The Length of Preincubation Times in Abbreviated Cytochrome P450 Time-dependent Inhibition Studies: One Size Fits All?

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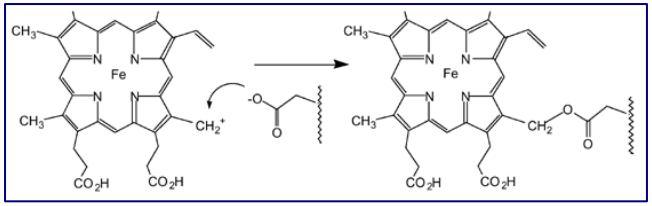
BD Biosciences Discovery Labware

In the Context of Drug-Drug Interactions, there are Generally Two Types of CYP Inhibition

- **Reversible Inhibition**
  - IC_{50} unchanged with incubation time
  - Most drugs are these
    - Competitive, noncompetitive, “mixed”



- **Time-Dependent Inhibition (TDI)**
  - Changes in IC_{50} with incubation time
  - Irreversible - covalently bound
    - Mechanism based inactivation
  - Quasi-Irreversible
    - Metabolite-intermediate complex [MIC]
  - Reversible
    - Metabolite more inhibitory than parent

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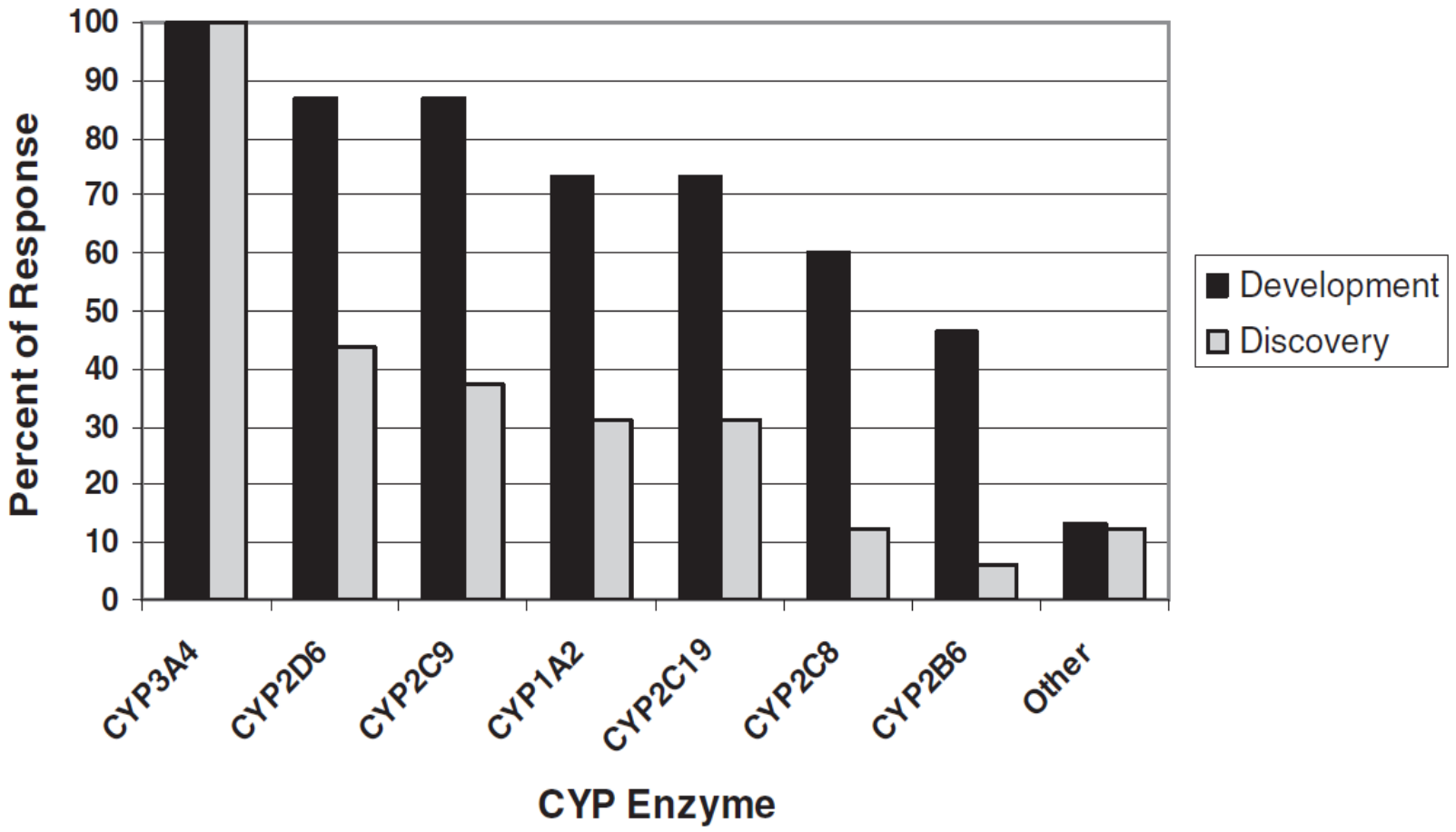
Consequences of TDI

- When TDI is the mode of inhibition, the inhibitory interaction will generally be greater over time following multiple dosing and be longer lasting after discontinuation

- Compared to reversible inhibition, prediction of inhibition response *in vivo* is more challenging

These Factors Drive Testing Behaviors



Percentage of respondents who test new chemical entities for their ability to cause time-dependent inhibition from a survey of 17 companies (2/3 large pharma) in March to April 2008 [Grimm SW et al (2009), Drug Metab. Dispos. 37:1355]

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Goals of Abbreviated Testing for TDI

- Detect TDI in a robust but simplified test
  - Use information to assess need for further investigation, such as determination of K_I/k_{inact} or testing in additional systems
- Avoid false negatives
  - TDI missed in assay, found later unexpectedly, after significant resources consumed – to be avoided
- Avoid false positives
  - TDI found in assay, but proved not to be inactivator upon subsequent testing
  - Chasing issues unnecessarily. A nuisance, but tolerable

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Regulatory Guidance - USFDA

"Time-dependent inhibition should be examined in standard in vitro screening protocols. A 30-minute pre-incubation of a potential inhibitor before the addition of substrate is recommended"

- *FDA DRAFT Guidance for Industry – Drug Interaction Studies (Sept. 2006)*

"TDI should be studied in standard in vitro screening protocols by pre-incubating the drug… before the addition of a substrate. Any time-dependent loss of initial product formation rate may indicate time-dependent inhibition, and definitive in vitro studies to obtain TDI parameters (i.e., k_{inact} and K_I). Details of this tiered approach were proposed by the PhRMA Drug Metabolism Technical Group (Grimm et al. 2009)."

- *FDA DRAFT Guidance for Industry – Drug Interaction Studies (Feb. 2012)*

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Regulatory Guidance - EMEA

- *"…If the inhibition is enhanced by pre-incubations, time-dependent inhibition may be present. In this situation kinact and KI should be determined"*

- Draft EMEA Guideline on the Investigation of Drug Interactions (April, 2010)

Time-dependent Inhibition Testing Practices

- A typical initial test is an "IC_{50} shift" assay
  - 47% of companies in 2009 PhRMA survey[1] use this model as initial TDI assessment
- 35% measure percent decline or inactivation rate at a single inhibitor concentration

0090-9556/09/3707-1355–1370$20.00
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DRUG METABOLISM AND DISPOSITION

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Perspective

The Conduct of in Vitro Studies to Address Time-Dependent Inhibition of Drug-Metabolizing Enzymes: A Perspective of the Pharmaceutical Research and Manufacturers of America

Scott W. Grimm, Heidi J. Einolf, Steven D. Hall, Kan He, Heng-Keang Lim, Kah-Hiing John Ling, Chuang Lu, Amin A. Nomeir, Eleanore Seibert, Konstantine W. Skordos, George R. Tonn, Robert Van Horn, Regina W. Wang, Y. Nancy Wong, Tian J. Yang, and R. Scott Obach

AstraZeneca Pharmaceuticals, Wilmington, Delaware (S.W.G.); Allergan Inc., Irvine, California (K.-H.J.L.); Amgen Inc., South San Francisco, California (G.R.T.); Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, Connecticut (E.S.); Bristol-Myers Squibb Co., Princeton, New Jersey (K.H.); Eisai Research Institute, Andover, Massachusetts (Y.N.W.); Eli Lilly and Company, Indianapolis, Indiana (S.D.H.); GlaxoSmithKline, King of Prussia, Pennsylvania (K.W.S.); Hoffmann-La Roche, Nutley, New Jersey (T.J.Y.); Johnson & Johnson Pharmaceutical Research Institute, Raritan, New Jersey (H.-K.L.); Merck Research Laboratories, Rahway, New Jersey (R.W.W.); Millennium Pharmaceuticals, Inc., Cambridge, Massachusetts (C.L.); Novartis Pharmaceuticals Corporation, East Hanover, New Jersey (H.J.E.); Pfizer Inc., Groton, Connecticut (R.S.O.); Sanofi-Aventis, Malvern, Pennsylvania (R.V.H.); and Schering-Plough Research Institute, Kenilworth, New Jersey (A.A.N.)

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ABSTRACT:

Time-dependent inhibition (TDI) of cytochrome P450 (P450) enzymes caused by new molecular entities (NMEs) is of concern because such compounds can be responsible for clinically relevant drug-drug interactions (DDI). Although the biochemistry underlying mechanism-based inactivation (MBI) of P450 enzymes has been generally understood for several years, significant advances have been made only in the past few years regarding how in vitro time-dependent inhibition data can be used to understand and predict clinical DDI. In this article, a team of scientists from 16 pharmaceutical research organizations that are member companies of the Pharmaceutical Research and Manufacturers of America offer a discussion of the phenomenon of TDI with emphasis on the laboratory methods used in its measurement. Results of an anonymous survey regarding pharmaceutical industry practices and strategies around TDI are reported. Specific topics that still possess a high degree of uncertainty are raised, such as parameter estimates needed to make predictions of DDI magnitude from in vitro inactivation parameters. A description of follow-up mechanistic experiments that can be done to characterize TDI are described. A consensus recommendation regarding common practices to address TDI is included, the salient points of which include the use of a tiered approach wherein abbreviated assays are first used to determine whether NMEs demonstrate TDI or not, followed by more thorough inactivation studies for those that do to define the parameters needed for prediction of DDI.

Pharmacokinetic drug-drug interactions (DDIs) can occur when one drug alters the metabolism of a coadministered drug. The outcome is an increase or decrease in the systemic clearance and/or bioavailability, and a corresponding change in the exposure to a coadministered drug. The clinical consequences of DDIs range from lack of therapeutic efficacy of a life saving drug to severe adverse drug reactions, including fatalities. Significant drug-drug interactions can lead to termination of development of otherwise promising new therapies, withdrawal of a drug from the market, or severe restrictions/limitations on its use (Wienkers and Heath, 2005). Because of the impact on patient health and safety, DDI was the subject of a position paper in 2003 by scientists from member companies of the Pharmaceutical Research and Manufacturers of America (PhRMA) that focused on

Article, publication date, and citation information can be found at http://dmd.aspetjournals.org.
doi:10.1124/dmd.109.026716.

ABBREVIATIONS: DDI, drug-drug interactions; PhRMA, Pharmaceutical Research and Manufacturers of America; FDA, Food and Drug Administration; P450, cytochrome P450; TDI, time-dependent inhibition; MBI; mechanism-based inactivation; MIC, metabolite-intermediate complex; NME, new molecular entity; IVIVE, in vitro-in vivo extrapolation; AUC, area under the curve; ADME, absorption, distribution, metabolism, and excretion; PK, pharmacokinetic; PBPK, physiologically based pharmacokinetics; MDMA, 3,4-methylenedioxymethamphetamine; SAR, structure activity relationships; HLM, human liver microsome.

[1]Grimm et al, 2009

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For Respondents Conducting the IC_{50} Shift…

- About half of respondents perform IC_{50} shift experiments by conducting the pre-incubation at a higher concentration of I and E and then diluting into incubation with probe substrate (like a typical k_{inact} experiment)

- …while the other half conduct the activity incubation by adding the probe substrate with no dilution step

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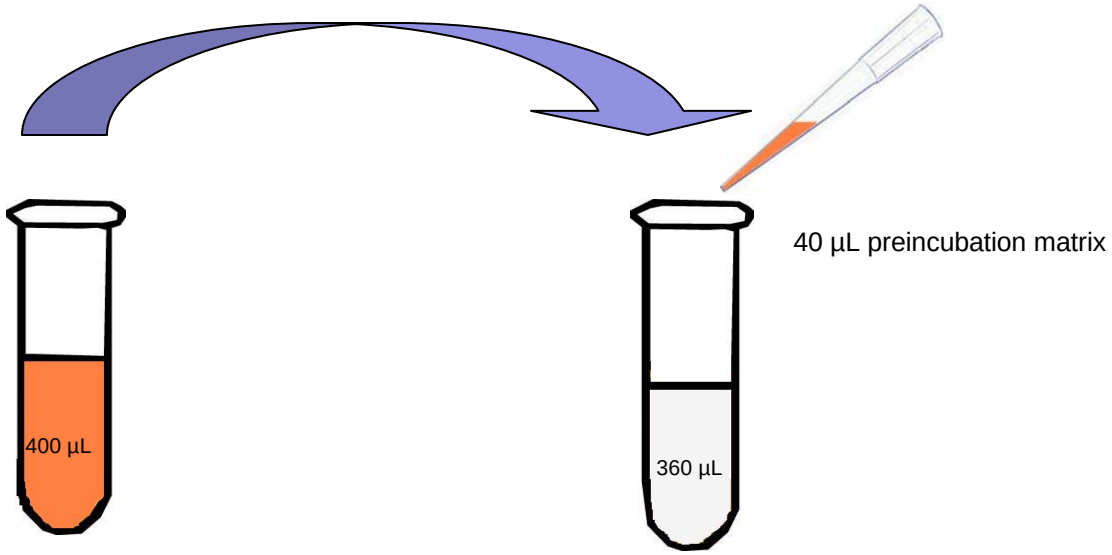
Dilution Method for IC_{50} Shift



Preincubation
10X Test article, n= 7, 0.5 log spacing*
10X Microsomal protein
1X NADPH regenerating system
Probe Substrate not present
30 min Preincubation time

Secondary Incubation different vessel
1X Test article, n = 7, 0.5 log spacing
1X Microsomal protein
1X NADPH regenerating system
Probe Substrate present
5 min incubation time

*This fold dilution is a typical value

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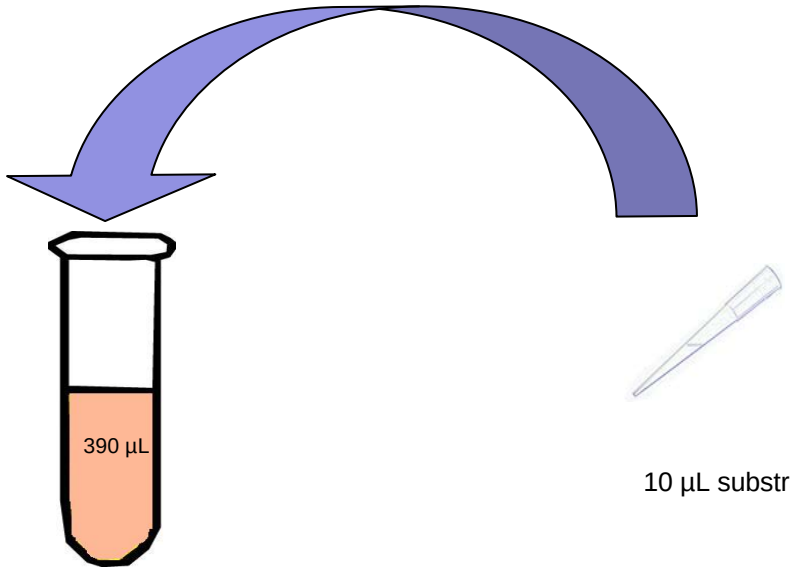
Non-dilution Method for IC_{50} Shift

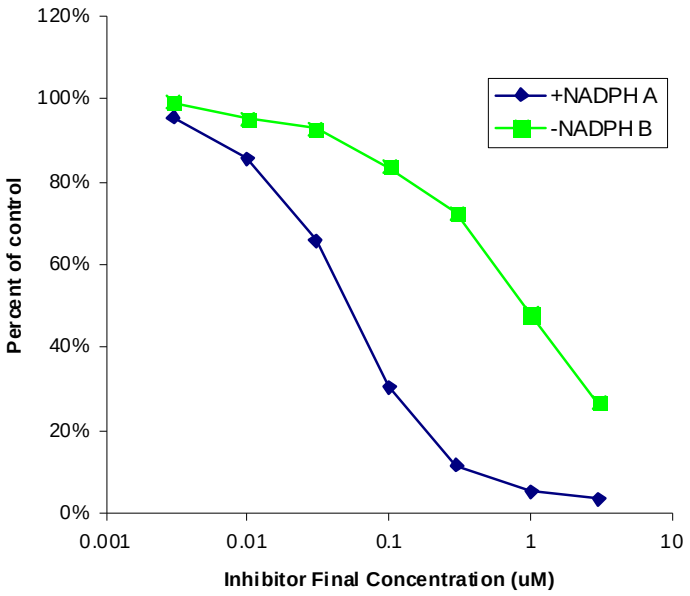


Preincubation
1X Test article, n= 7, 0.5 log spacing
1 X Microsomal protein
1X NADPH regenerating system
Probe Substrate not present
30 min Preincubation time

Secondary Incubation same vessel
1X Test article, n = 7, 0.5 log spacing
1X Microsomal protein
1X NADPH regenerating system
Probe Substrate present
5 min Incubation time

IC_{50} Shift Defined

- Simple ratio of IC_{50} values
  - "Plus NADPH" IC_{50} value as denominator
  - "Minus NADPH" IC_{50} value as numerator (or direct IC_{50} value)
- If the ratio is > than cut-off value, this indicates TDI
- A recommended cut-off value is 1.5 to 2-fold (Grimm et al, 2009)
- Note: Comparison of percent inhibition data is also important, particularly when inhibition or solubility limitations preclude calculation of an IC_{50} value

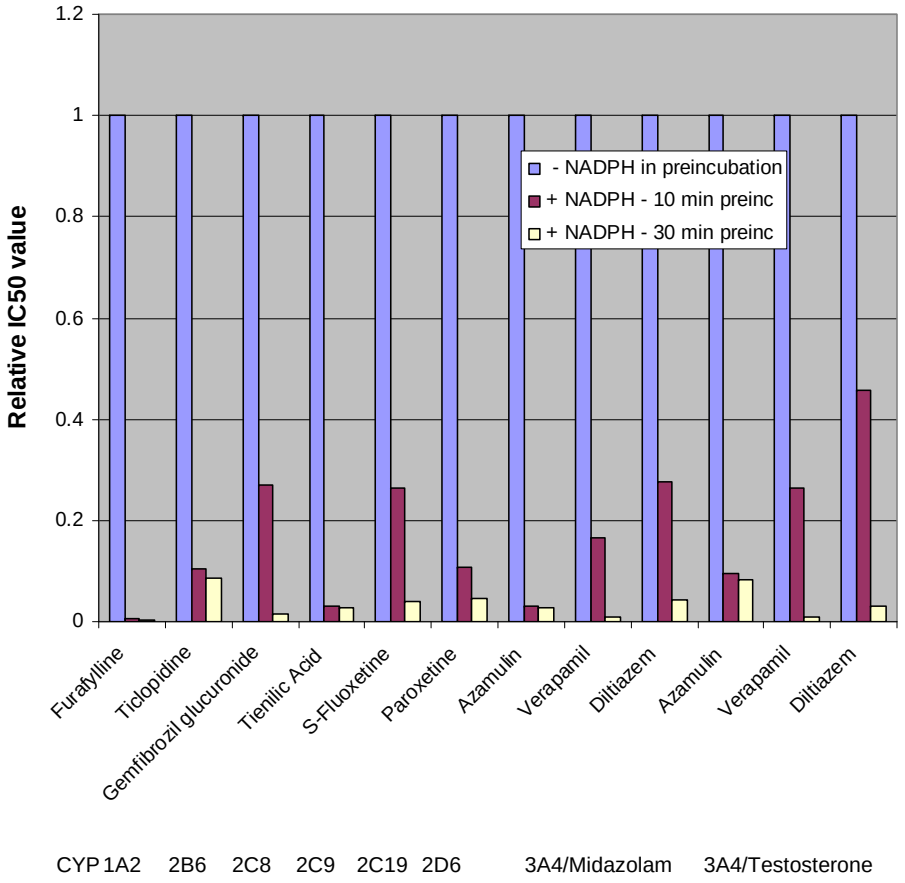


How Long to Preincubate?

- A 30 min preincubation period is often used
- Why?
  - There is a general assumption that this is adequate to permit detection of TDI
  - It was recommended in 2006 draft FDA guidance

Comparison of 10 and 30 Min Preincubation

- Results from our laboratory demonstrate that comparing pre-incubation times of 10 to 30 minutes distinguish rapid from slow acting time-dependent inhibitors[1].
- We found significant decreases in shifted IC_{50} values in the interval 10 to 30 minutes for some, but not all compounds, suggesting the 30 min period is arbitrary and possibly too short



1 - Perloff et al (2009) Xenobiotica, 39:99-112; US patent 7,968,314

Longer Incubation Times to Enhance Sensitivity in Detecting TDI

Case Study with Diltiazem

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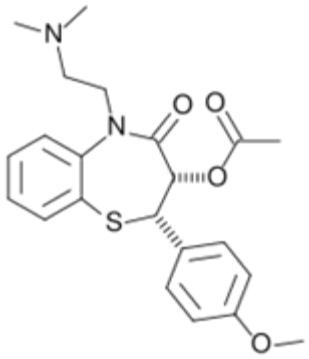
Sequential Metabolism of Diltiazem is Responsible for Time-Dependent Inhibition of CYP3A

- N-desmethyl metabolite is a more potent inactivator than parent diltiazem
- Further oxidation to N-hydroxydesmethyl diltiazem leads to MIC and TDI

	K_{inact}	K_I
N-desmethyl diltiazem	0.047	1.1
Diltiazem	0.012	0.48



Adapted from Zhao et al (2007) Sequential Metabolism Is Responsible for Diltiazem-Induced Time-Dependent Loss of CYP3A. Drug Metab Dispos 35:704.

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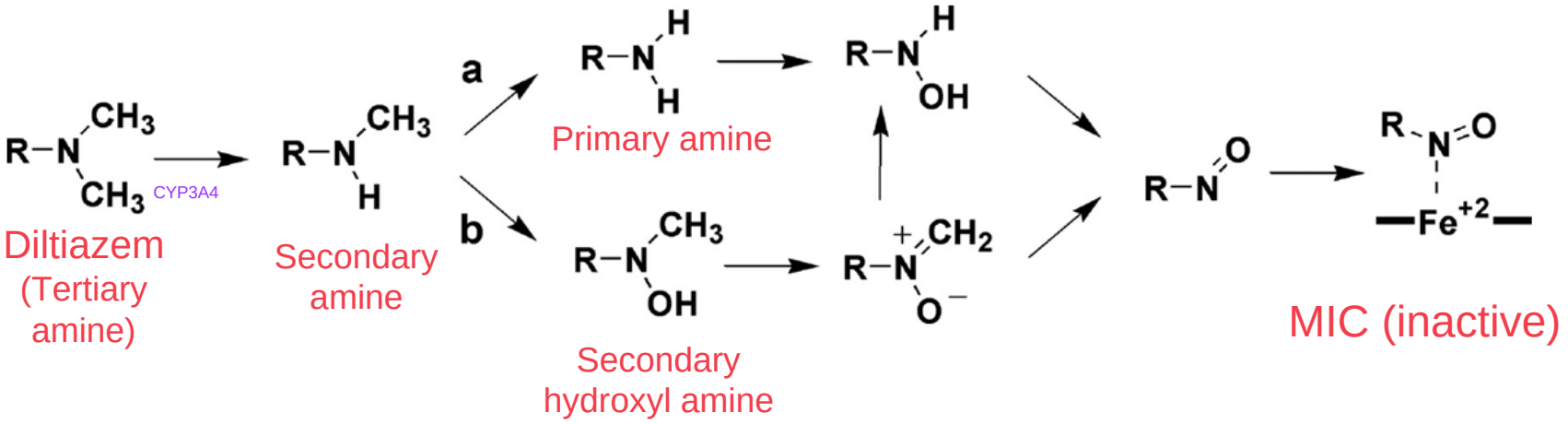
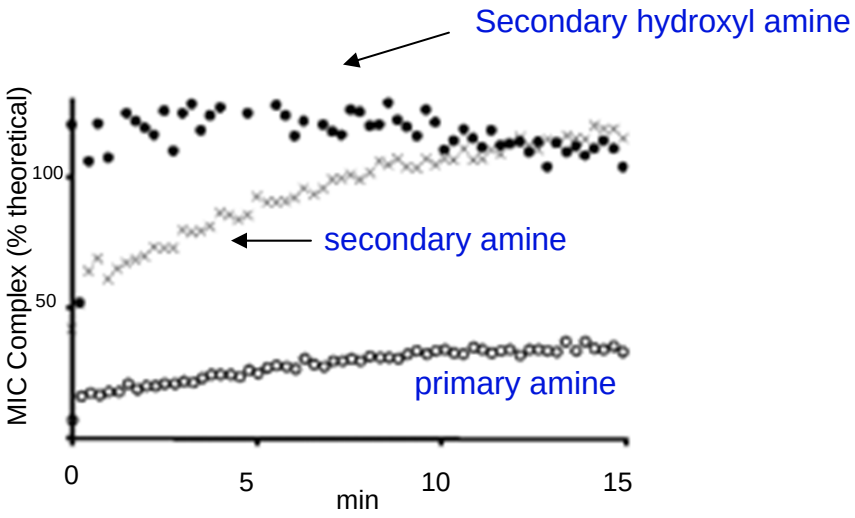
Sequential Metabolism Leading to TDI





- Conditions that promote <u>sequential metabolism</u> are expected to drive MIC formation
- This would include higher [I], higher protein (because v ~ E), and longer preincubation times

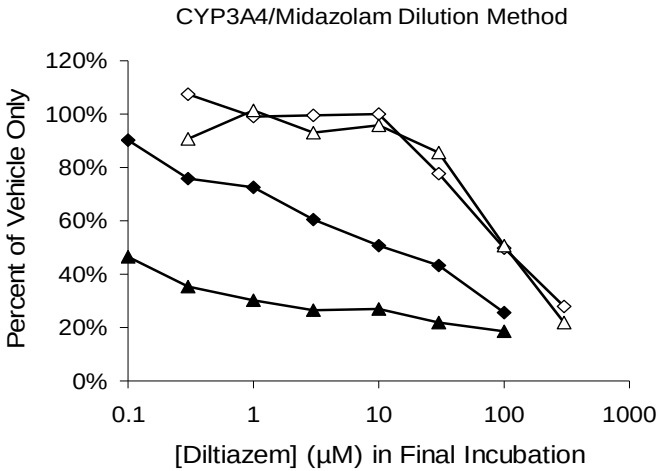
Hansen et al (2010) Sequential Metabolism of Secondary Alkyl Amines to Metabolic-Intermediate Complexes: Opposing Roles for the Secondary Hydroxylamine and Primary Amine Metabolites of Desipramine, (S)-Fluoxetine, and N-Desmethyldiltiazem Drug Metab Dispos. 38:963-972

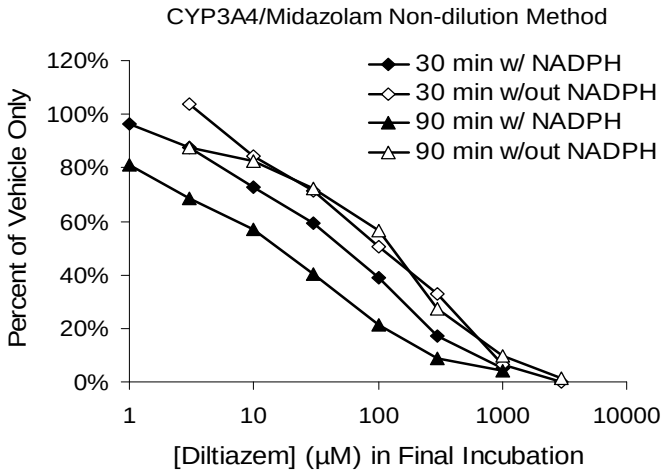
Effect of Increasing Preincubation Times in the Dilution and Non-dilution Method IC50 Shift Assay for Diltiazem for CYP3A4 with Testosterone and Midazolam as Substrates

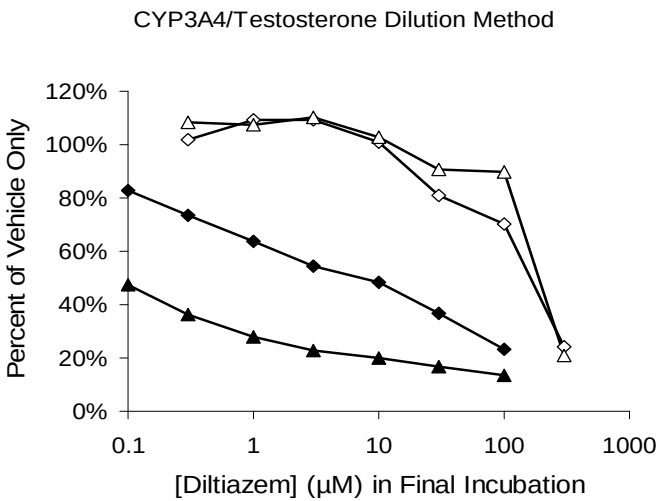
			Dilution method			Non-dilution method		
Substrate	Precincubation time	Exp[1]	IC50 (- NADPH)	IC50 (+NADPH)	Shift	IC50 (- NADPH)	IC50 (+NADPH)	Shift
Midazolam	30 min	1	109	10	11	100	45	2.2
Testosterone	30 min	1	151	5.7	27	74	29	2.5
Midazolam	90 min	1	107	0.02	5711	98	13	7.5
Testosterone	90 min	1	197	0.04	4545	183	13	14
Midazolam	3 min	2	82	56	1.5	117	123	0.9
Midazolam	10 min	2	108	46	2.4	146	98	1.5
Midazolam	30 min	2	123	20	6.1	151	80	1.9
Midazolam	3 min	3	87	59	1.5	111	96	1.2
Midazolam	10 min	3	74	34	2.2	132	109	1.2
Midazolam	30 min	3	92	13	6.8	150	74	2.0
Midazolam	90 min	3	154	0.10	1493	234	41	5.7

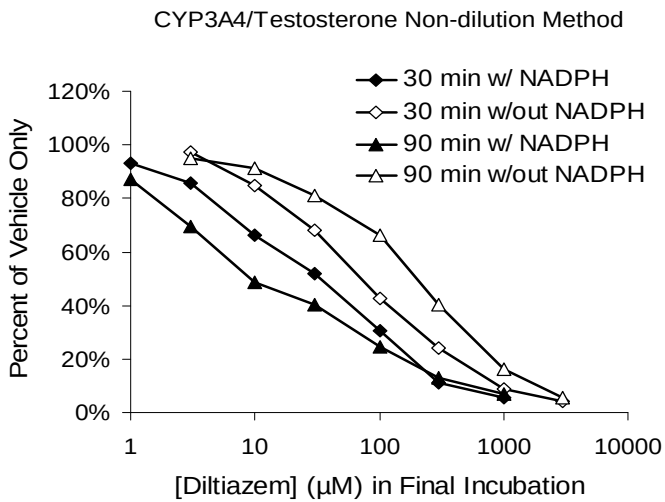
[1] - Experiment number

Effect of Extended Incubation Times in the Dilution and Non-dilution Method IC50 Shift Assay - Diltiazem









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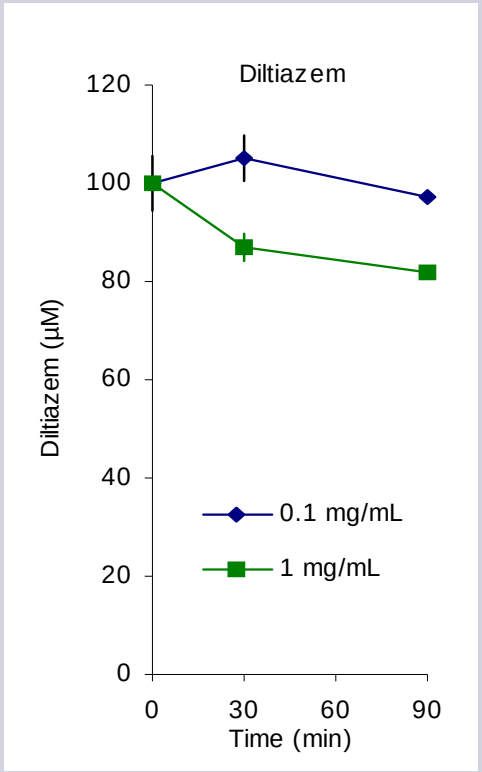
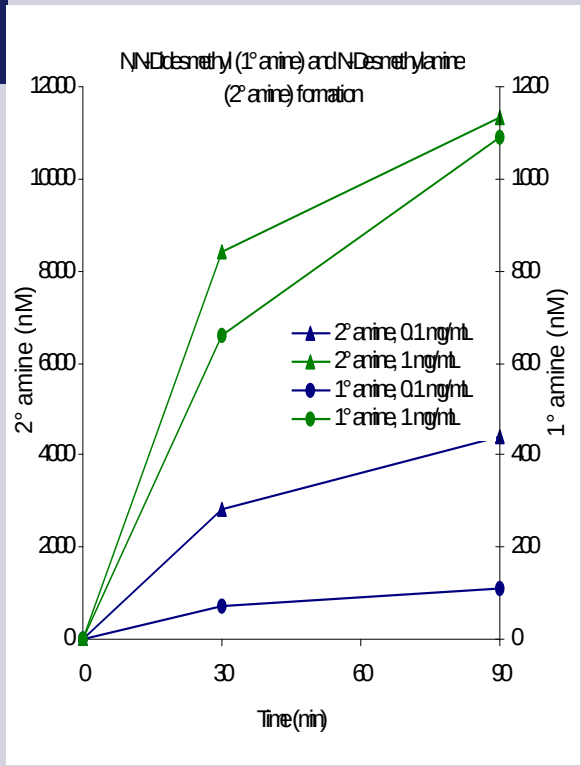
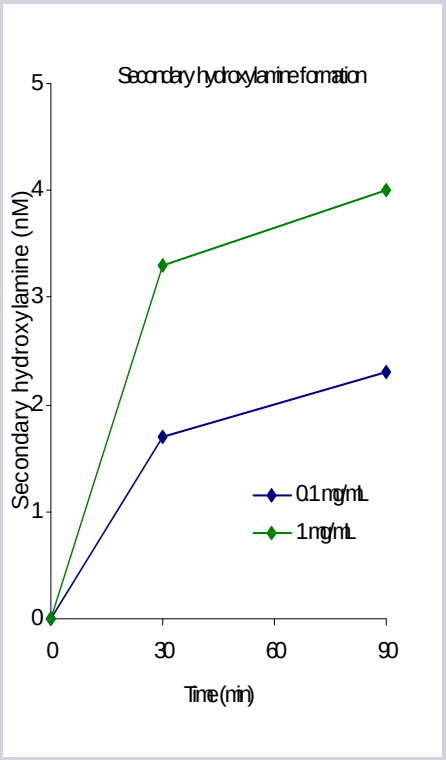
Metabolism of 100 µM Diltiazem in 0.1 or 1 mg/mL HLM







Confirmation that the longer incubation times as well as higher protein concentrations are driving metabolism

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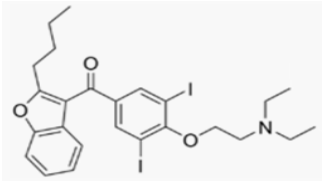
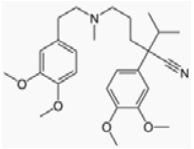
Other Examples

Inhibitor	Precincubation time	Exp[1]	Dilution method			Non-dilution method		
			IC50 (-NADPH)	IC50 (+NADPH)	Shift	IC50 (-NADPH)	IC50 (+NADPH)	Shift
Amiodarone	10 min	1	49	19	2.6	46	40	1.2
Amiodarone	30 min	1	51	6.7	7.5	61	22	2.7
Amiodarone	90 min	1	21	0.93	23	55	4.5	12
Amiodarone	10 min	2	81	34	2.4	40	25	1.6
Amiodarone	30 min	2	28	11	2.5	41	15	2.7
Amiodarone	90 min	2	13	0.8	16	30	4.0	7.5
Verapamil	3 min	1	26	15	1.7	41	31	1.3
Verapamil	10 min	1	29	7.5	3.9	40	20	2.0
Verapamil	30 min	1	26	0.54	48	37	10	3.6
Verapamil	3 min	2	21	11	1.9	29	21	1.4
Verapamil	10 min	2	18	3.7	4.8	28	12	2.2
Verapamil	30 min	2	23	0.32	70	30	4.1	7.2
Verapamil	90 min	2	28	0.03	962	25	1.2	21.0
Ketoconazole	3 min	1	0.0092	0.0113	0.8	0.0153	0.0144	1.1
Ketoconazole	10 min	1	0.0100	0.0114	0.9	0.0099	0.0107	0.9
Ketoconazole	30 min	1	0.0096	0.0126	0.8	0.0088	0.0113	0.8

[1] - Experiment number



Amiodarone



Verapamil

Negative control

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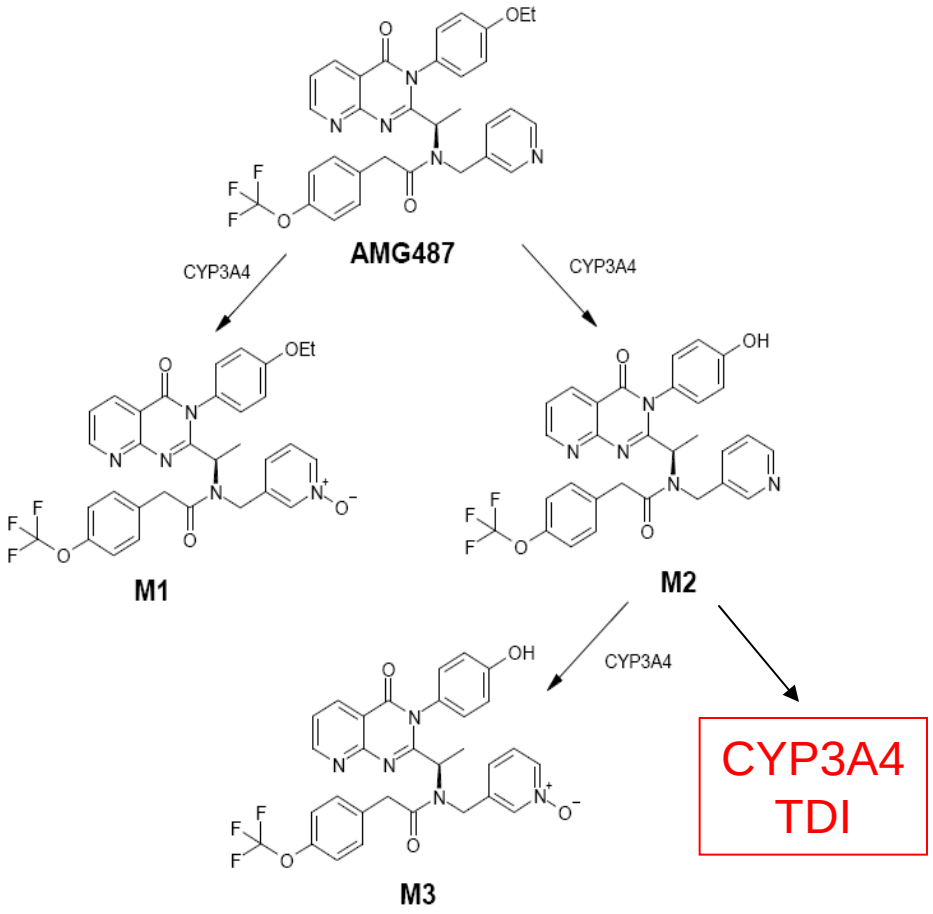
AMG487

- A potent and selective CXCR3 antagonist, displayed a dose- and time-dependent reduction in oral clearance in a Phase I multiple dose clinical study.
- One explanation for this observation is time-dependent inhibition of CYP3A4, the enzyme primarily responsible for AMG487 metabolism
- The major phenol metabolite (M2), but not parent AMG487 shows TDI of CYP3A4 *in vitro*



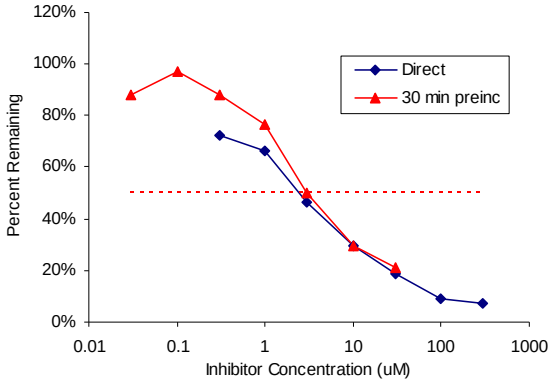
Tonn GR, et al. (2009) An inhibitory metabolite leads to dose- and time-dependent pharmacokinetics of (R)-N-{1-[3-(4-ethoxy-phenyl)-4-oxo-3,4-dihydro-pyrido[2,3-d]pyrimidin-2-yl]-ethyl}-N-pyridin-3-yl-methyl-2-(4-trifluoromethoxy-phenyl)-acetamide (AMG 487) in human subjects after multiple dosing Drug Metab Dispos 37:502-513.

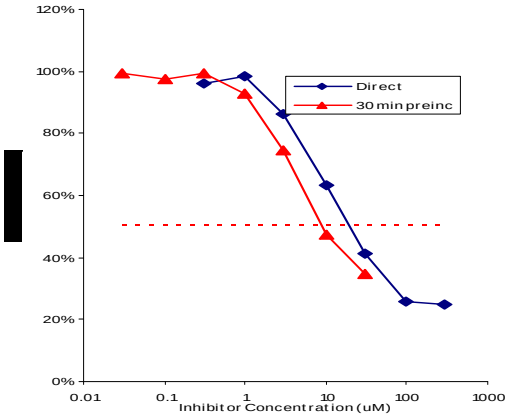
AMG487 and M2 with 30 Min Preincubation

Midazolam

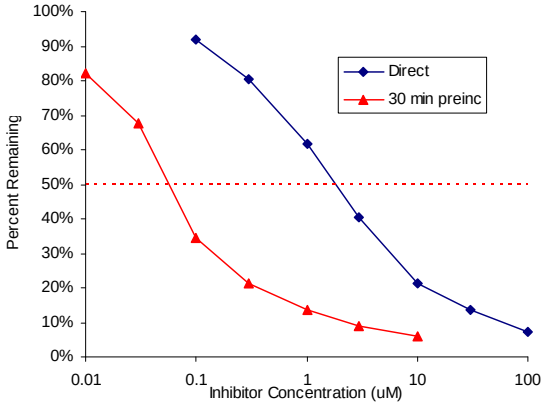
Testosterone

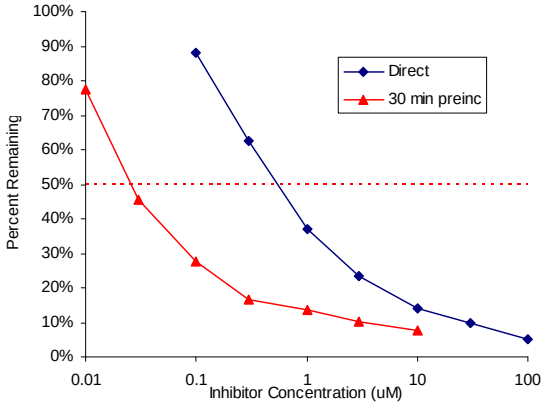
AMG487





M2





Hypothesis: Driving metabolism with higher protein and longer preincubation time will permit detection of TDI of AMG487

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AMG487 - Dilution and Substrate Addition Method (30 and 90 min Preincubation)

30 Minute Preincubation		Substrate Addition method			Dilution method		
Substrate	Inhibitor	IC50 (µM) (-NADPH)	IC50 (µM) (+NADPH)	IC50 Shift	IC50 (µM) (-NADPH)	IC50 (µM) (+NADPH)	IC50 Shift
Midazolam	AMG487	8.3	5.7	1.5	7	5.2	1.4
Testosterone	AMG487	20	22	0.9	26	25	1.1

90 Minute Preincubation		Substrate Addition method			Dilution method		
Substrate	Inhibitor	IC50 (µM) (-NADPH)	IC50 (µM) (+NADPH)	IC50 Shift	IC50 (µM) (-NADPH)	IC50 (µM) (+NADPH)	IC50 Shift
Midazolam	AMG487	9.2	2.9	3.2	8.0	2.7	2.9
Testosterone	AMG487	27	30	1.1	30	21	1.4

- Longer preincubation gave higher shifts
- No difference between dilution and non-dilution method

Henne et al (2012) Sequential Metabolism of AMG 487, a Novel CXCR3 Antagonist, Results in Formation of Quinone Reactive Metabolites that Covalently Modify CYP3A4 Cys239 and Cause Time-Dependent Inhibition of the Enzyme. Drug Metab Dispos. Manuscript in review

Summary and Conclusions

- The time-course of the "IC_{50} shift" was examined and extended beyond the conventional 30 min preincubation period to examine the effect on assay sensitivity.
- All compounds tested exhibited increases in IC_{50} shift when incubating for 90 min compared to 30 min.
- The increase occurred in both the dilution and non-dilution methods, but the increase was much larger for diltiazem and verapamil in the dilution method.

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Summary and Conclusions

- In all cases, the increase in shift was attributable to a lowering of the +NADPH IC_{50} values.
- The finding of larger shifts at 90 min in both the dilution and non-dilution methods demonstrate enzyme inactivation is not complete at the conventional 30 min period
- The data suggest broad application of extended preincubation times represents a simple method to enhance sensitivity of the IC_{50} shift assay in detection of TDI and may improve opportunities to de-risk compounds

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- Eric Gangl
- Kirk Henne (Amgen)

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Questions?

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