XENOTECH

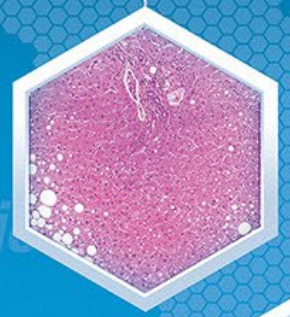
A BioIVT Company

25 Years

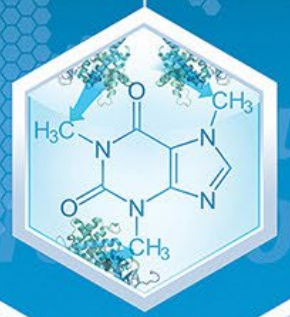
ADME | DRUG-DRUG INTERACTION | DMPK

CONTRACT RESEARCH & TEST SYSTEM EXPERTISE

Cell & Tissue-Based Products

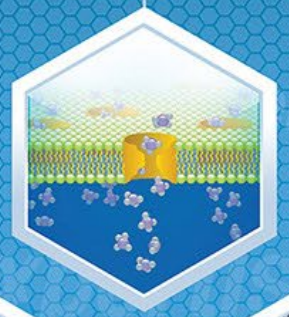


Reaction Phenotyping

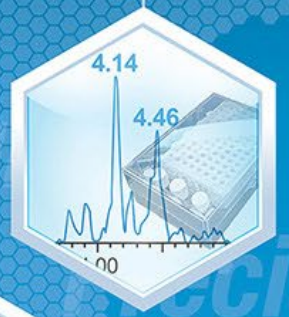


Enzyme Induction & Inhibition

Drug Transporters



Metabolite ID & Production



Screening

Pharmacokinetics & QWBA

Radiolabeling

Bioanalytical

Suicide by Binding: Putting Time-Dependent Inhibition of CYP Enzymes into Perspective

Brian W. Ogilvie, Ph.D.
VP Scientific Consulting
XenoTech, LLC
Kansas City, Kansas
Phone: 913.438.7450
Email: bogilvie@xenotechllc.com

September 2, 2020

Presentation Outline

1. Defining types of time-dependent inhibition (TDI) and their importance

2. In vitro approaches to evaluate various types of TDI:

- Screening vs. definitive studies

3. Approaches to predicting clinically-relevant DDIs from TDI

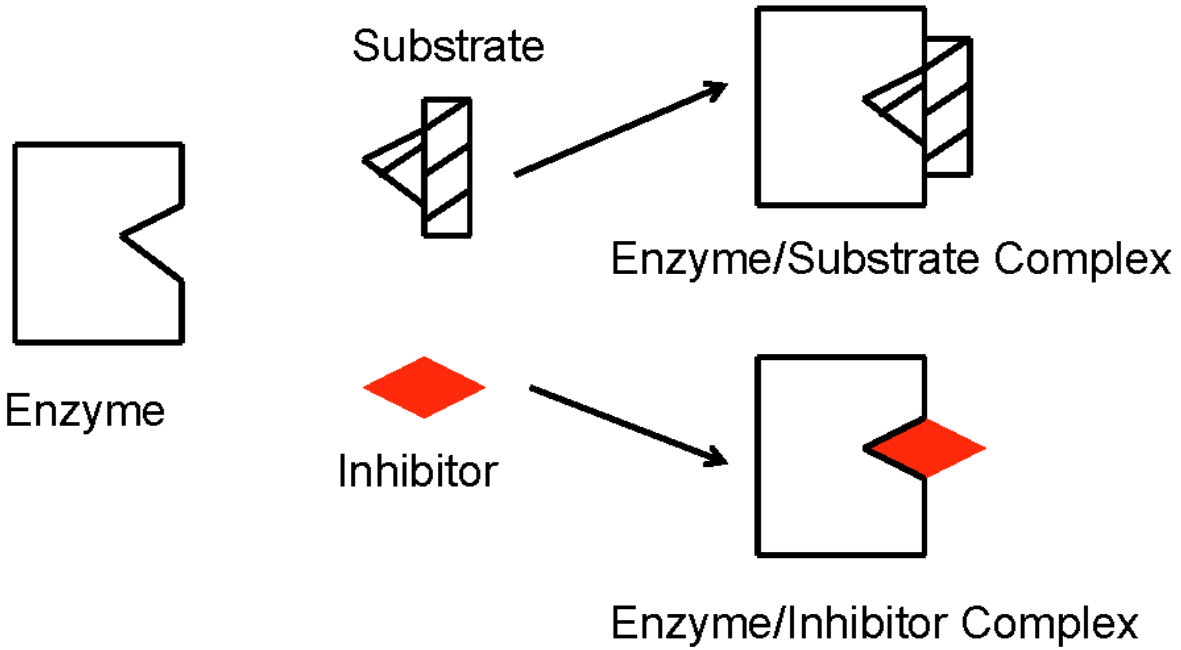
4. Case Examples:

- Drugs that only cause TDI in vitro (System-dependent effects)

- Drugs that cause clinically-relevant TDI

TDI of CYP enzymes: What it *isn't*

- TDI of CYPs is *not* simple direct or reversible inhibition (but there can be both)
- Reversible inhibition is most often competitive and ***can*** cause clinically significant **pharmacokinetic** drug-drug interactions (DDIs)
- The inhibitor and substrate bind to the same site on the enzyme.
  The inhibitor is often a substrate with higher affinity.
- The effect is reversible and decreases as the inhibitor is cleared
- Competitive inhibition can be overcome by increasing substrate concentration



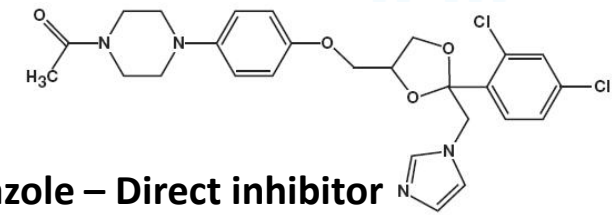
TDI of CYP enzymes: Some definitions

- TDI of CYP enzymes ***can*** cause clinically significant **pharmacokinetic** DDIs that are often of greater clinical concern than direct inhibition due to potentially long-lasting effects, even after the TDI is withdrawn
- If a xenobiotic inhibits CYPs in a time-dependent manner, it is typically first *metabolized* (i.e., it usually is "metabolism-dependent", too) to one or more species that:
  1. Is a more potent direct inhibitor than the parent
     - **Reversible TDI**
       Examples include ***R*-fluoxetine** and **amiodarone**

TDI of CYP enzymes: Some definitions (continued)

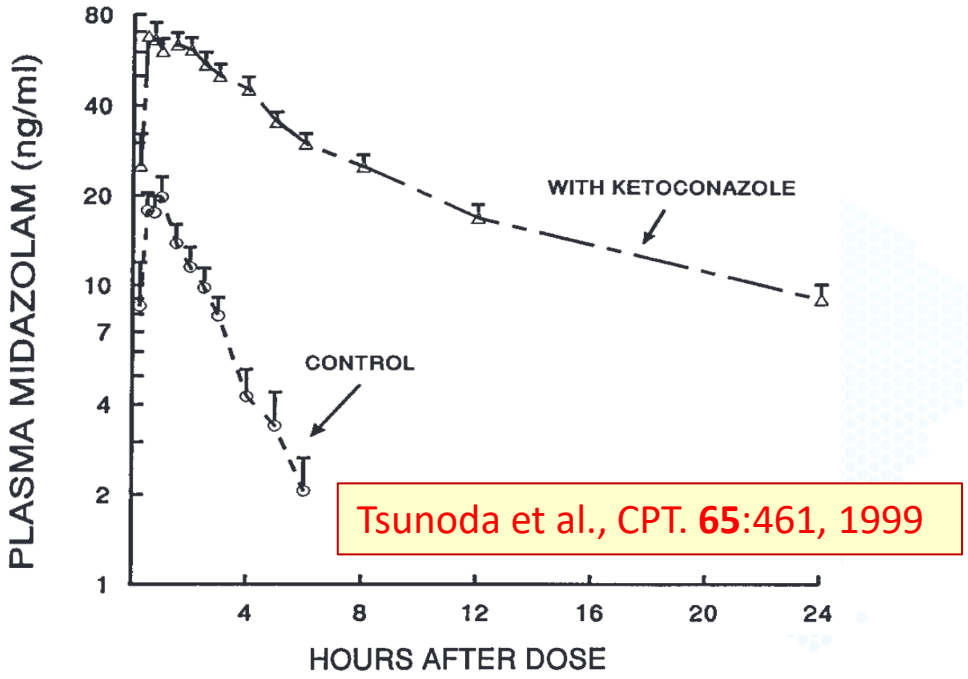
- If a xenobiotic inhibits CYPs in a time-dependent manner, it is typically first *metabolized* to a species that:
  2. Does not leave the active site and binds irreversibly at or near the active site
     - Referred to as **irreversible TDI**, mechanism-based inhibition or **a "suicide substrate".**
       Examples include **mibefradil**, **mifepristone**, and **gemfibrozil glucuronide. Various subtypes exist (e.g., heme destruction, apoprotein adduction, etc.)**.
  3. Coordinates very tightly to the heme iron of cytochrome P450
     - Quasi-irreversible inhibition - due to formation of a metabolite-inhibitory complex (MIC) Examples include **erythromycin**, **clarithromycin**, ***S*-fluoxetine**.
       Essentially irreversible over the time-frame that an enzyme exists. Irreversible inhibition persists until new enzyme is synthesized
- **But irreversible TDI may not be a showstopper for a lead candidate**

On the surface, it can be difficult to see the difference

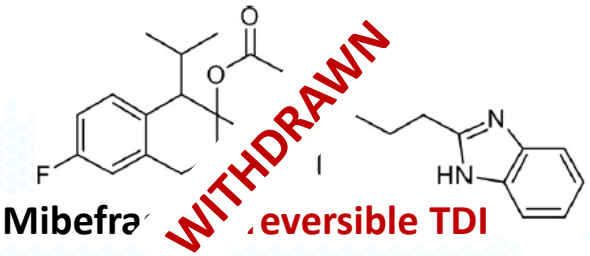


Ketoconazole – Direct inhibitor

Ketoconazole increased midazolam AUC by ~15-fold

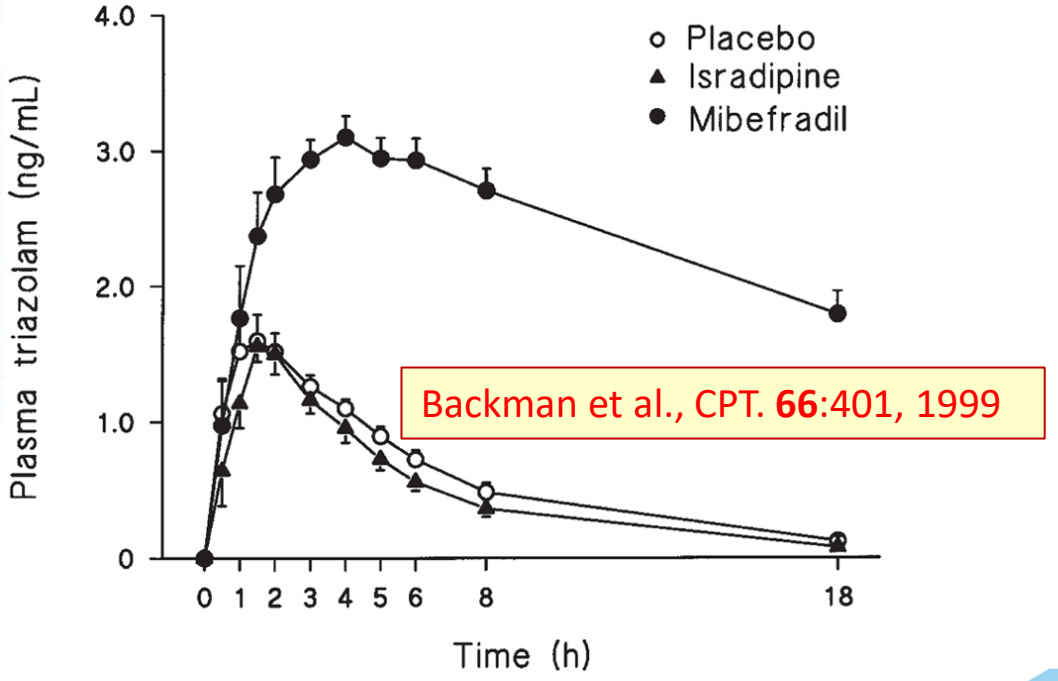


Tsunoda et al., CPT. **65**:461, 1999



Mibefra[illegible] WITHDRAWN [illegible]eversible TDI

Mibefradil increased triazolam AUC by ~9-fold



Backman et al., CPT. **66**:401, 1999

Presentation Outline

1. Defining types of time-dependent inhibition (TDI) and their importance
2. In vitro approaches to evaluate various types of TDI:

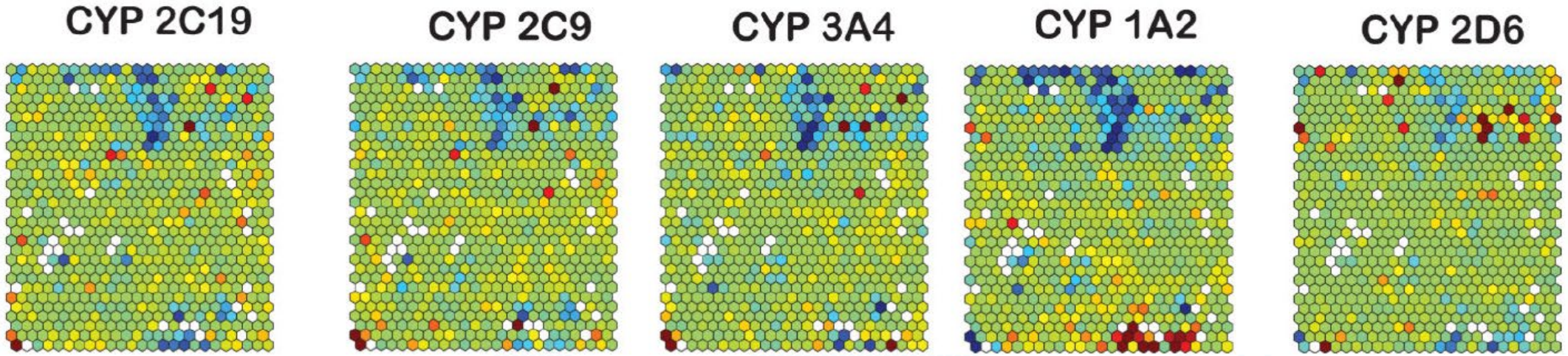
- Screening vs. definitive studies

3. Approaches to predicting clinically-relevant DDIs from TDI
4. Case Examples:

- Drugs that only cause TDI in vitro (System-dependent effects)
- Drugs that cause clinically-relevant TDI

Direct CYP inhibition screening

- High throughput screens (HTS) originally focused on direct inhibition only



- "As no pre-incubation . . . was included, this database will . . . miss mechanism-based inhibitors" {of 17,000 compounds}

Veith et al., *Nat Biotech*. **27**:1050, 2009

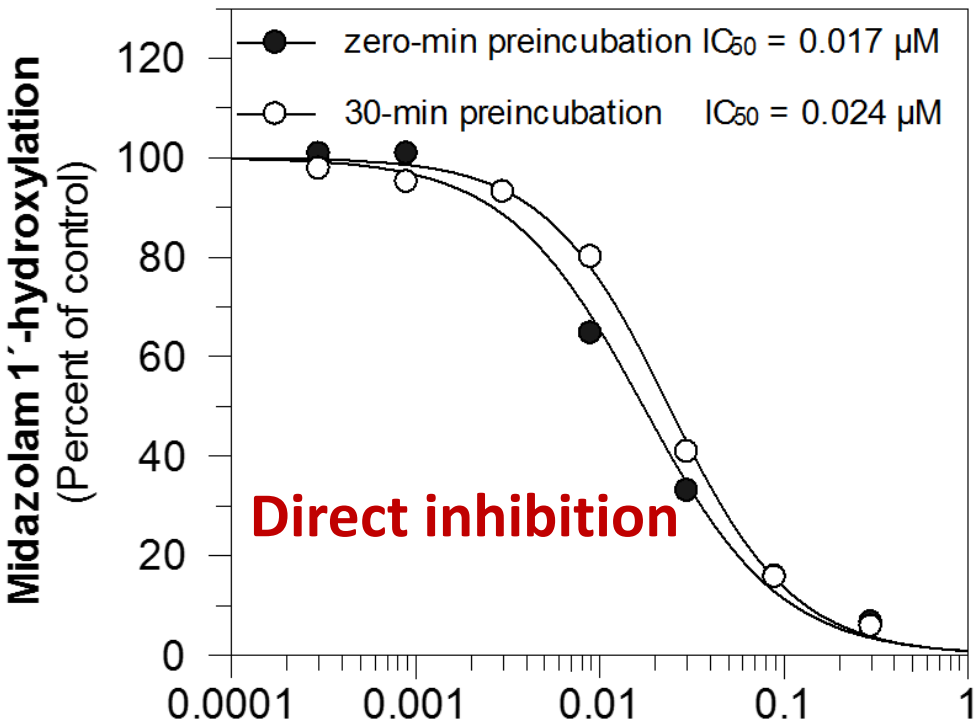
- This error was widely recognized in the mid 2000s:
"Although reversible . . . inhibition testing is well established for predicting the drug-drug interaction potential of clinical candidates, time-dependent inhibition (TDI) has become the focus of drug designers only recently"

Zimmerlin et al., *DMD*. **39**:1039, 2011

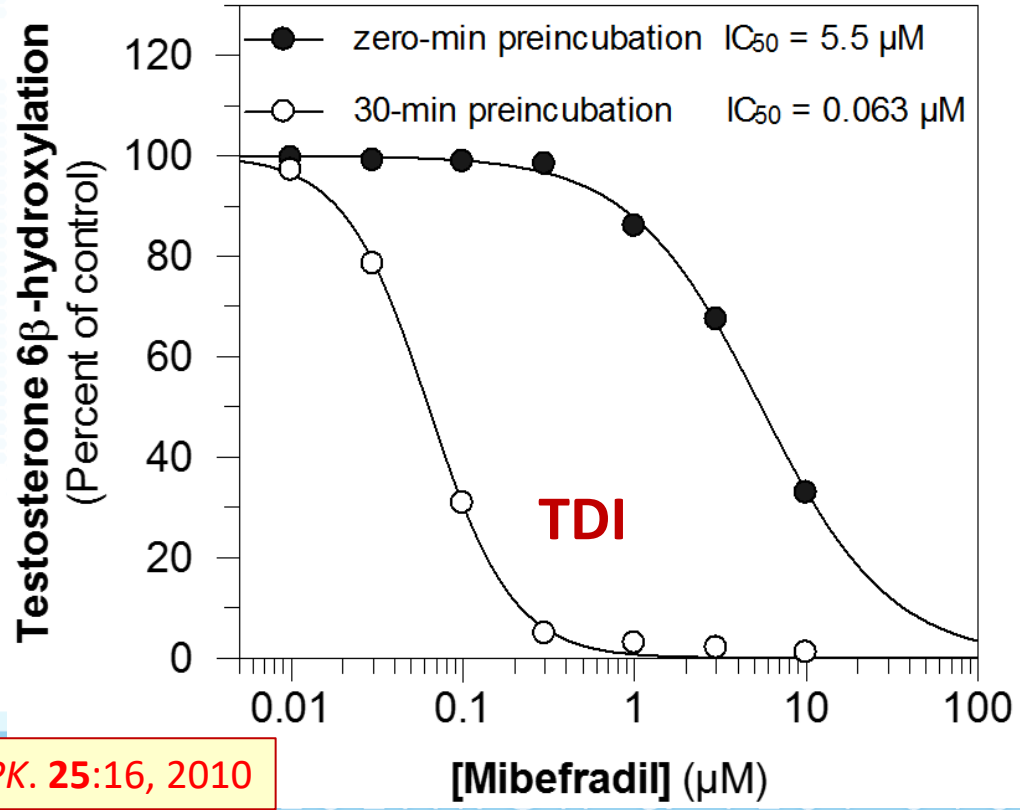
Direct and TDI CYP screening

- Most contemporary CYP inhibition screens incorporate evaluation of TDI
  - The IC_{50} shift assay is an extension of classic HTS, simply adding NADPH during a pre-incubation

HLM + Ketoconazole



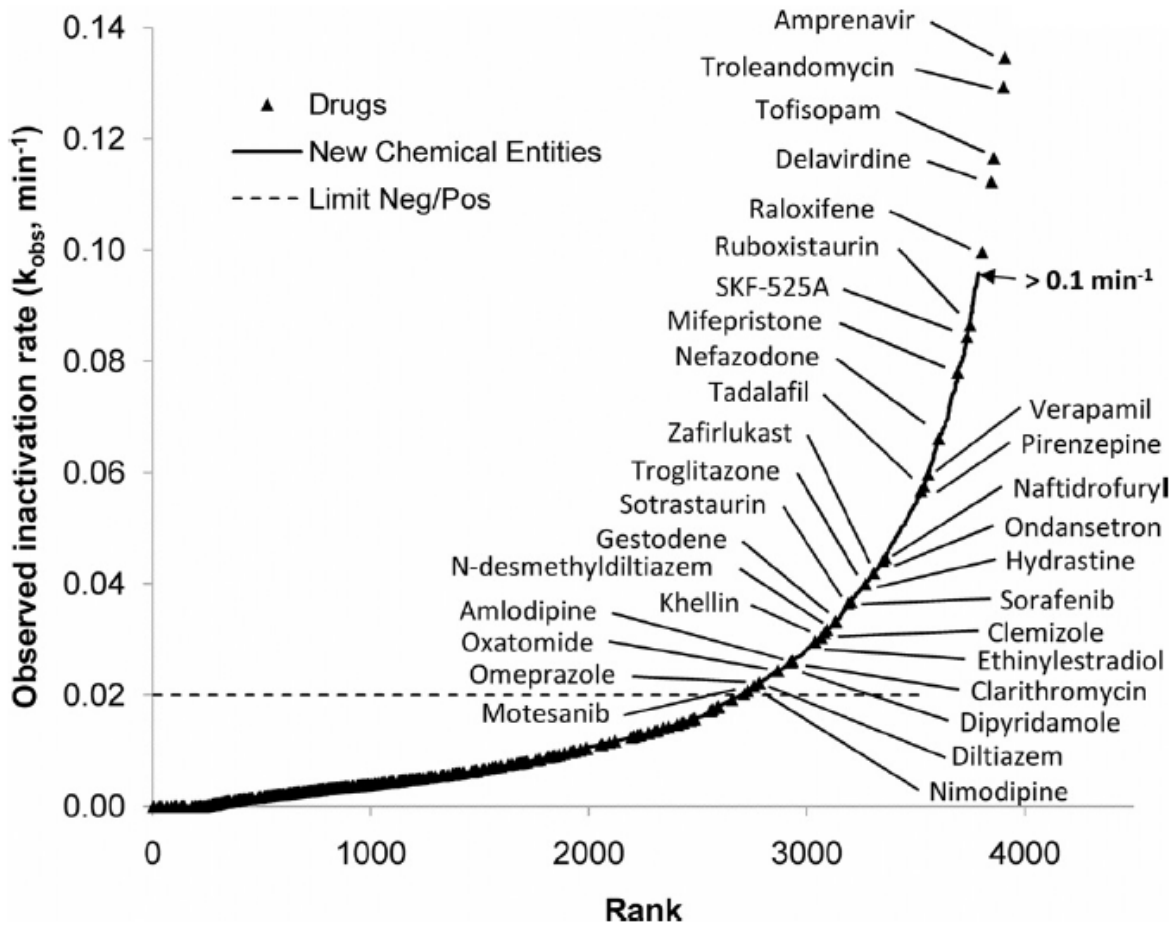
HLM + Mibefradil



Parkinson et al., *DMPK*. **25**:16, 2010

Direct and TDI CYP screening

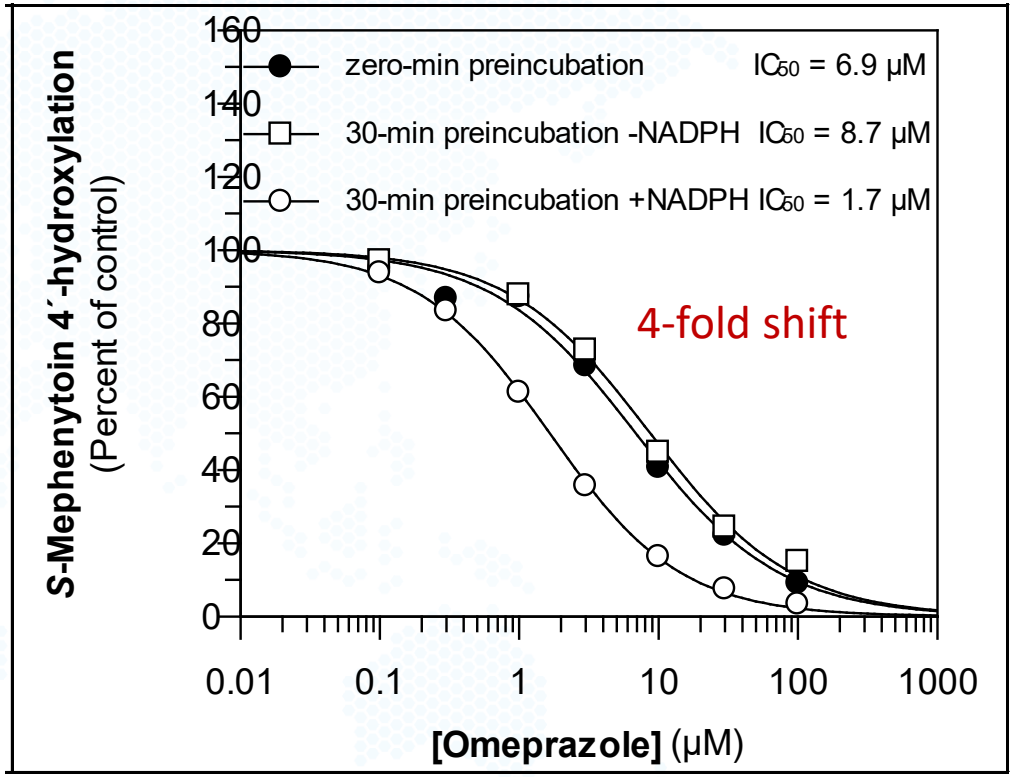
- Single concentration HTS screens have also been developed to rank-order TDI
- Novartis used 10 µM for >4000 drugs and NCEs, with 0 and 30 min pre-incubation to approximate a rate of inactivation (k_{obs} in min^{-1})
- The positive/negative cutoff was set at a k_{obs} = 0.02 min^{-1}
- 71% of registered drugs flagged as TDIs were not flagged in the direct inhibition screen



Zimmerlin et al., *DMD*. **39**:1039, 2011

Definitive direct and TDI IC_{50} shift assays

- XenoTech's definitive IC_{50} shift design measures in one assay:
  1. Direct (Zero-min preincubation)
  2. TDI control (30-min preincubation **-** NADPH)
  3. TDI (30-min preincubation **+** NADPH)



- Controls = ± solvent control, positive control inhibitors for direct and TDI
- [Marker substrate] ≈ K_m or S_{50}
- [HLM] ≤ 0.1 mg/mL to minimize partitioning
- Substrate incubation (5 min) short relative to pre-incubation (30 min)

But we still don't know if this is *irreversible* TDI

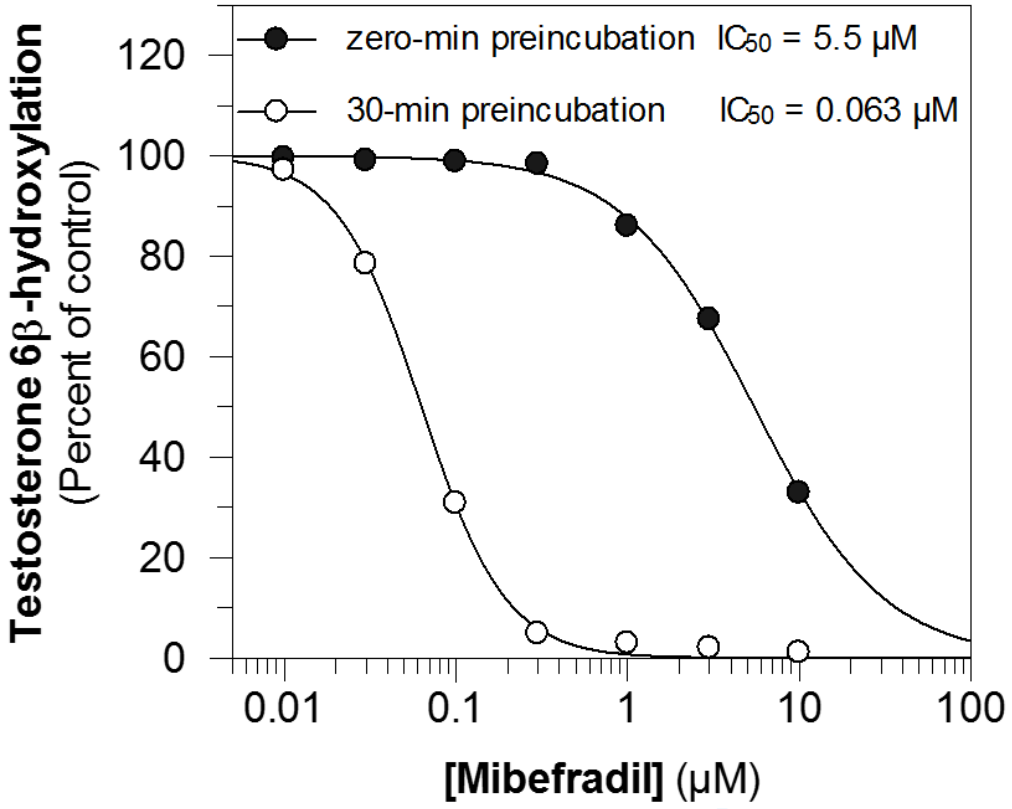
Ogilvie et al., *DMD*. **39**:2020, 2011

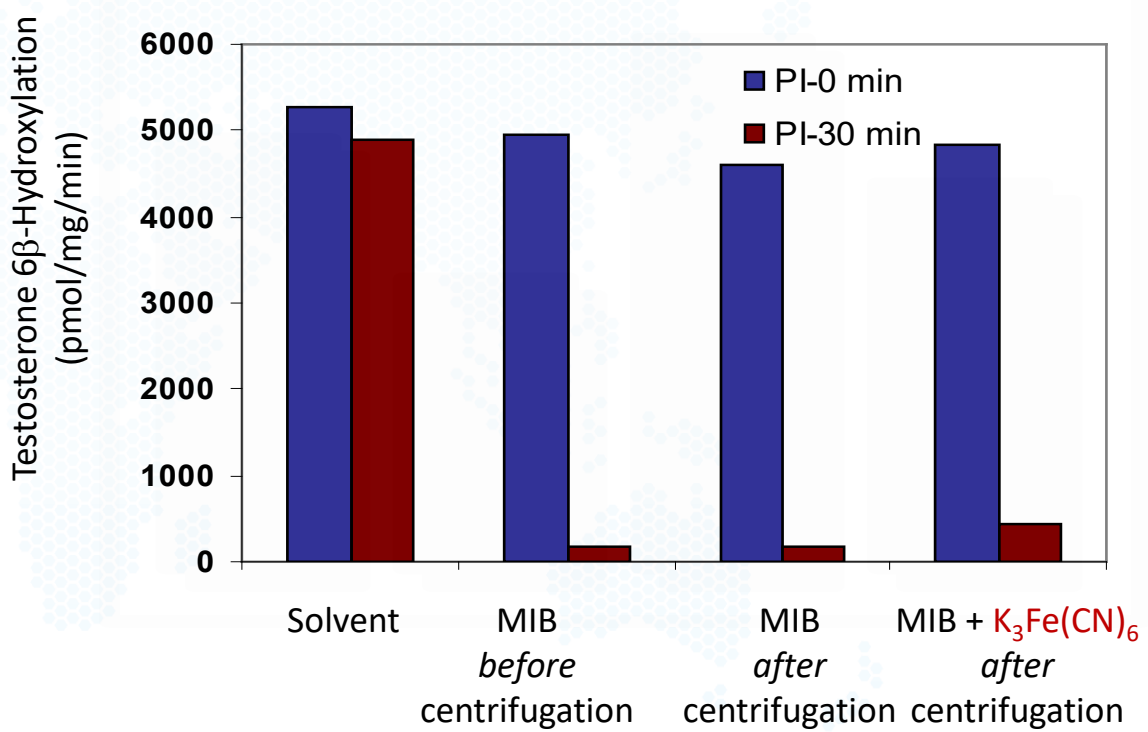
TDI Reversibility by ultracentrifugation

- Provides qualitative answers for better decision-making
- One concentration of drug (chosen from the IC_{50} shift assay), pre-incubated with NADPH, [substrate] = 10 K_m & HLM [0.1 mg/mL] (typically 30 - 120 min)
- After pre-incubation, samples are ultracentrifuged to remove unbound drug
- Typically 4 groups of samples:
  - Group A: Solvent control
  - Group B: Preincubated only (replicates IC_{50})
  - Group C: Preincubated and microsomes are re-isolated
  - Group D: Preincubated; microsomes are treated with 2 mM $K_3Fe(CN)_6$ (to dissociate quasi-irreversible metabolite-inhibitor complexes) prior to centrifugation

TDI Reversibility by ultracentrifugation

Ultracentrifugation does not reverse TDI:
Irreversible TDI of CYP3A4 by mibefradil (2 µM)

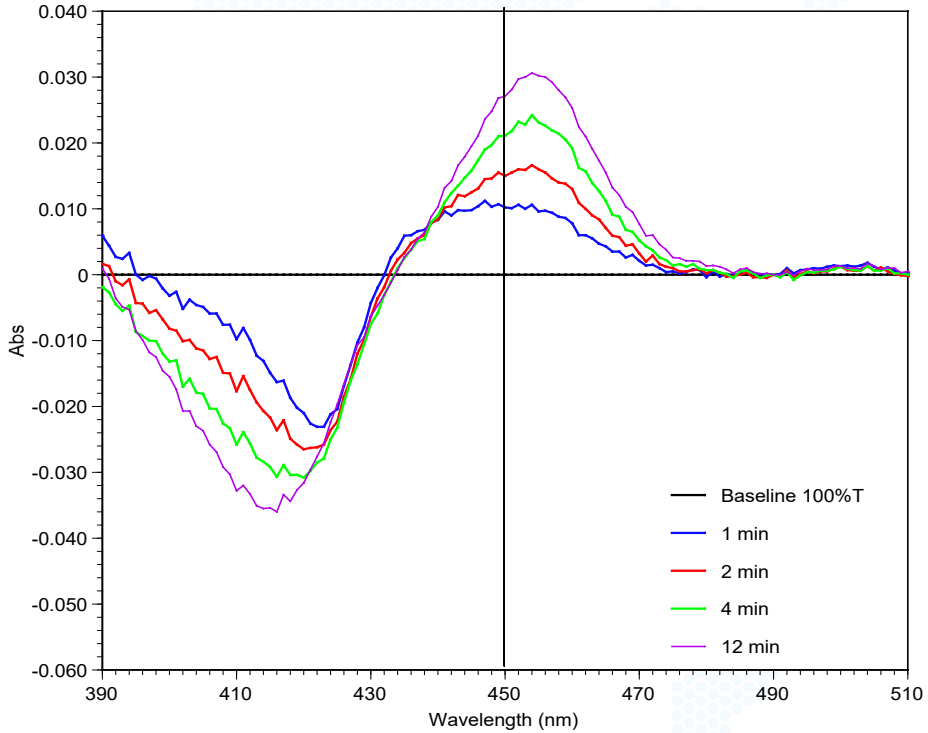




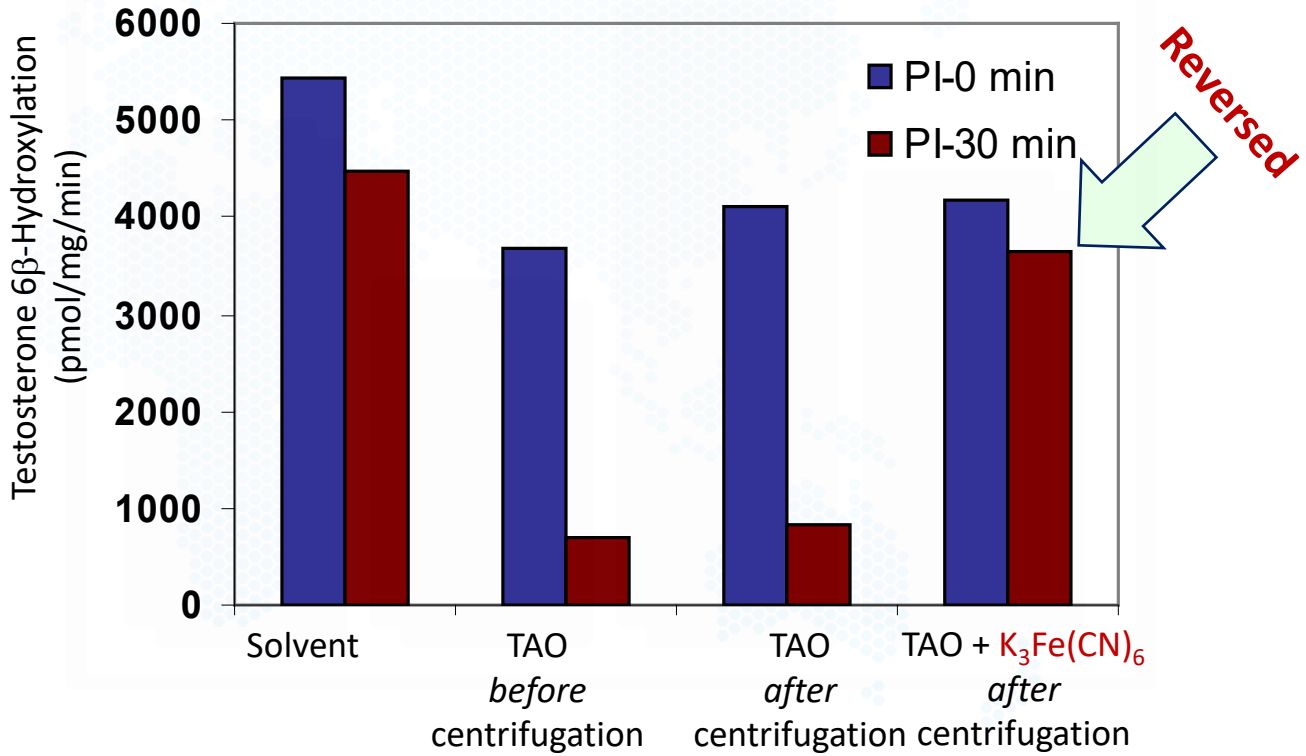
Mechanism of irreversible TDI of CYP3A4 by mibefradil shown to be heme destruction. Foti et al., *DMD*. **39**:1188, 2011

TDI Reversibility by ultracentrifugation

Ultracentrifugation does not reverse TDI, except in presence of $K_3Fe(CN)_6$:
Quasi-irreversible TDI of CYP3A4 by troleandomycin (25 µM)

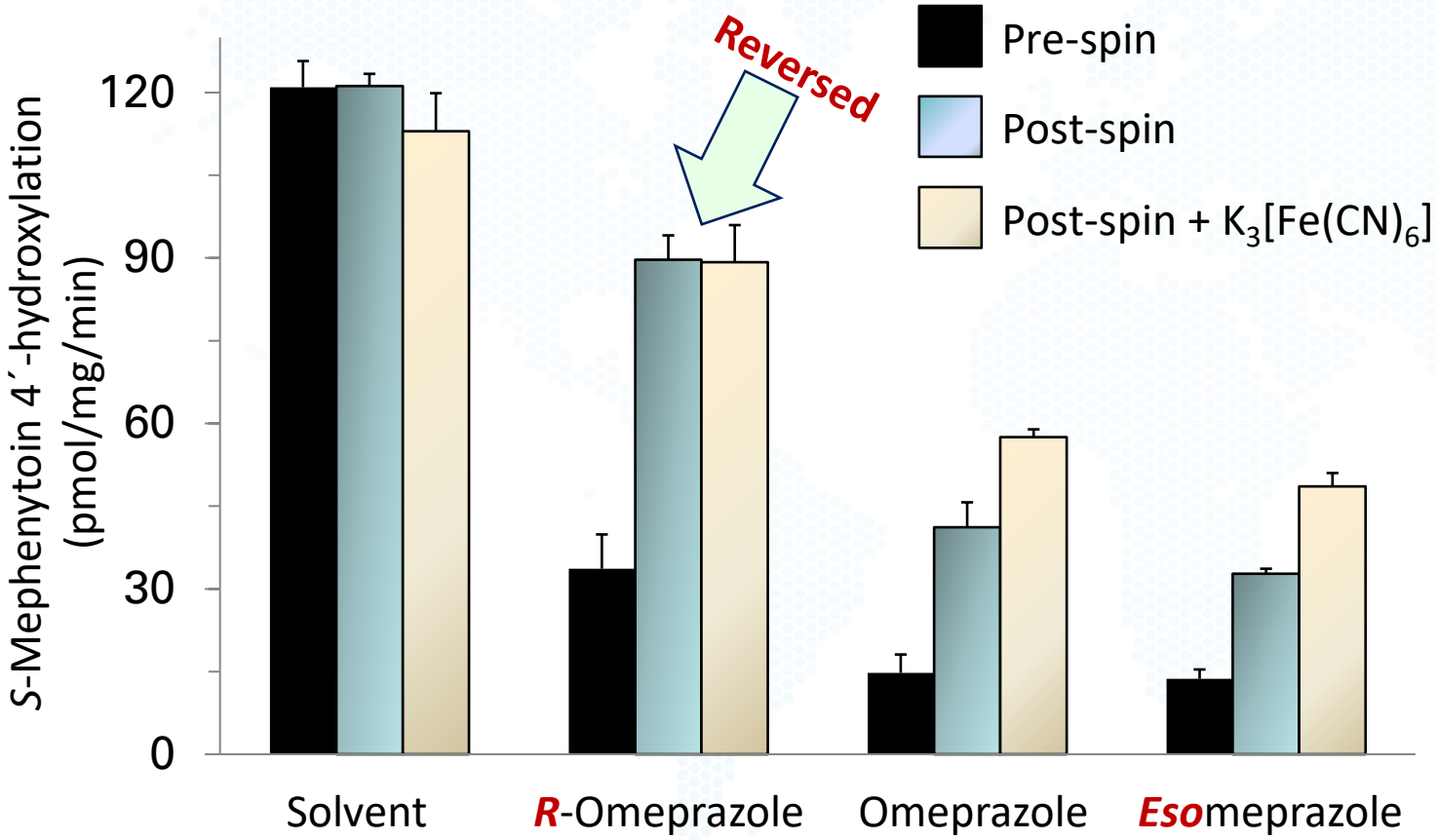


MIC formation (peak at ~455 nm) with TAO during an incubation with NADPH-fortified HLM (with high CYP3A4 content)



TDI Reversibility by ultracentrifugation

Ultracentrifugation has opposite effects on CYP2C19 TDI by omeprazole enantiomers



Esomeprazole is an *irreversible* TDI of CYP2C19, but *R*-omeprazole is a reversible TDI.

If these were drug candidates, and the *R*-enantiomer had activity, it may allow for an internal decision to prioritize it.

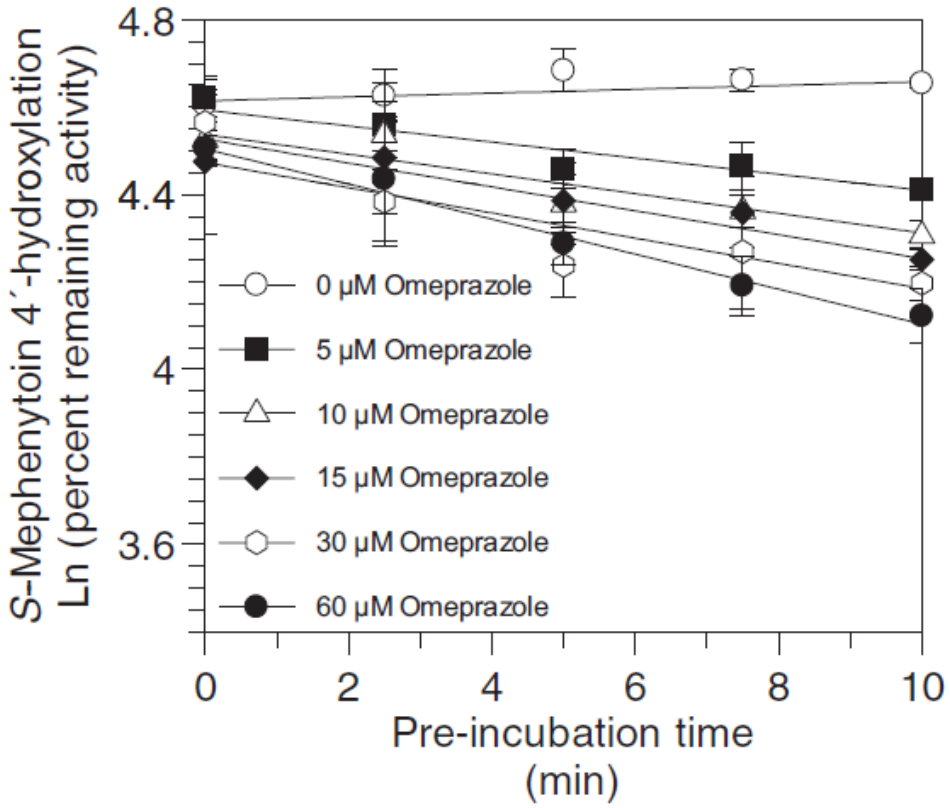
Ogilvie BW. (2015) An In Vitro Investigation Into the Mechanism of the Clinically Relevant Drug-Drug Interaction Between Omeprazole or Esomeprazole and Clopidogrel. University of Kansas, 289 pages

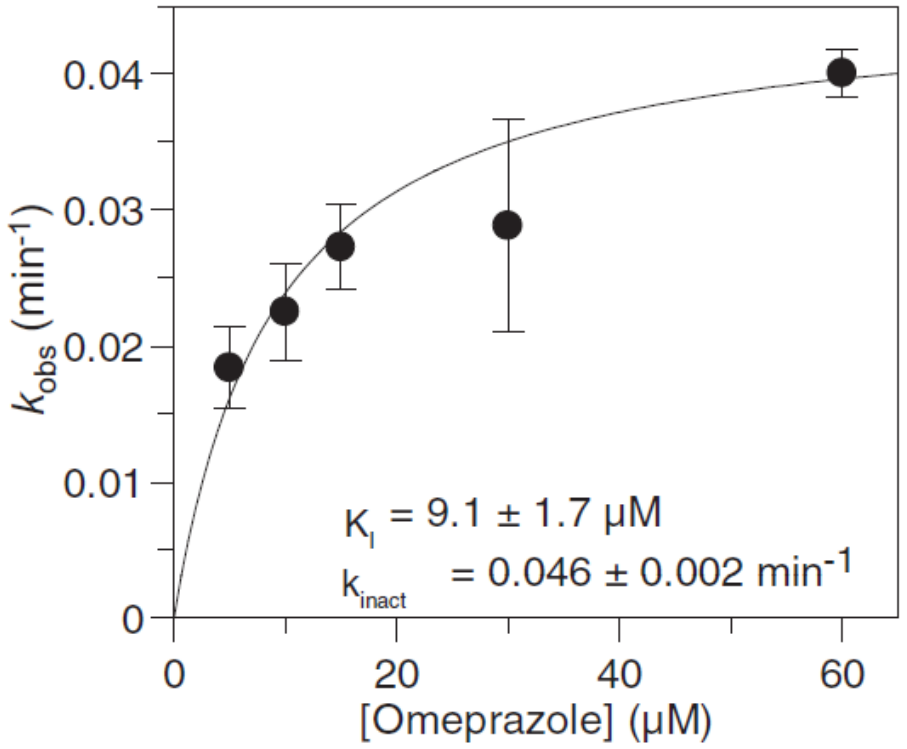
Definitive k_{inact} /K_I determinations for irreversible TDI

- Provides quantitative answers to put irreversible TDI into clinical context
- Typically 5 inhibitor concentrations + solvent control
- Typically 4-5 preincubation time points + zero-min preincubation
- Should be performed at saturating marker substrate concentrations (e.g., 10 x K_m) if solubility permits to minimize any direct competitive inhibition
- Usually incorporates a 10- to 30-fold dilution to decrease remaining test drug and metabolite concentrations
- Values can be used in basic, mechanistic static or mechanistic dynamic (i.e., PBPK) modelling to predict clinical impact on a co-administered drug

Definitive k_{inact} /K_I determinations for irreversible TDI

Pre-incubation: 2.5 mg/mL protein, diluted to 0.1 mg/mL. 5 min substrate incubation





Ogilvie et al., *DMD*. **39**:2020, 2011

Presentation Outline

1. Defining types of time-dependent inhibition (TDI) and their importance

2. In vitro approaches to evaluate various types of TDI:

- Screening vs. definitive studies

3. Approaches to predicting clinically-relevant DDIs from TDI

4. Case Examples:

- Drugs that only cause TDI in vitro (System-dependent effects)

- Drugs that cause clinically-relevant TDI

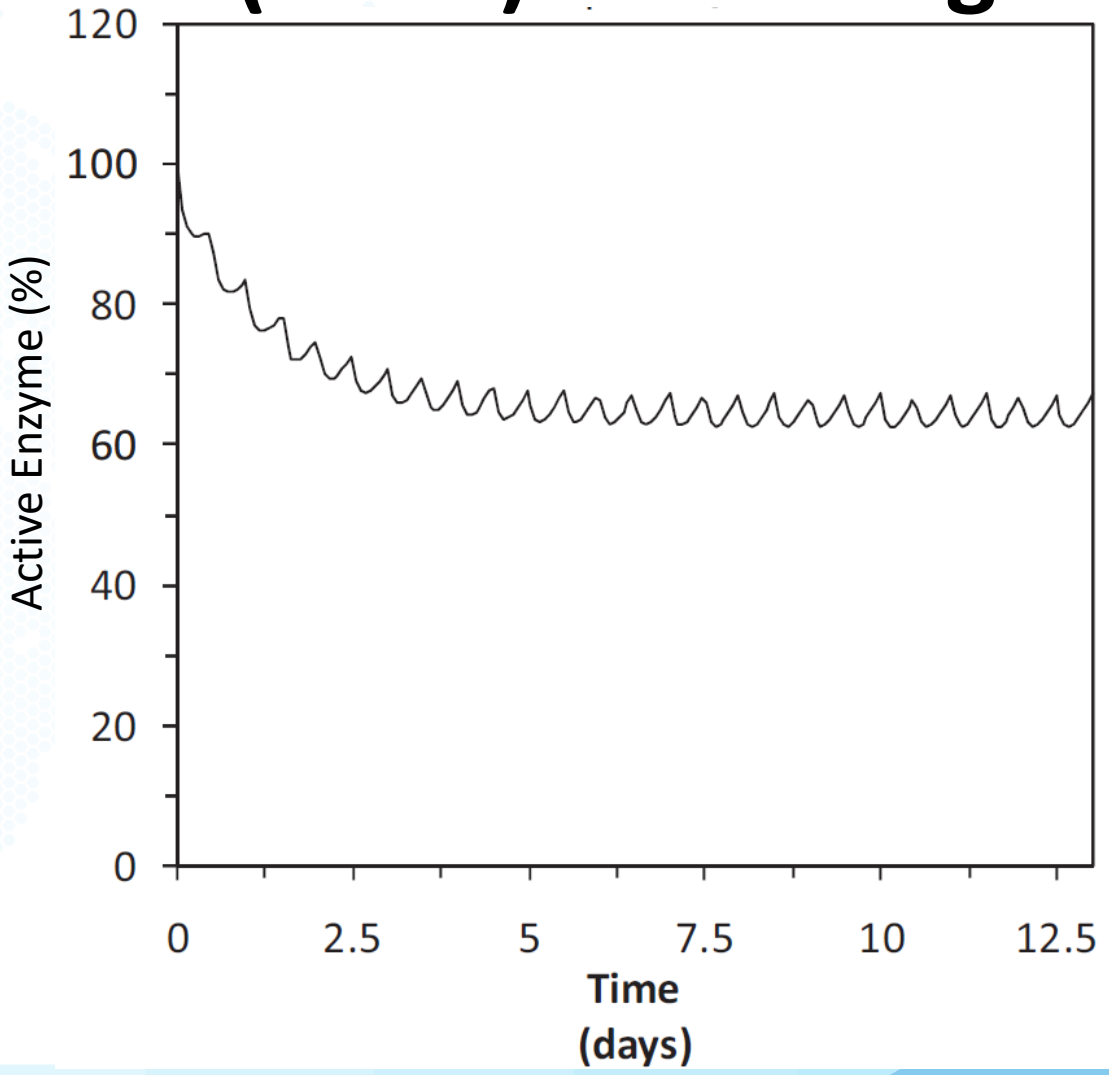
Basic interpretation of irreversible inhibition of hepatic CYP enzymes

Agency	Equation (as written)	Unbound or total concentration?	Cutoff $\frac{k_{obs} + k_{deg}}{k_{deg}}$	Comment
FDA (2020)	$K_{obs} = \frac{k_{inact} \cdot 50 \cdot I_{max,u}}{K_{I,u} + 50 \cdot I_{max,u}}$	Unbound C_{max} **Unbound** K_I	≥ 1.25	Same
PMDA (2018)	$K_{obs} = \frac{k_{inact} \cdot 50 \cdot [I]}{K_I + 50 \cdot [I]}$	Unbound C_{max} Not specified for K_I	≥ 1.25	Same
EMA (2013)	$K_{obs} = \frac{k_{inact} \cdot [I]}{K_I + [I]}$ (Note that here, [I] is 0.1 x dose/250ml)	Unbound C_{max} Not specified for K_I	≥ 1.25	Same cutoff, different equation

Note: PMDA and EMA recommend estimating unbound [I] in vitro due to non-specific binding, but not included in equations.

Example of mechanistic dynamic (PBPK) modelling

- Simulation of time-dependent changes in active CYP2C19 after 14 days of omeprazole (40 mg b.i.d.)
- The Simcyp Simulator version 10.2 was used to assess the time-varying effect of repeated omeprazole administration on active CYP2C19 over ~2 weeks
- The simulated increase in *S*-mephenytoin AUC was 1.45-fold, similar to the 2.21-fold observed increase in moclobemide AUC upon omeprazole coadministration



Ogilvie et al., *DMD*. **39**:2020, 2011

Presentation Outline

1. Defining types of time-dependent inhibition (TDI) and their importance

2. In vitro approaches to evaluate various types of TDI:

- Screening vs. definitive studies

3. Approaches to predicting clinically-relevant DDIs from TDI

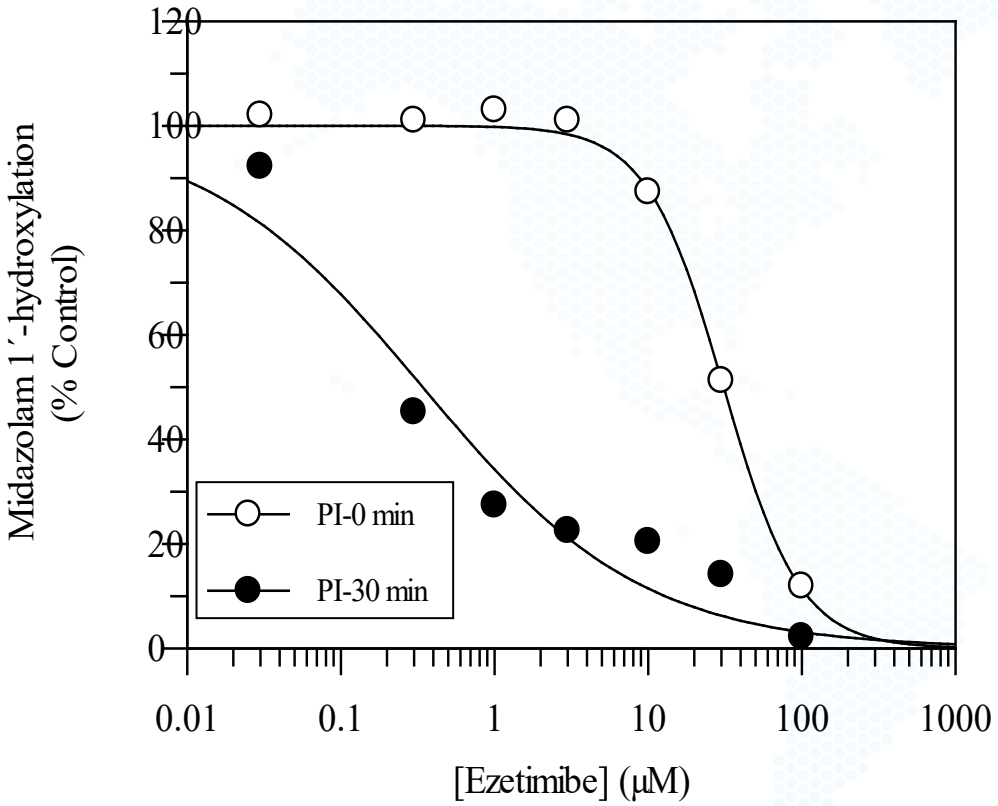
4. Case Examples:

- Drugs that only cause TDI in vitro (System-dependent effects)

- Drugs that cause clinically-relevant TDI

Drugs that only cause TDI in vitro (system-dependence)

- Inhibition of midazolam 1´-hydroxylation (CYP3A4/5) by ezetimibe in **HLM**



Zero-min IC_{50} = 31 µM
30- min IC_{50} = 0.34 µM

Ezetimibe is an effective TDI of CYP3A4 in human liver microsomes (~100-fold shift in IC_{50} value);

however

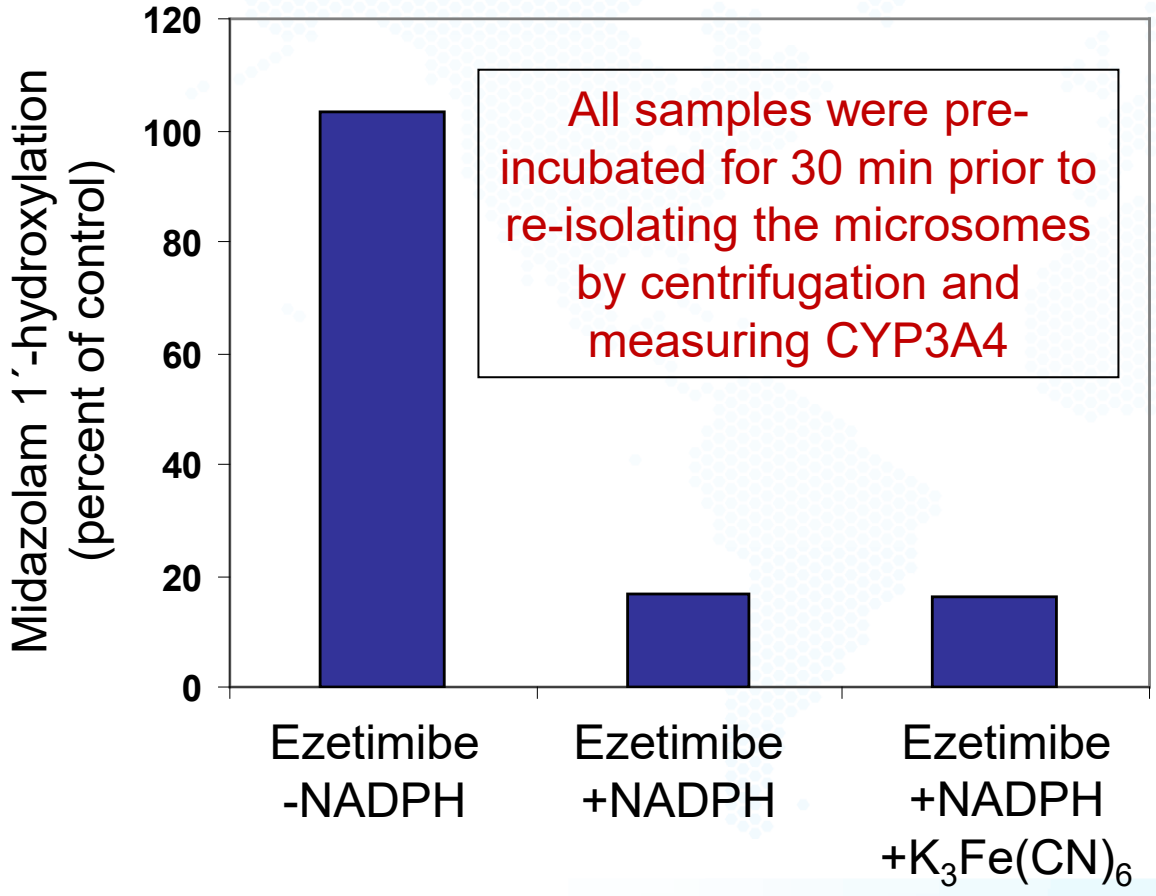
ezetimibe does NOT cause clinically significant inhibition of CYP3A4

WHY?

Parkinson et al., *DMPK*. **25**:16, 2010

Drugs that only cause TDI in vitro (system-dependence)

- Inhibition of midazolam 1´-hydroxylation (CYP3A4/5) by ezetimibe (10 µM) in re-isolated human liver microsomes



Ezetimibe is an irreversible TDI of CYP3A4 in human liver microsomes;

however

it does not cause clinically significant inhibition of CYP3A4

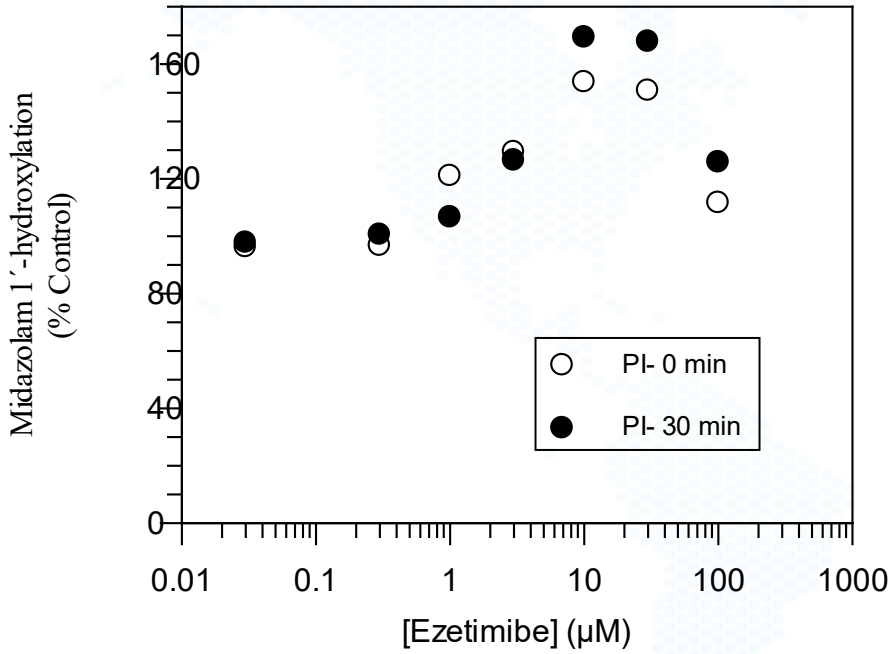
WHY?

Parkinson et al., *DMPK*. **25**:16, 2010

Drugs that only cause TDI in vitro (system-dependence)

- Inhibition of midazolam 1´-hydroxylation (CYP3A4/5) by ezetimibe

Human hepatocytes



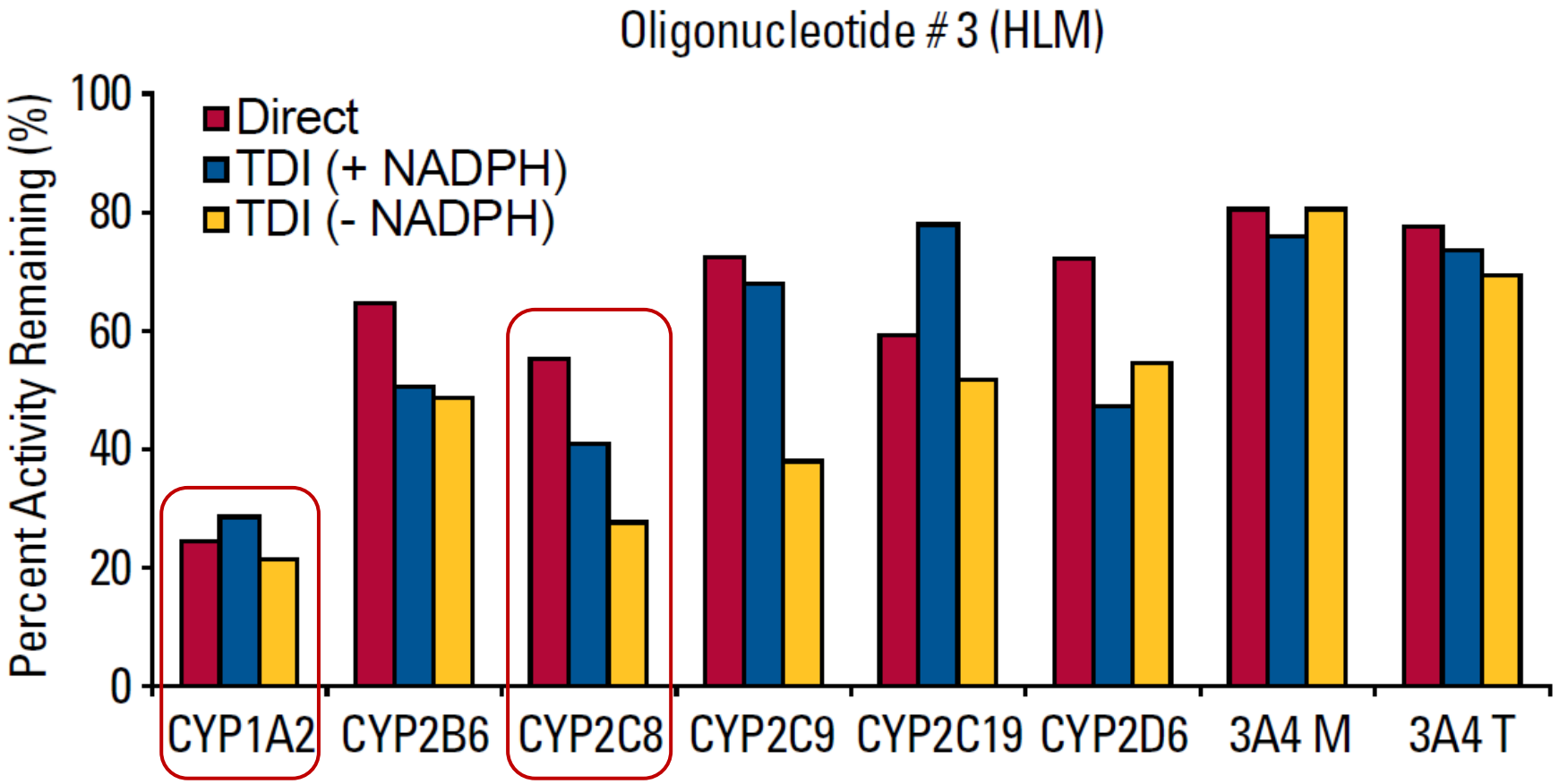
No inhibition

- Lack of clinical CYP3A4 inhibition by ezetimibe
- Ezetimibe is a potent metabolism-dependent inhibitor of CYP3A4 in human liver microsomes
- In hepatocytes, glucuronidation protects CYP3A4 – consistent with clinical results

Parkinson et al., *DMPK*. **25**:16, 2010

Drugs that only cause TDI in vitro (system-dependence)

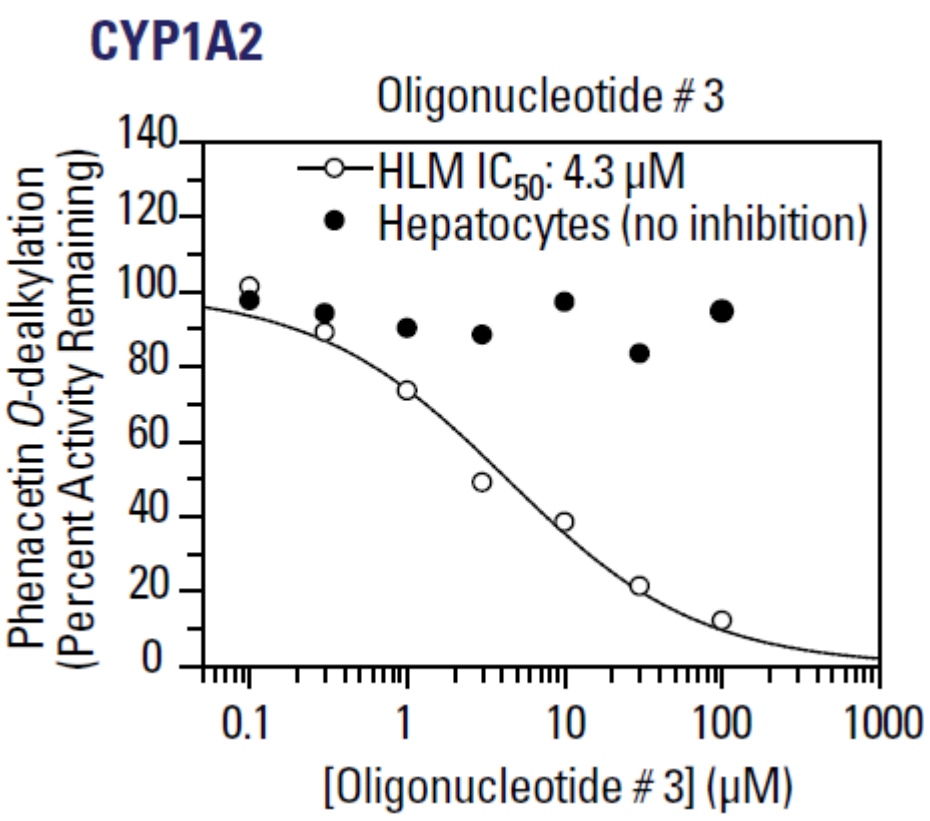
- Direct and TDI of CYPs by proprietary phosphorothioate oligonucelotides in HLM

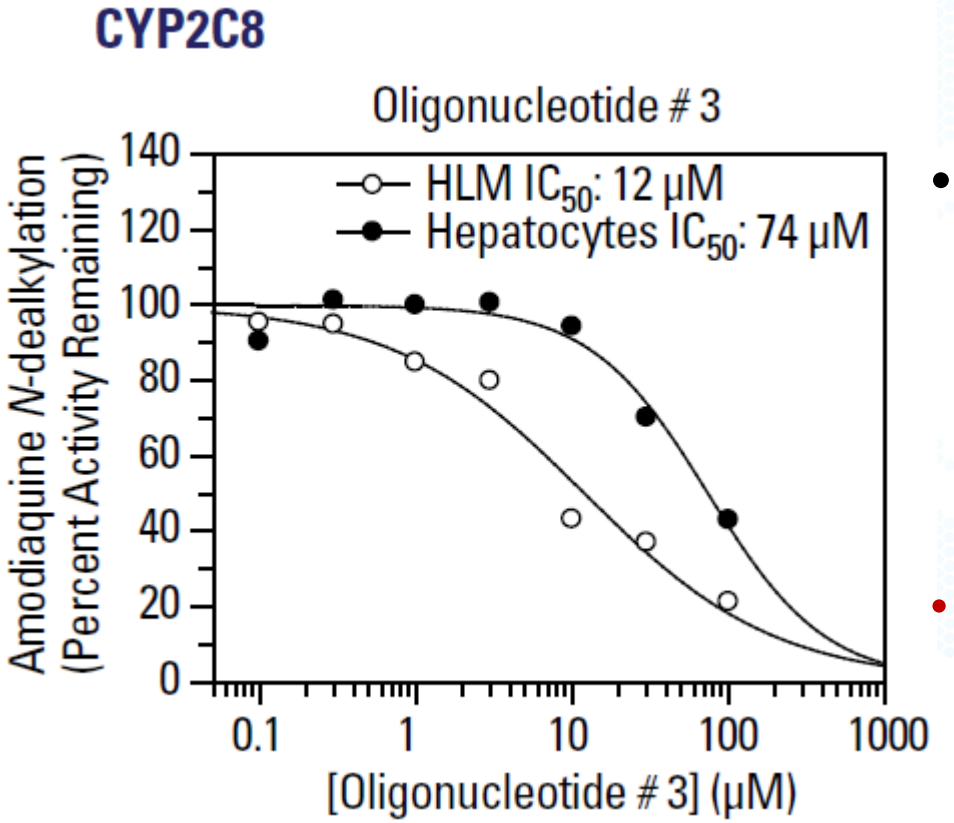


Adapted from Kazmi … Ogilvie, *DMD* **46**:1066, 2018

Drugs that only cause TDI in vitro (system-dependence)

- Inhibition of CYPs by proprietary phosphorothioate oligonucelotides in HLM and hepatocytes





- Similar results were observed with the investigational oligonucleotide, imeltestat.
- See Kazmi et al., *DMD* **47**:9, 2019

Adapted from Kazmi … Ogilvie, *DMD* **46**:1066, 2018

Presentation Outline

1. Defining types of time-dependent inhibition (TDI) and their importance

2. In vitro approaches to evaluate various types of TDI:

- Screening vs. definitive studies

3. Approaches to predicting clinically-relevant DDIs from TDI

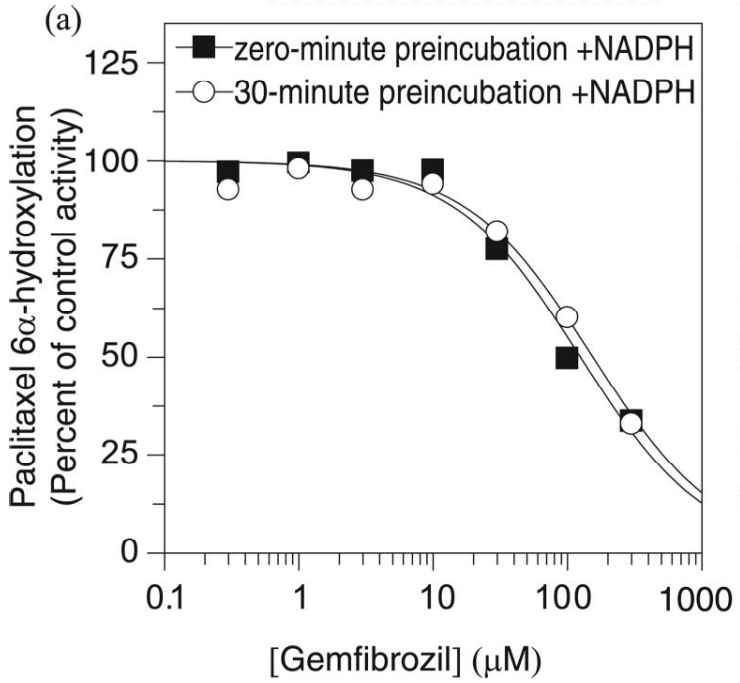
4. Case Examples:

- Drugs that only cause TDI in vitro (System-dependent effects)

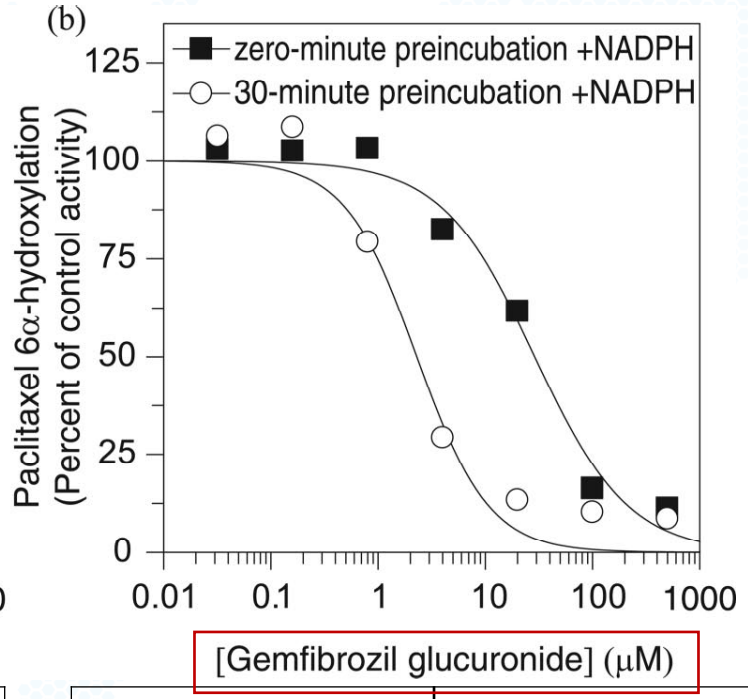
- Drugs that cause clinically-relevant TDI

Approved, marketed drugs that cause clinically-relevant TDI

- Gemfibrozil – metabolized to an acyl glucuronide that causes irreversible TDI of CYP2C8



0 min IC_{50}	30 min IC_{50}
120 μM	150 μM



0 min IC_{50}	30 min IC_{50}
24 μM	1.8 μM

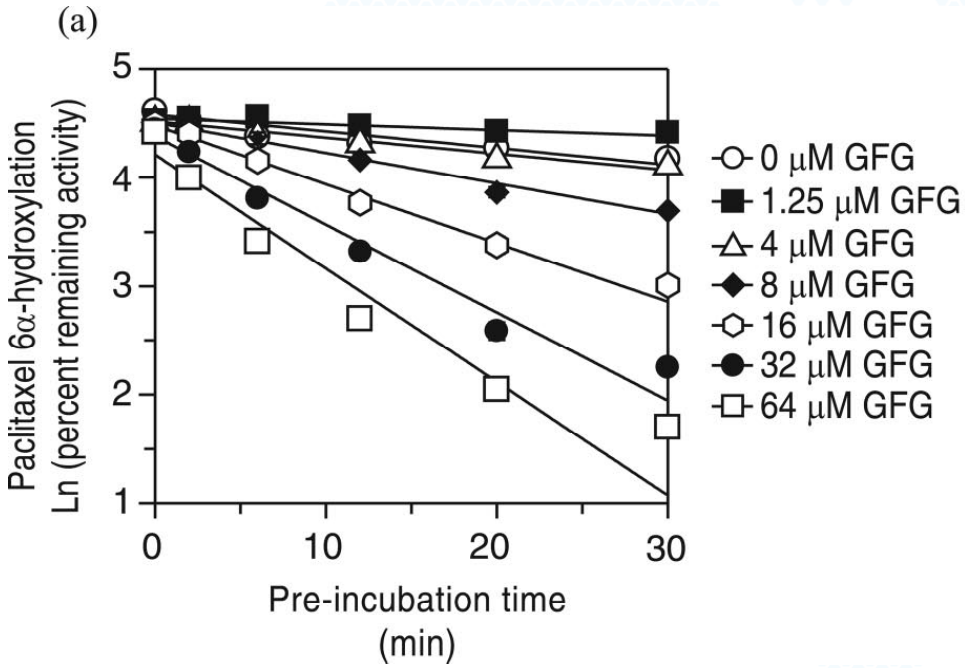
Fatal interactions occurred with cerivastatin, which is a CYP2C8 substrate.

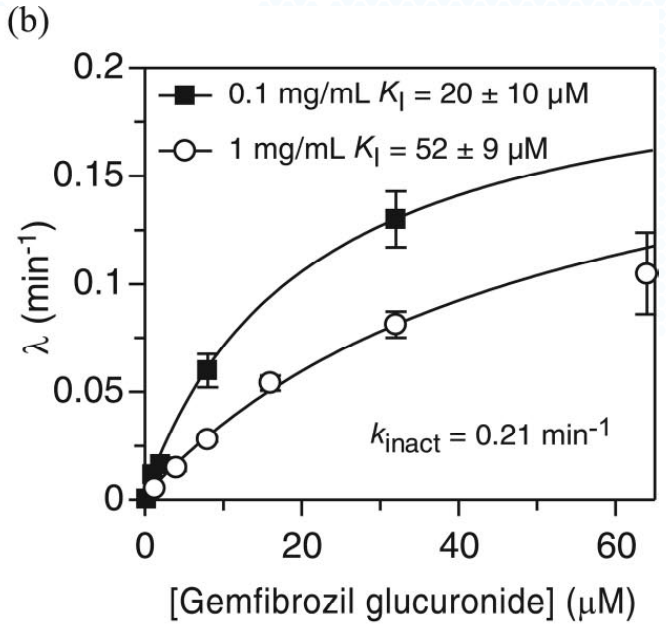
Cerivastatin, not gemfibrozil, was withdrawn in 2001.

Ogilvie et al., *DMD* **34**:191, 2006

Approved, marketed drugs that cause clinically-relevant TDI

- Gemfibrozil – metabolized to an acyl glucuronide that causes irreversible TDI of CYP2C8





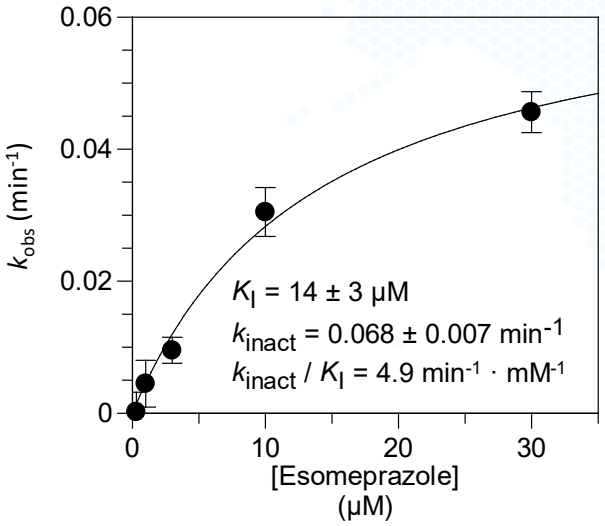
Gemfibrozil is still marketed, with labelling that states it is a strong CYP2C8 inhibitor and dosing reductions of CYP2C8 substrates may be required.

Ogilvie et al., *DMD* **34**:191, 2006

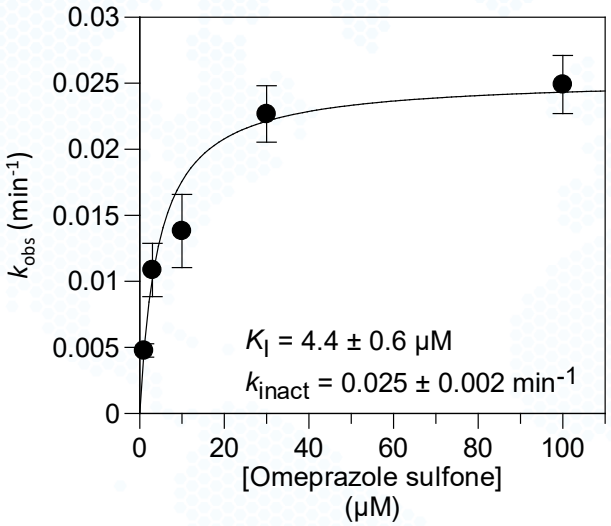
Approved, marketed drugs that cause clinically-relevant TDI

- Esomeprazole and its major metabolites irreversibly inhibit CYP2C19

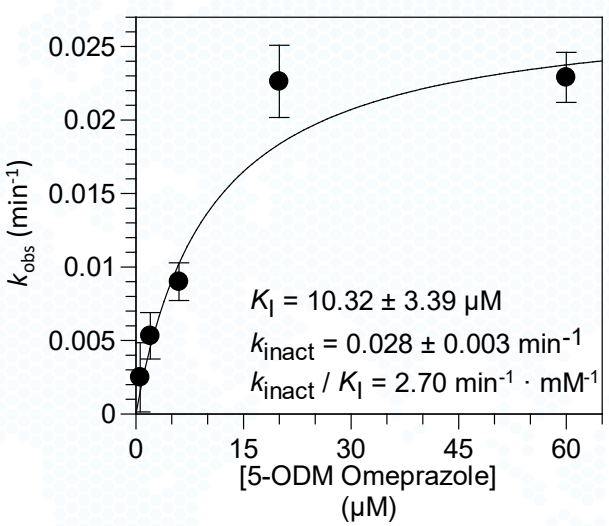
Esomeprazole



Omeprazole sulfone



5-*O*-desmethylomeprazole



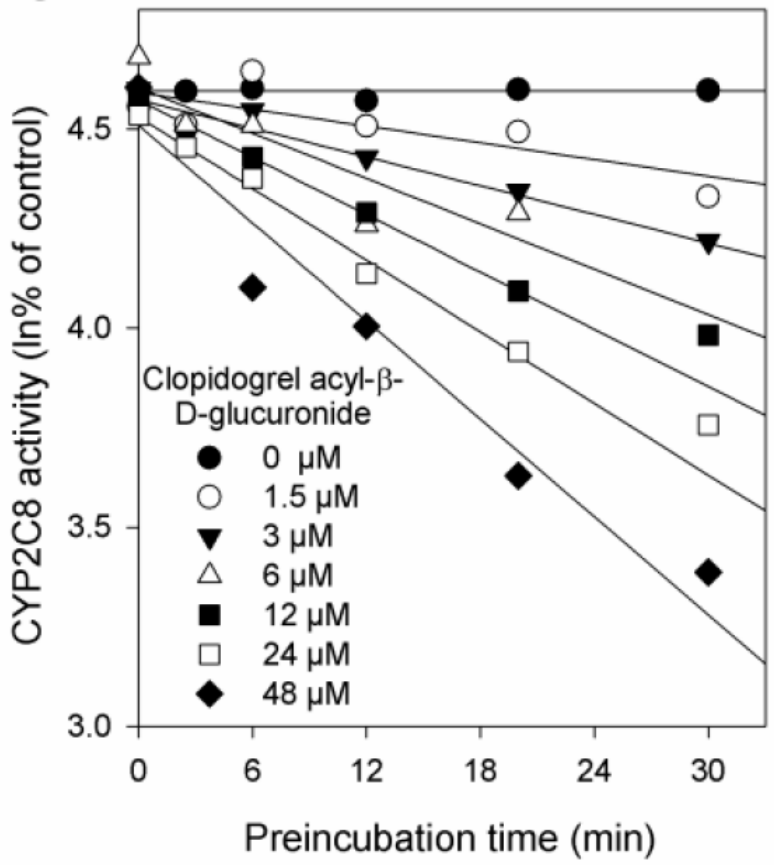
Esomeprazole is still marketed (including OTC), with labelling stating: "Clopidogrel is metabolized to its active metabolite in part by CYP2C19. **Avoid concomitant administration".**

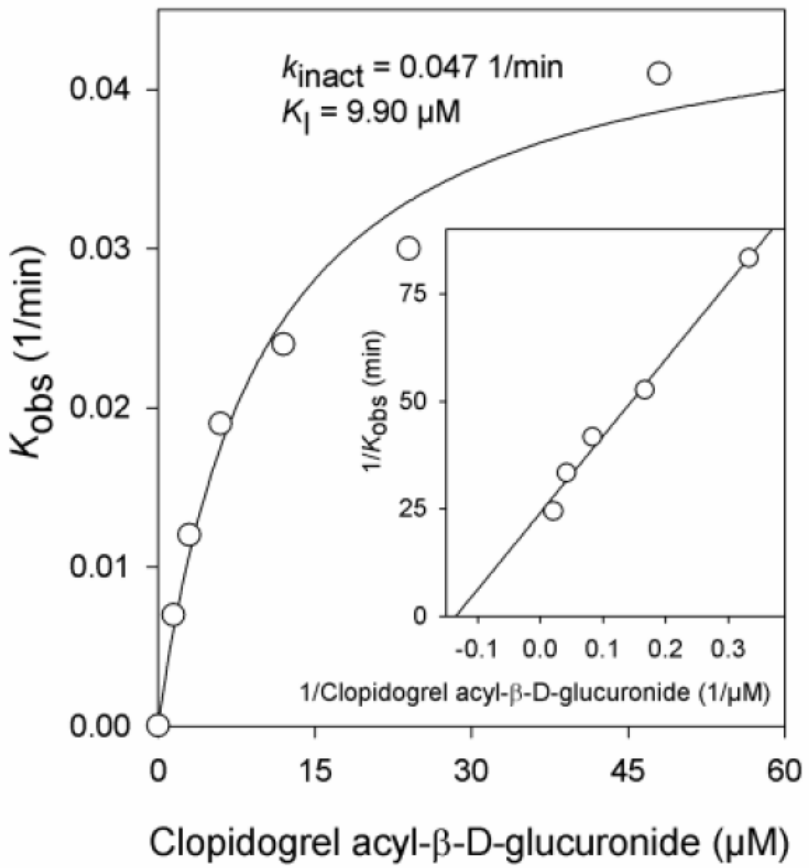
Compound	K_I (µM)	k_{inact} (min^{-1})	k_{inact} / K_I (min^{-1} · mM^{-1})
Esomeprazole	3.23 ± 0.35	0.0481 ± 0.0018	14.9 ± 1.7
Omeprazole sulfone	5.50 ± 1.94	0.0211 ± 0.0016	3.85 ± 1.39
5-*O*-Desmethylomeprazole	10.3 ± 3.4	0.0278 ± 0.0033	2.70 ± 0.94

Ogilvie BW. (2015) An In Vitro Investigation Into the Mechanism of the Clinically Relevant Drug-Drug Interaction Between Omeprazole or Esomeprazole and Clopidogrel. University of Kansas, 289 pages

Approved, marketed drugs that cause clinically-relevant TDI

- Clopidogrel, like gemfibrozil, is converted to a glucuronide that causes potent irreversible TDI of CYP2C8.



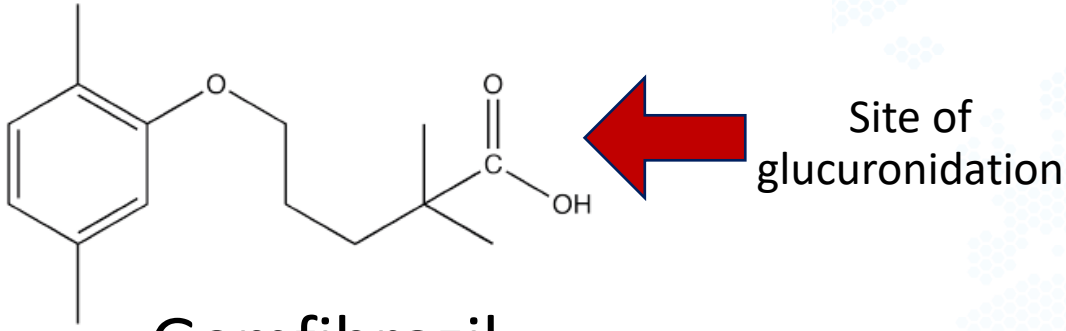


Clopidogrel is still marketed, with 2019 labelling noting that "acyl-β-glucuronide metabolite of clopidogrel is a strong inhibitor of CYP2C8. [Clopidogrel] can increase the systemic exposure to **drugs that are primarily cleared by CYP2C8, thereby needing dose adjustment and appropriate monitoring".**

Tornio … Backman, *CPT* **96**:498, 2014

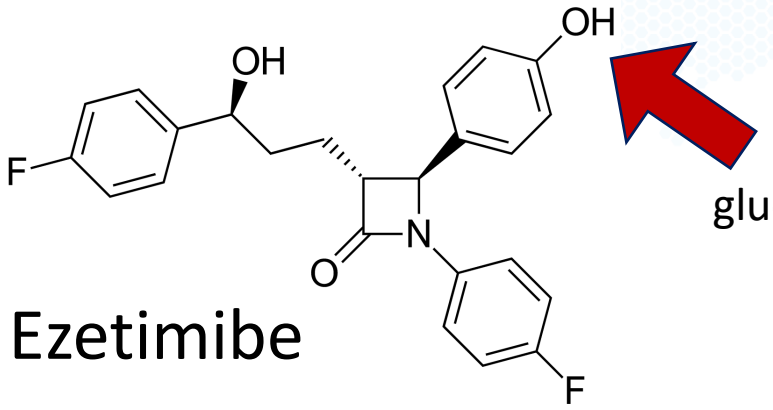
Potentially opposite effects of glucuronidation

- Consider phase II metabolism in some cases



Site of glucuronidation

Gemfibrozil



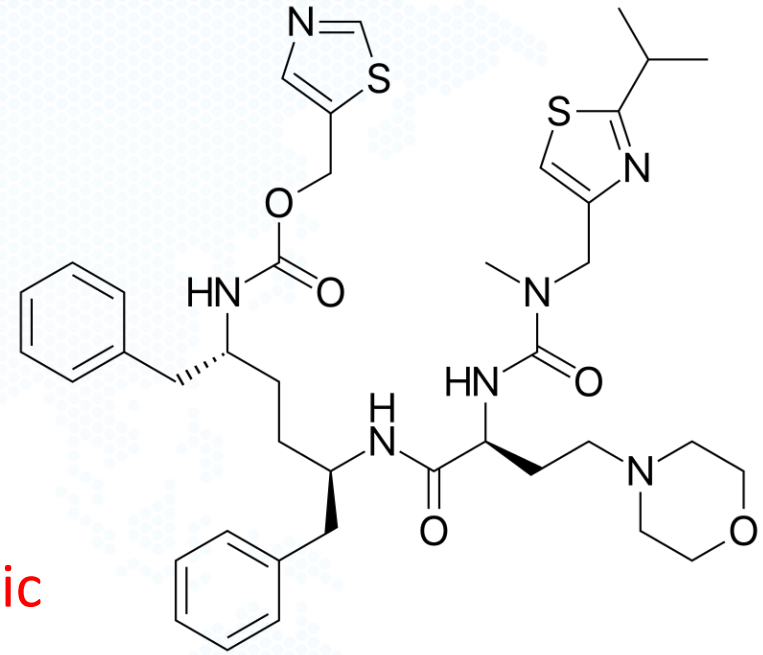
Site of glucuronidation

Ezetimibe

- Clinically, gemfibrozil causes significant TDI of CYP2C8, but *not* in HLM **(false -)**
- Ezetimibe is a good TDI of CYP3A4 in HLM (i.e., 100-fold shift in IC_{50} value), but it does *not* cause clinically significant inhibition of CYP3A4 **(false +)**
- In the case of gemfibrozil, its glucuronide (a major metabolite) actually causes irreversible TDI of CYP2C8 Ogilvie et al., *DMD* **34**:191, 2006
- In the case of ezetimibe, the glucuronide does *not* inhibit CYP3A4
- Opposite results (TDI vs. protection from inhibition) can occur due to glucuronidation Parkinson et al., *DMPK*. **25**:16, 2010

Finally - Irreversible inhibition can even be a benefit

- The curious case of cobicistat
- An approved drug with no pharmacological action (marketed as Tybost)
  - Not an anti-HIV drug – used to "boost" the exposure of anti-HIV drugs (i.e., elvitegravir, emtricitabine, tenofovir, darunavir, atazanavir)
  - Label: Mechanism of action – "a mechanism-based inhibitor of cytochrome P450 3A (CYP3A)"
  - Indication: "CYP3A inhibitor indicated to increase systemic exposure of atazanavir or darunavir (once daily dosing regimen) in combination with other antiretroviral agents in the treatment of HIV-1 infection in adults"
  - Increases midazolam AUC by 1,800% after 14 days of dosing
- Also inhibits CYP2D6, and several transporters



Cobicistat (Tybost®) Approved 2014

Conclusions

- Both CYP inhibition screening *and* definitive studies for candidate drugs and their metabolites have become increasingly sophisticated in their ability to detect TDI.
- Methods for discerning reversible from irreversible TDI of CYPs were discussed, as well as approaches to put the in vitro results into context.
- Simply because a signal for TDI is found, even if later shown to be irreversible, it does not necessarily mean the end of the road for these suicide substrates. Modelling may provide evidence that such inhibition is unlikely to be clinically relevant, or the data could be an in vitro artifact upon further investigation.
- Examples were provided of approved marketed drugs that cause irreversible TDI of one or more CYPs. This inhibition is managed through labelling changes, but is not typically a contemporary reason for withdrawal from the market.

Thank you for watching!

Questions? Get in touch through the Contact Us tab on our website

Please contact your regional account manager if you are interested in placing CYP inhibition, induction, reaction phenotyping, drug transporter or other studies or have interest in high-quality test systems for your assays