing enzymes; 0.1X dose/250ml for CYP3A4 in intestine)	-
Duration of treatment	Unspecified	"Generally 3 days"; any less must be well-justified	-

Experimental

Item	FDA Guidance (Draft)	EMA Guidance (Final)	Degree of alignment
Frequency of media change	Unspecified	"Changed regularly" with at least daily addition	-
Assessment of test article concentration in the media	Unspecified	Yes; encouraged at several time points on last day of treatment, unless loss has previously been shown to be negligible or tested prior and compensated for by media change or by drug addition	-
Assessment of binding to protein in the media	Unspecified	Yes, unless incubations are run serum-free or it has been previously demonstrated that degree of plasma protein binding is low	-
Assessment of non-specific binding	Unspecified	"The possibility of non-specific binding should also be taken into account."	-
Endpoints recommended	mRNA	mRNA, and if enzyme stabilization is suspected as a mechanism of induction, enzyme activity should also be measured	+

Experimental Detail

Item	FDA Guidance (Draft)	EMA Guidance (Final)	Degree of alignment
Positive control inducer	Omeprazole & Lansoprazole for CYP1A2; phenobarbital for 2B6; rifampicin for CYP2C8, 2C9, 2C19 and 3A4; a range of concentrations are provided	20 µM RIF for PXR; ≤ 100 nM CITCO for CAR; 50 µM Omeprazole for AhR; 50 µM dexamethasone for GR	+/-
Number of positive control inducers	One	One; Two when using the RIS correlation method or Mechanistic Static Model	+/-
Vehicle control	Required	Required	+
Negative control (non-inducing drug)	Required	Not required	-
Guidance on the QC of hepatocyte test system	Unspecified	Performance of the positive control; Viability of cells should be ≥ 80% at start of the incubation; Viability at end of the incubation should not deviate markedly from other donors	-

Data Interpretation

Item	FDA Guidance (Draft)	EMA Guidance (Final)	Degree of alignment
Definition of inducer, Basic model/method	At least one donor exceeds the predefined threshold (e.g. R values is < 0.9)	Response is more than 100% increase in mRNA over the vehicle and the increase is concentration dependent	-
Definition of non-inducer	All donors exceed the predefined threshold (e.g. R values is ≥ 0.9)	Response of mRNA over vehicle is <100% and is less than 20% of the response of the positive control	-

"Basic Model" vs "Basic method" – notable differences

FDA

- "Basic Model"
- Relies on "R3" (or "threshold") calculation
  - Must have EC50 & Emax
  - Must have [I]
    - Maximal total systemic inducer concentration in plasma
- No guidance provided for non-CYP3A4 and when parameter cannot be obtained
- R value < 0.9 is positive
- No comparison to positive control

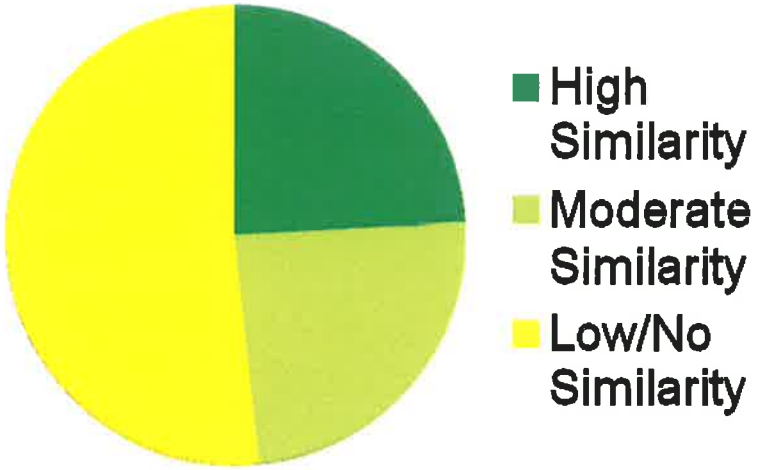
EMA

- "Basic Method"
- Simple fold-induction endpoint
- > 2-fold and concentration dependent is positive
- Positive control used to define the donor response for negative result

Data Interpretation

Item	FDA Guidance (Draft)	EMA Guidance (Final)	Degree of alignment
Evaluation models permitted	Basic Model, Mechanistic Static or Dynamic models including PBPK	Basic method required, followed by RIS correlation method or Mechanistic static method if positive in the Basic method and EC50/Emax can be determined	+/-
Guidance to interpret data that does not yield an EC50 and Emax?	No	Yes	-
Guidance to interpret down-regulation?	No	Yes; if down-regulation observed in vitro, effect should be studied in vivo	-
Criteria for down-regulation	Unspecified	50% decrease in mRNA, not attributable to cytotoxicity	-
Use nominal or unbound concentration in the assay for evaluation of response?	Unspecified	Unbound	-
Positive control inducer	Not used in the quantitative evaluation	Used to determine reliability of response in the model and to interpret a negative finding of induction	-

Summary – Degree of similarity among 29 parameters



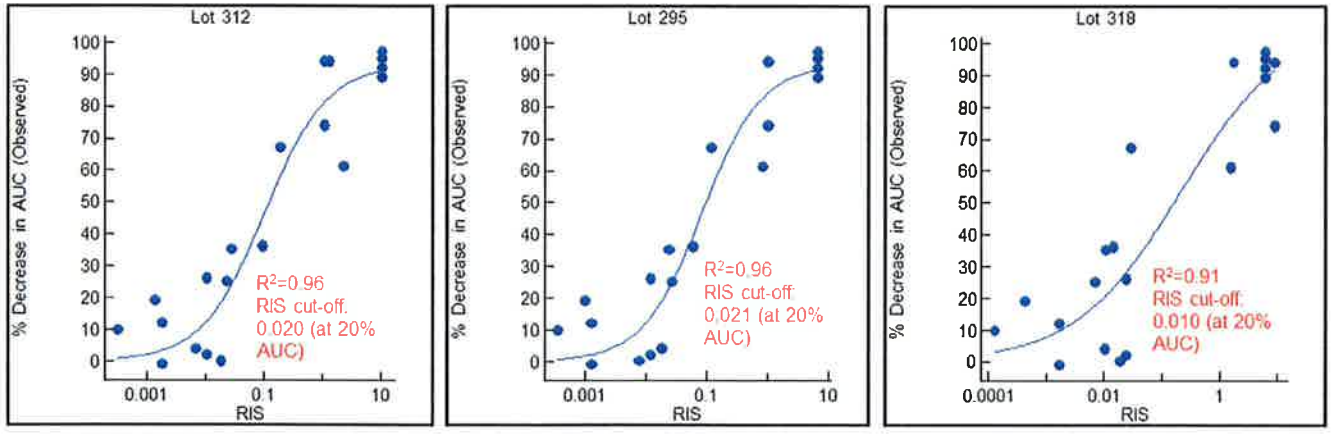
- Most parameters had low or no similarity in content
  - Some bias because "unspecified" counted as low/no
  - 9 incidents of this in FDA guidance
- This may change as the FDA guidance becomes final

Conclusions

- There are notable differences in the guidance documents concerning parameters for testing enzyme induction *in vitro*
- The number of differences suggests there are further opportunities for harmonization and standardization
- Overarching themes of model choice, concepts of dose-response modeling and conservative practices aimed ultimately at patient safety are essentially identical

Gentest[SM] Contract Services

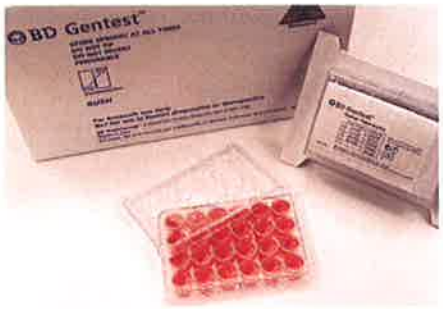
- Over 10 years performing induction studies in hepatocytes to support discovery efforts and regulatory submissions
- Enzyme inhibition, transport, metabolism studies
- ISO9001:2008
- Many services available GLP



Calibration Curve of RIS (relative induction score) vs Observed AUC Change.

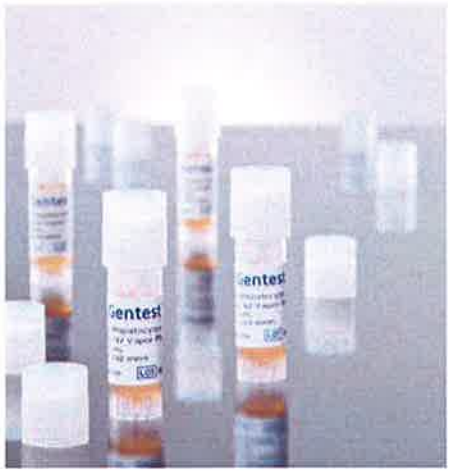
- Detail of these graphs are explained in PDF poster "Evaluation of mRNA EC50 And Emax as Endpoints in Human Hepatocyte Induction Studied to Predict Clinical Inducers and Non-Inducers of CYP3A4".

Gentest™ Hepatocytes



Fresh Hepatocytes with and without Matrigel Overlay™ plated on BioCoat™ Collagen I

- Available in 6, 12, 24, 48 and 96-well formats



Plateable Cryopreserved Hepatocytes
Qualified for:

- Induction
- Metabolism
- Transport

Thank you for your attention

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