Underprediction of Aldehyde Oxidase (AO)–Mediated Drug Metabolism: Important Considerations for In Vitro Assessment

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Outline

- Introduction to aldehyde oxidase (AO)
- Examples of early clinical development termination of AO substrate drugs
- Areas of concern: Challenges in poor prediction
- Strategies to overcome poor prediction
- Summary and important highlights

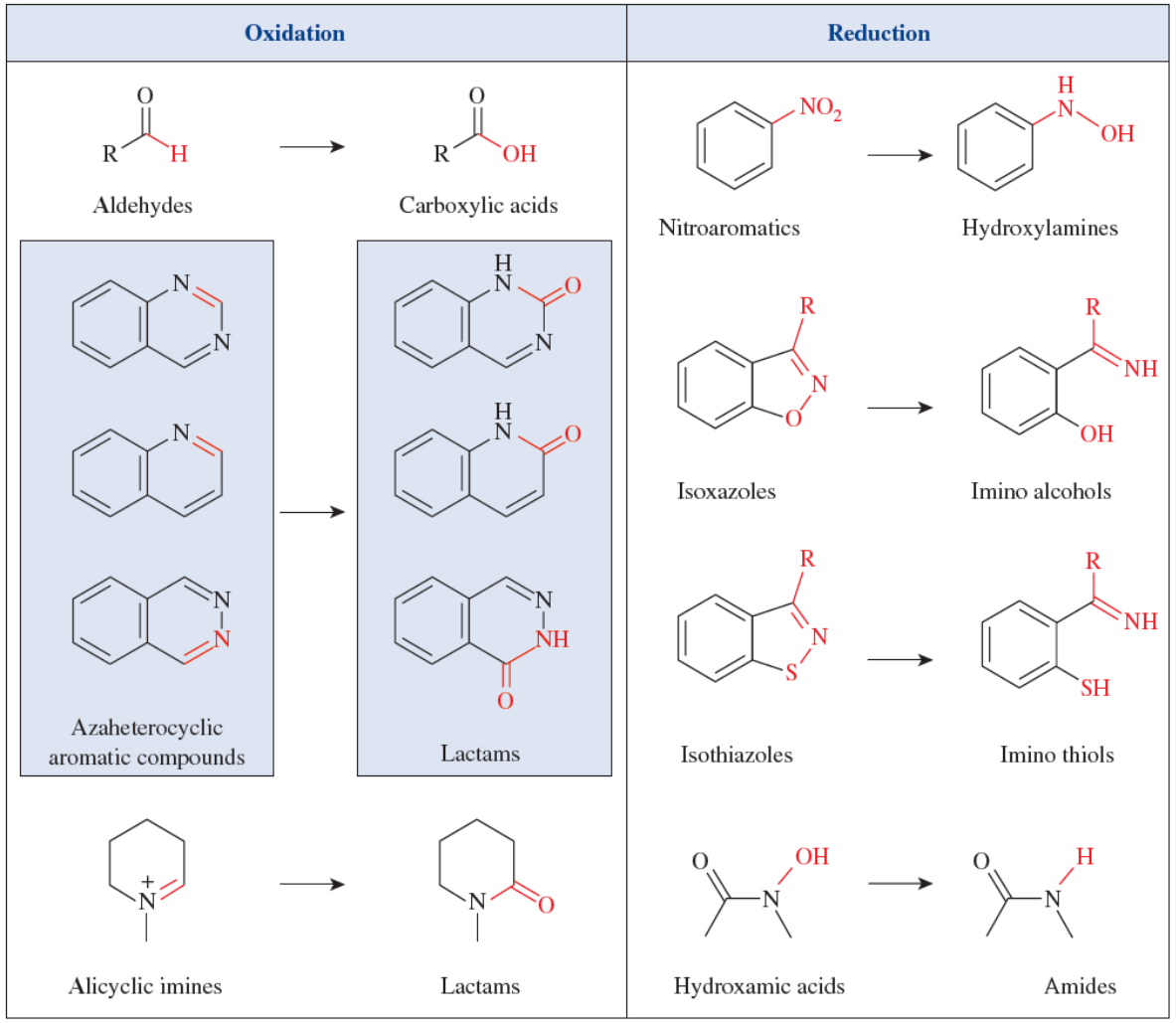
AO: Non-CYP Pathway in Drug Metabolism

- AO is a molybdenum cofactor (MoCo)-containing drug-metabolizing enzyme localized in the cytosolic fraction, expressed at high levels in liver
- Attractive strategy to avoid CYP-mediated metabolism:
  - Replacing a carbon in aromatic and non-aromatic carbocycles with heteroatoms results in lowering electron density of molecules and in decreasing their lability towards P450-mediated aromatic oxidation
  - Introduction of electron-deficient moieties result in increased susceptibility to AO mediated metabolism (e.g., introduce nitrogen)
    - CYPs tend to oxidize carbon atoms with high electron density
    - But AO tends to oxidize carbon atoms with low electron density
  - Correlation between high lipophilicity and CYP; but no such correlation for AO metabolism
  - Specific structural features alone are a good indicator of AO turnover

AO-Mediated Metabolism

- Oxidation of some aldehydes to the corresponding carboxylic acid – preferentially aromatic (vanillin to vanillinic acid)
- Oxidation of carbon atom adjacent to nitrogen within the heteroaromatic ring systems
- Reductive ring-opening metabolic pathways (zonisamide, ziprasidone)
- Amide hydrolysis (GDC-0834)
- Physiological functions largely unknown, although endogenous compounds such as retinaldehyde, nicotinamide, and pyridoxal are substrates of AO

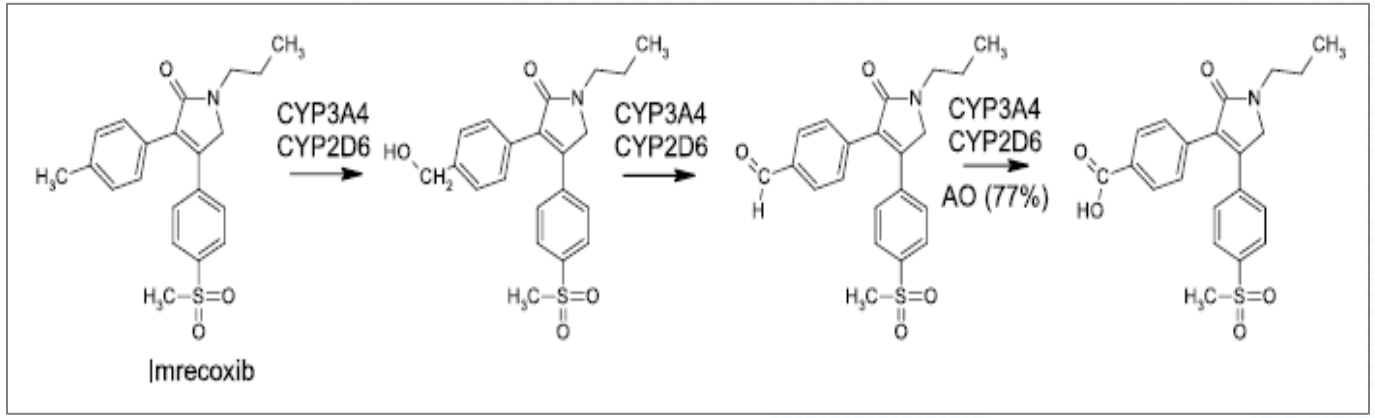
AO substrate classes



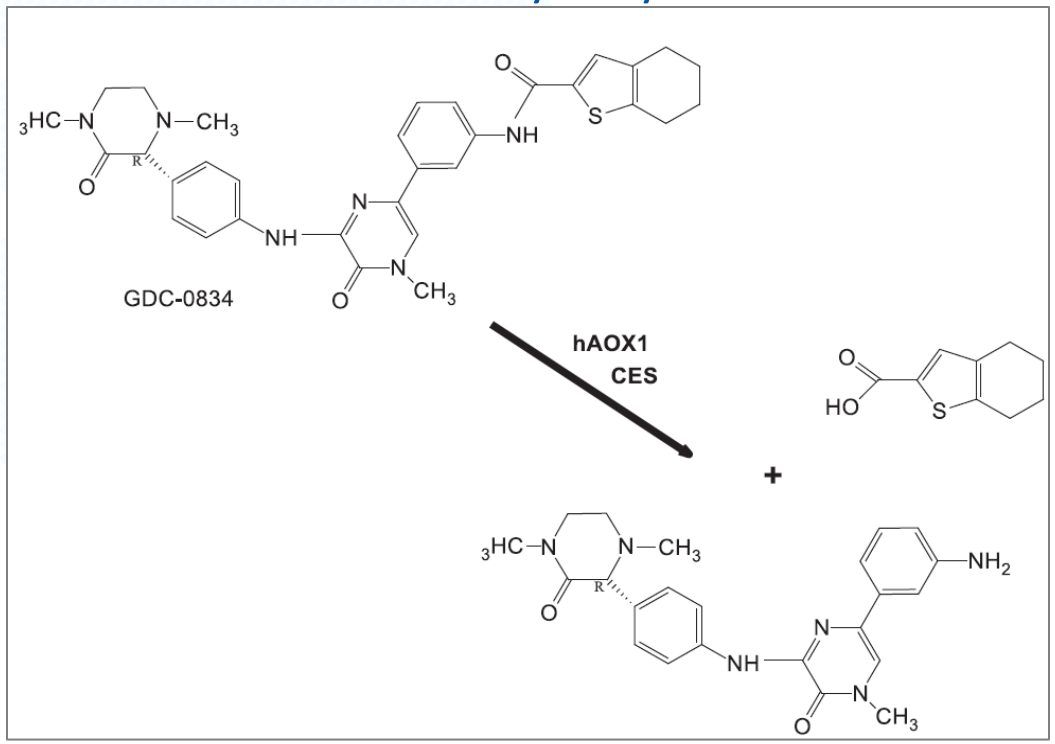
Parkinson et al.; Casarett & Doull's Toxicology: The Basic Science of Poisons, 9th edition; 2018

AO drug substrates – Aldehyde oxidation, Reduction, Amide hydrolysis

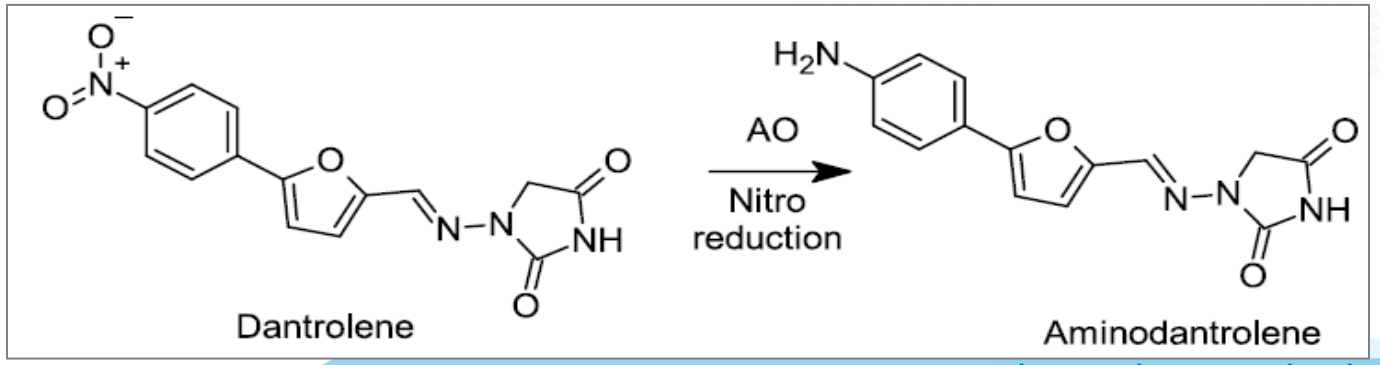
Intermediate aldehyde oxidation



Amide hydrolysis

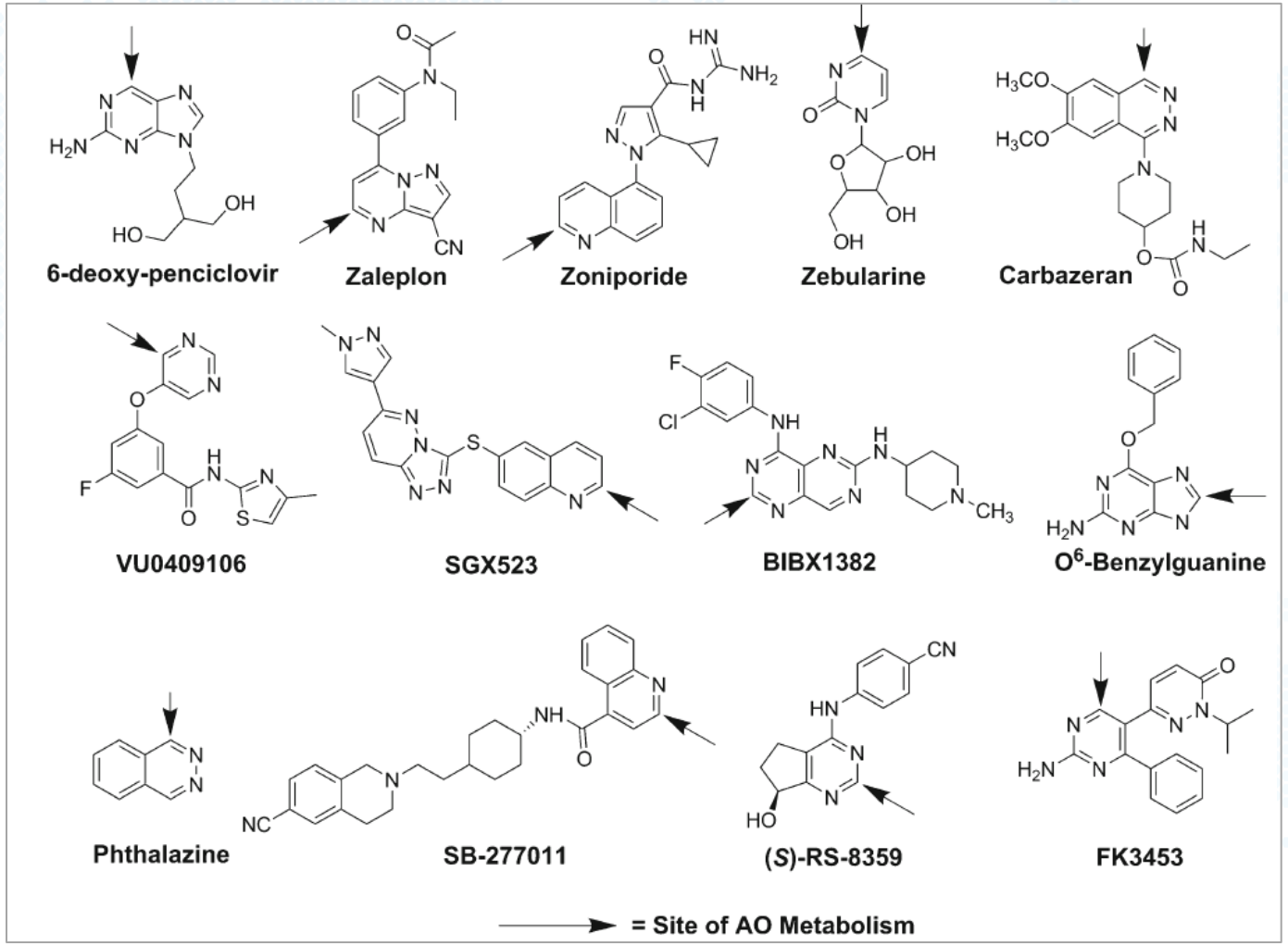


Nitro reduction



Manevski et al. J. Med. Chem.; 2019

AO drug substrates - Azaheterocycle oxidation



Argikar et al.; The AAPS Journal; 2016

Poll question #2

Primary Concern

Early termination of several clinical programs of AO substrate drugs due to numerous challenges in predicting human AO-mediated metabolism

Main difficulties in clinical development

- Low bioavailability duo to rapid metabolism compared to preclinical species
- Detection of novel metabolite not seen in preclinical phase
- Dose limiting toxicity due to AO metabolite in clinic

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Clinical Failure Example 1:
Low bioavailability and dose limiting toxicity

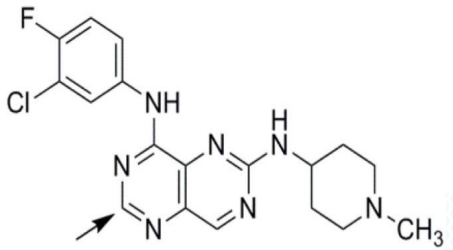
PERGAMON

European Journal of Cancer 38 (2002) 1072–1080

European Journal of Cancer
www.ejconline.com

Phase I and pharmacokinetic study of BIBX 1382 BS, an epidermal growth factor receptor (EGFR) inhibitor, given in a continuous daily oral administration

Ch. Dittrich[a,*], G. Greim[b], M. Borner[c], K. Weigang-Köhler[b], H. Huisman[d], A. Amelsberg[e], A. Ehret[e], J. Wanders[d], A. Hanauske[f], P. Fumoleau[g] for the EORTC Early Clinical Studies Group (ECSG)



BIBX1382

- Target plasma levels could not be reached in humans
- Dose-limiting increase of liver enzymes
- Additional preclinical studies showed that BIBX 1382 BS is metabolized by a hepatic AO to M404/9.3
- Only observed in cynomolgus and rhesus monkeys, but not in in mice and rats used for preclinical experiments and does not even exist in dogs
- Due to low bioavailability of BIBX 1382 BS and the detection of a pharmacologically inactive metabolite, this trial was discontinued

Clinical Failure Example 2:
Renal toxicity due to insoluble AO metabolite

Published OnlineFirst March 5, 2015; DOI: 10.1158/1078-0432.CCR-14-3258

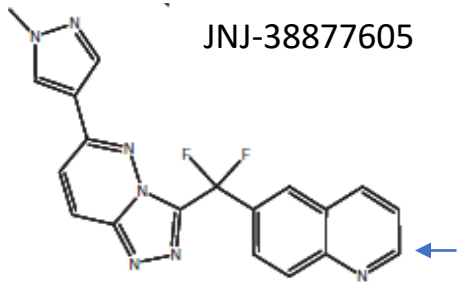
Cancer Therapy: Clinical

Clinical Cancer Research

The c-Met Tyrosine Kinase Inhibitor JNJ-38877605 Causes Renal Toxicity through Species-Specific Insoluble Metabolite Formation

Martijn P. Lolkema[1,2], Hilde H. Bohets[3], Hendrik-Tobias Arkenau[1], Ann Lampo[3], Erio Barale[3], Maja J.A. de Jonge[2], Leni van Doorn[2], Peter Hellemans[3], Johann S. de Bono[1], and Ferry A.L.M. Eskens[2]

[1]Phase I Unit, Royal Marsden NHS Foundation Trust, Surrey and London, United Kingdom. [2]Department of Medical Oncology, Erasmus MC, Cancer Institute Rotterdam, Rotterdam, the Netherlands. [3]Janssen Research and Development, Beerse, Belgium.



JNJ-38877605

- Even at subtherapeutic doses, mild though recurrent renal toxicity
- Renal toxicity not observed in preclinical studies in rats and dogs
- Additional toxicology studies in rabbits demonstrated JNJ-38877605 induced species-specific renal toxicity
- Renal crystals revealed M1/3 and M5/6 metabolites
- These main culprit insoluble metabolites were generated by aldehyde oxidase

Clinical Failure Example 3:
AO metabolite as a perpetrator of drug drug Interaction

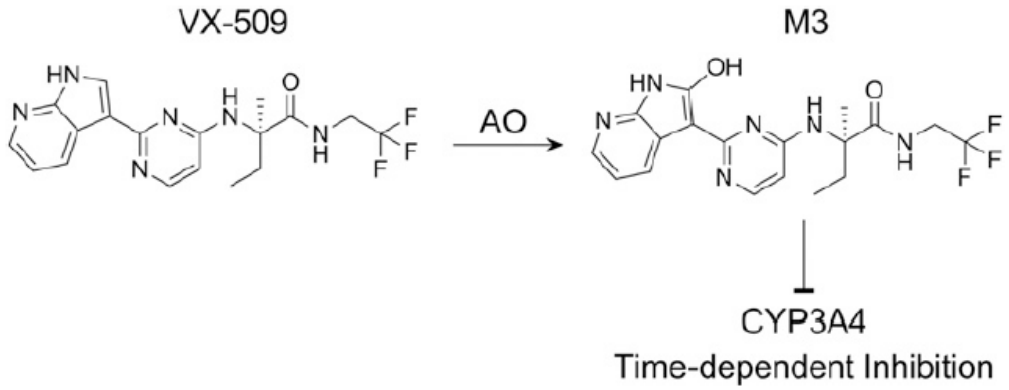
1521-009X/44/8/1286–1295$25.00
DRUG METABOLISM AND DISPOSITION
http://dx.doi.org/10.1124/dmd.116.071100
Drug Metab Dispos 44:1286–1295, August 2016

Special Section on Emerging Novel Enzyme Pathways in Drug Metabolism

VX-509 (Decernotinib)-Mediated CYP3A Time-Dependent Inhibition: An Aldehyde Oxidase Metabolite as a Perpetrator of Drug-Drug Interactions[S]

Craig Zetterberg, Francois Maltais, Leena Laitinen, Shengkai Liao, Hong Tsao, Ananthsrinivas Chakilam, and Niresh Hariparsad

Drug Metabolism and Pharmacokinetics (C.Z., L.L., S.L., H.T., A.C., N.H.) and Department of Chemistry (F.M.), Vertex Pharmaceuticals Inc.. Boston. Massachusetts



- Studies in HLM suggested a low risk for CYP3A4-mediated DDI
- VX-509 increased the area under the curve of midazolam, atorvastatin, and methyl-prednisolone by approximately 12.0-, 2.7-, and 4.3-fold, respectively
- Metabolite identification studies using human liver cytosol indicated that VX-509 is converted to an oxidative metabolite by AO, which is the perpetrator of the DDIs

List of Drugs shown AO-Related Challenges

compound	reaction type	observed challenges
carbazeran BIBX1382 FK3453 Lu AF09535 RO-1	oxidation of azaheterocycle	high metabolic clearance in humans due to oxidations by AO
JNJ-38877605	oxidation of azaheterocycle	renal toxicity in humans due to accumulation of insoluble AO metabolite; renal toxicity had not been observed in nonclinical studies in rats and dogs
SGX523		high clearance in humans due to AO metabolism; renal toxicity in humans due to accumulation of insoluble AO metabolite
momelotinib	oxidation of azaheterocycle	disproportionate human metabolite due to oxidations by AO [metabolites in safety testing (MIST) issue]
BILR 355	oxidation of azaheterocycle	disproportionate human metabolite due to oxidations by AO (MIST issue)

Manevski et al. J. Med. Chem.; 2019

List of Drugs shown AO-Related Challenges cont..

compound	reaction type	observed challenges
imidazoquinoline-containing cancer Osaka thyroid kinase inhibitors	oxidation of azaheterocycle	rapid metabolism by AO in rats prevents pharmacological studies
pyridine-containing toll-like receptor subtype 7 agonist	oxidation of azaheterocycle	rapid metabolism by AO in rats prevents toxicological studies
VX-509 (decernotinib)	oxidation of azaheterocycle	AO metabolite is a time-dependent inhibitor of CYP3A4, leading to clinically relevant drug–drug interactions
dantrolene	reduction of nitro group	bioactivation via AO-mediated nitro reduction and subsequent liver injury
imrecoxib	aldehyde oxidation	in vitro–in vivo disconnect in metabolite abundance due to AO-mediated aldehyde oxidation
GDC-0834	hydrolysis of amide	high metabolic clearance in humans due to AO-mediated amide hydrolysis

Manevski et al. J. Med. Chem.; 2019

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Factors causing underprediction : Species differences

- Humans only have one active AO; rats, mice, rabbits up to 4; dogs, cats, pigs have none; chimpanzees have similar expression to humans
- Significant species difference in expression, activity and/or inhibition potential of AO (Beedham et al. 1987; Sahi et al. 2008; Dalvie et al. 2010; 2013)

Table 1. The table lists the complement of functionally active aldehyde oxidases expressed in the liver and other tissues of humans, chimpanzees and popular animal experimental models.

Animal species	Liver isoenzyme(s)	Other isoenzyme(s)	Pseudogenes
Human	AOX1	-	*AOX3, AOX3L1*
Chimpanzee	AOX1	-	*AOX3, AOX3L1*
Rhesus monkey	AOX1	AOX3l1	*AOX3, AOX4*
Guinea pig	AOX1	AOX4, AOX3l1	-
Dog	-	AOX4, AOX3l1	*AOX1, AOX3*
Cat	-	AOX3l1	AOX1, AOX4
Pig	-	AOX3L1	*AOX1, AOX3, AOX4*
Mouse	AOX3, AOX1	AOX4, AOX3l1	-
Rat	AOX3, AOX1	AOX4, AOX3l1	-
Rabbit	AOX3, AOX1	AOX4, AOX3l1	-

The number and type of inactive aldehyde oxidase pseudogenes are also indicated. Please note that the data in mice and rats refer to the C57BL/6J and Wistar strains, since variability of AOX isoenzyme expression has been observed in other strains, as discussed in the article.

Garattini and Terao. Expert Opin Drug Metab Toxicol 8:487-503; 2012

- No single species can reliably predict human AO-metabolism for all AO substrates
- Preclinical animal models to humans extrapolation may not be accurate

Factors causing underprediction : Species differences

Zoniporide metabolism

In vivo

Metabolites	Healthy Male Volunteers		
	Urine	Feces	Total
M1	16	36	52
M2		17	17

Metabolites	% Dose Excreted		
	Sprague-Dawley Rats		
	Urine	Feces	Total
M1	10	25	35
M2			

Metabolites	Beagle Dog		
	Urine	Feces	Total
M1			
M2			

Dalvie et al. Drug Metab Dispos 38:641–654, 2010

S9 fraction

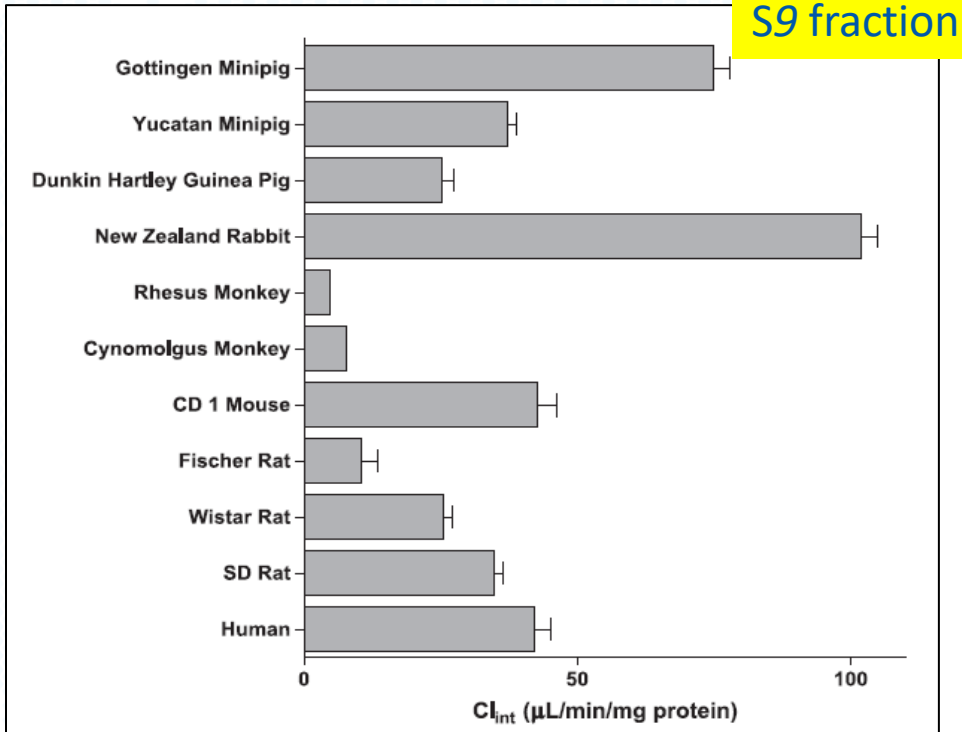


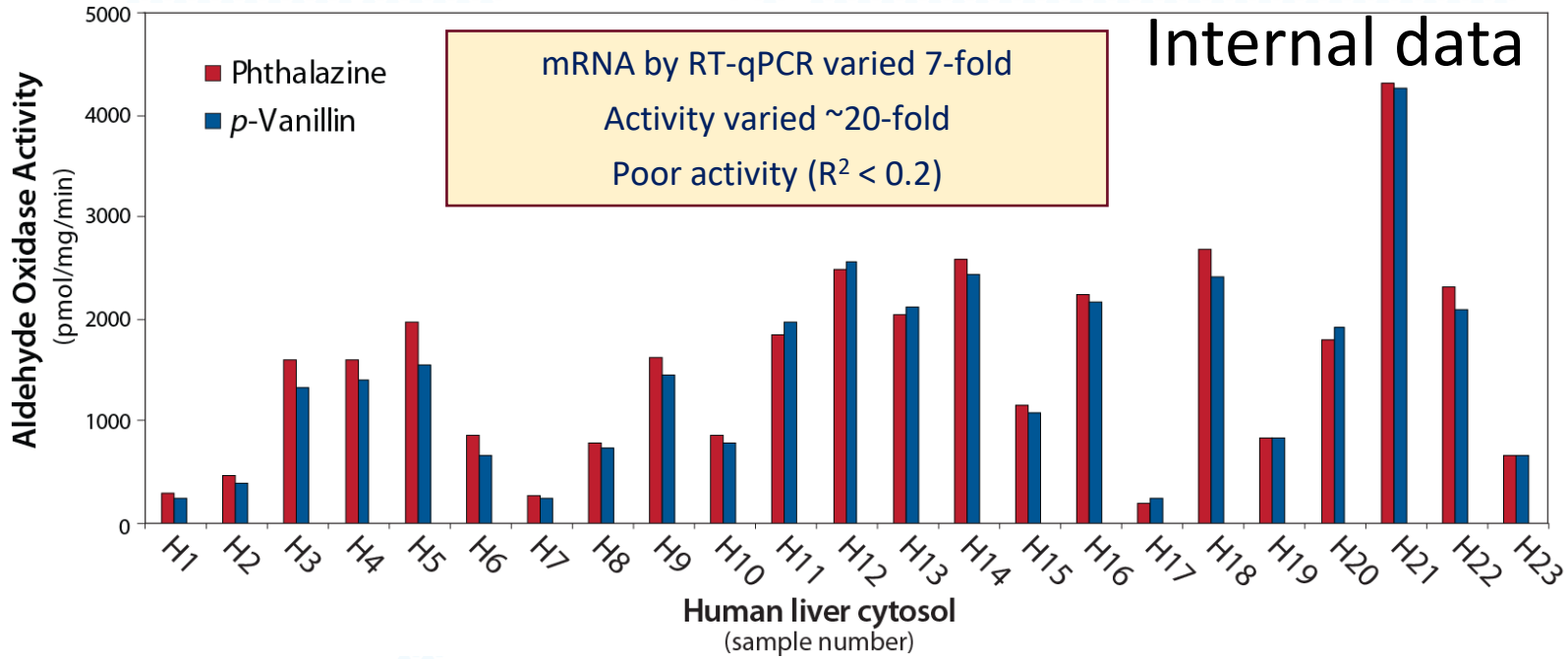
Figure 4. Comparison of the Cl_{int} for 2-oxozoniporide (M1) formation among species.

Dalvie et al. Xenobiotica 43: 399–408, 2013

Factors causing underprediction:

Instability in vitro; High donor-to-donor variation

- Loss in AO activity within 24 hours after isolation of hepatocytes (average loss of 42%, range 15%–81%) (Hutzler et al. 2014).
- Donor variability in CL_{int} in fresh or cryopreserved hepatocytes, (5- to 8-fold) (Hutzler et al. 2014).

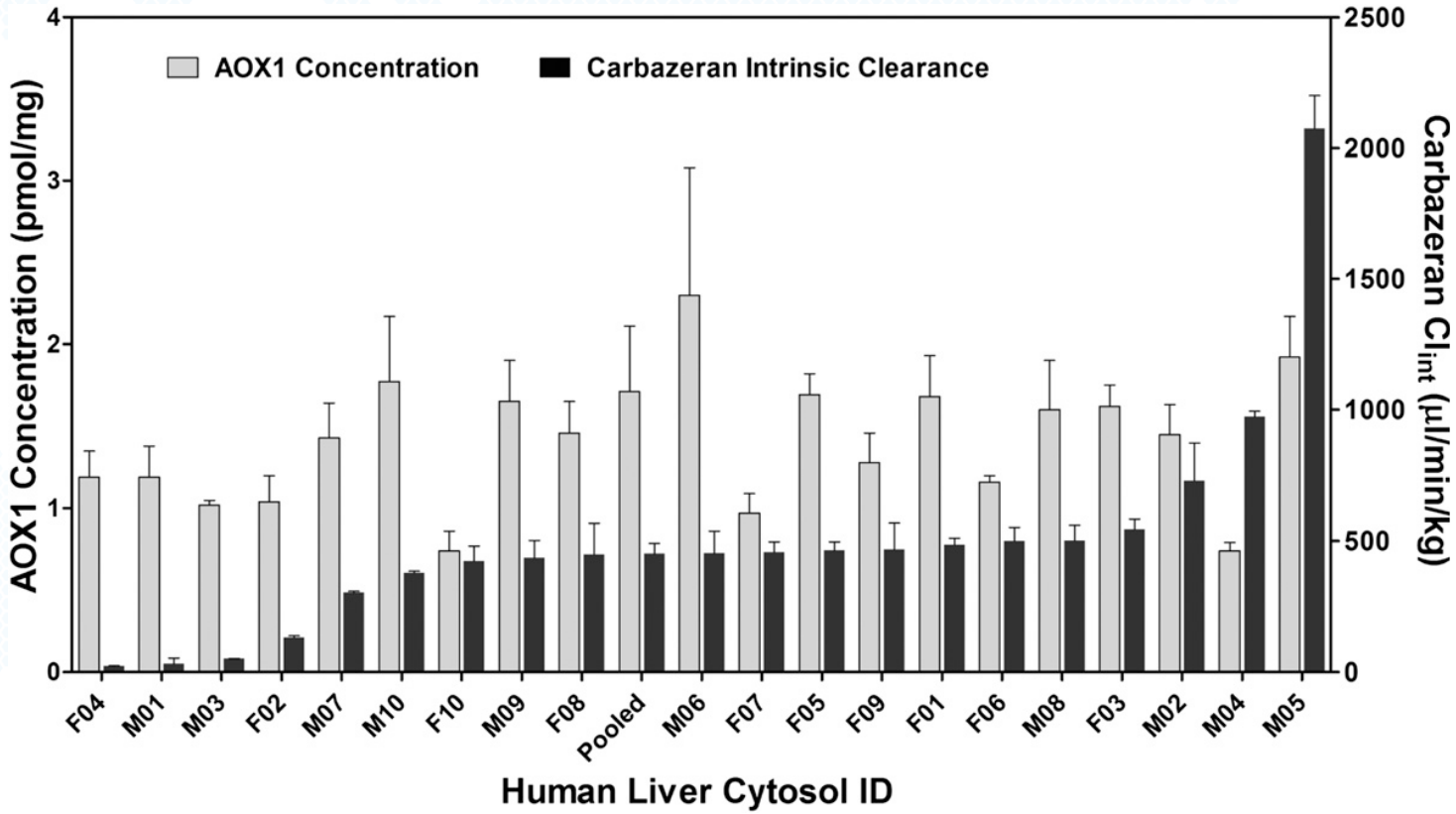


- Magnitude of variability depends on the substrate and method (e.g., metabolite formation or substrate depletion) (Hutzler et al. 2014).

Factors causing underprediction:
Poor correlation with active enzyme and protein levels

- ~ 2–4 fold difference in hAOX1 protein levels, however, 19-, 43- and 90-fold variability in CL_{int} for phthalazine, zoniporide, and carbazeran across 20 individuals (50:50 ♂:♀) (Fu et al., 2013)

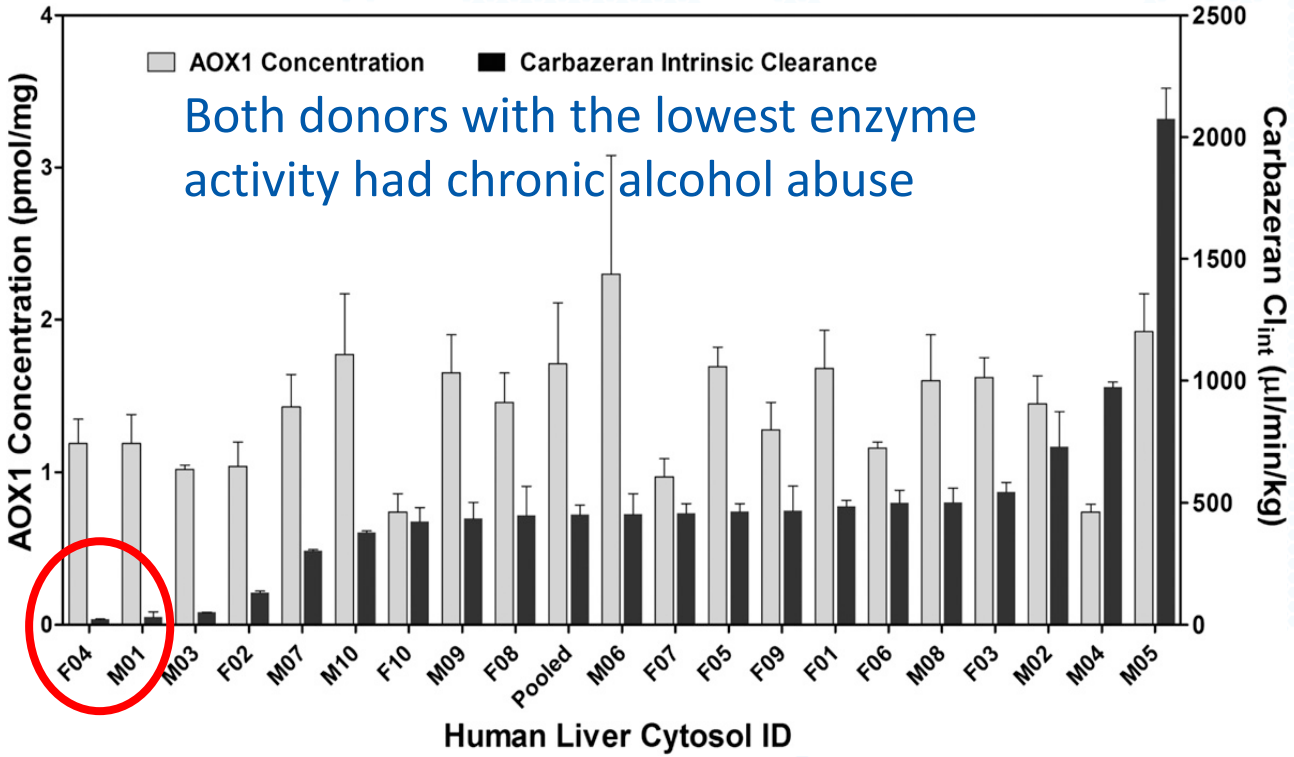
- 1.5-fold range in V_{max} for DACA (N-[(2-dimethylamino)ethyl]acridine-4-carboxamide) oxidation across three lots of pooled human liver cytosol from the same vendor (Barr et al., 2013)



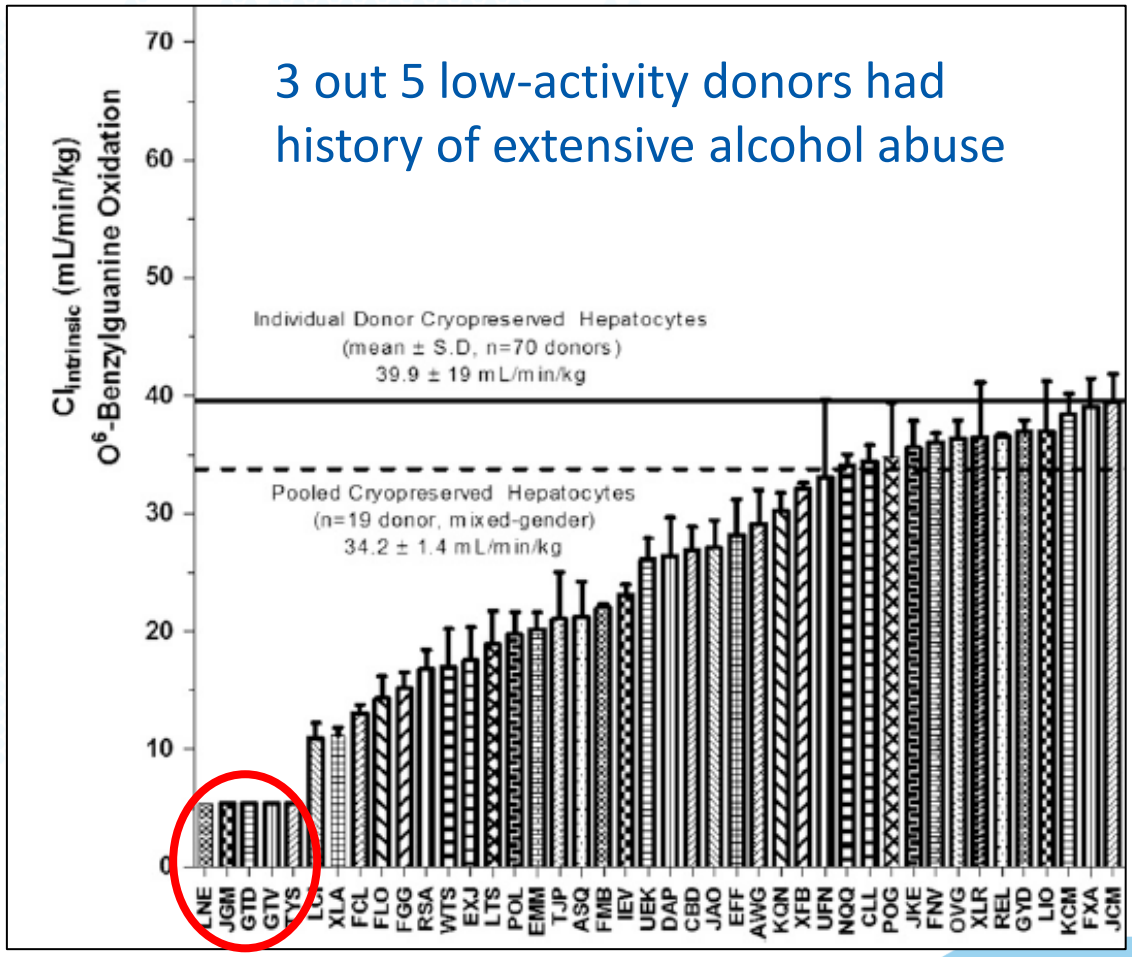
Fu et al., Drug Metab Dispos 41:1797–1804, 2013

Factors causing underprediction:

Chronic alcohol consumption

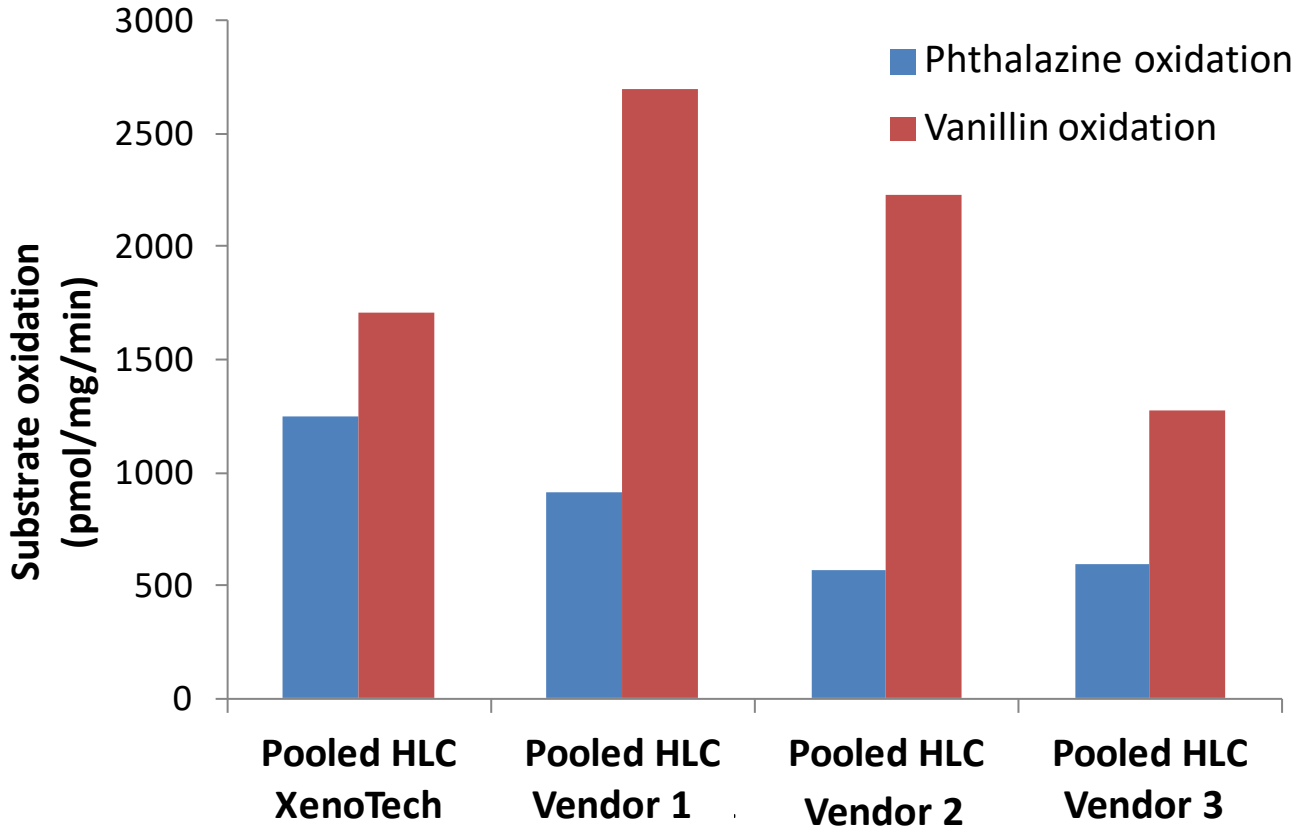


Fu et al., Drug Metab Dispos 41:1797–1804, 2013



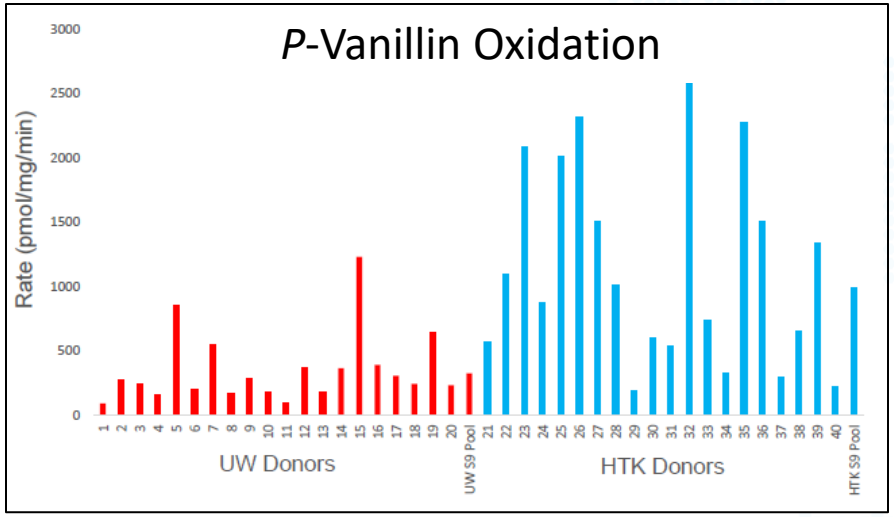
Hutzler et al., Drug Metab Dispos 42:1090–1097, 2014

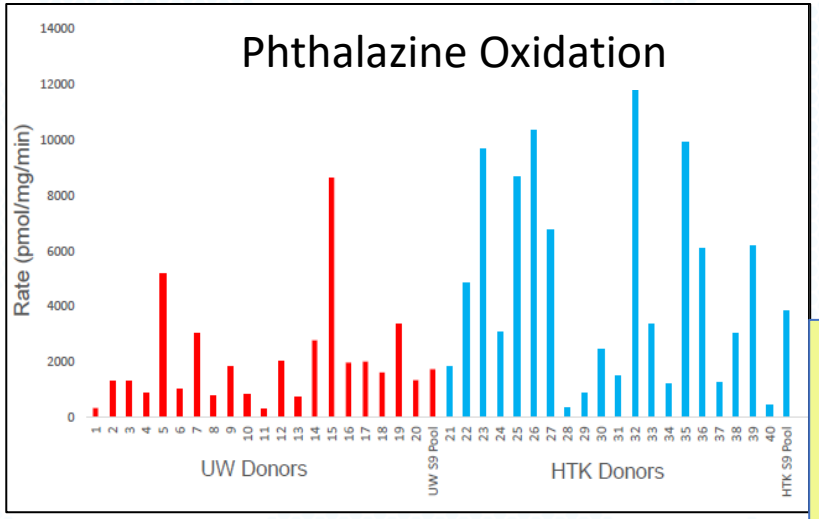
Factors causing underprediction: Substrate specificity

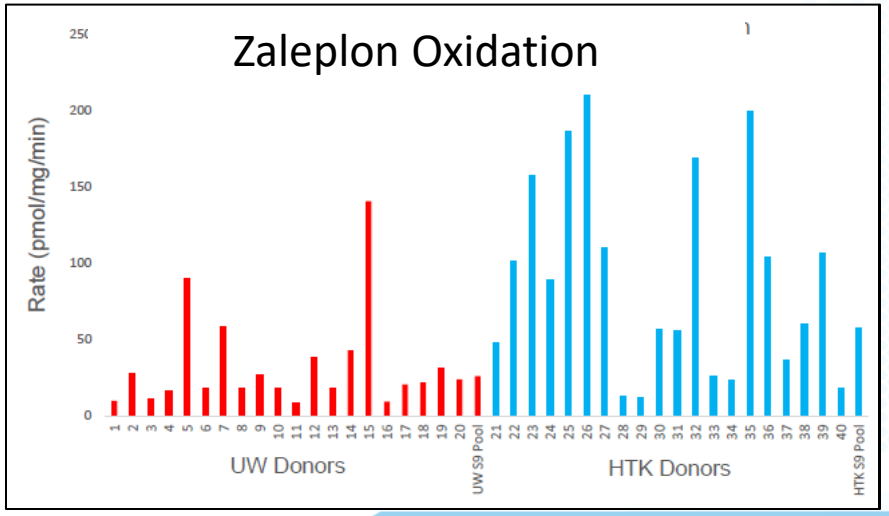


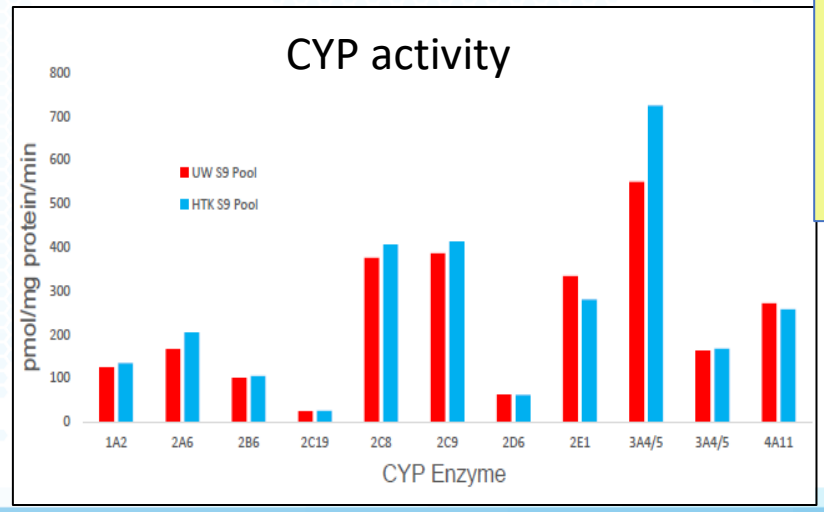
Within the same experiment, vendor comparisons can be substrate-dependent, rates within approximately 2-fold of one another

Factors causing underprediction: Organ preservation solution









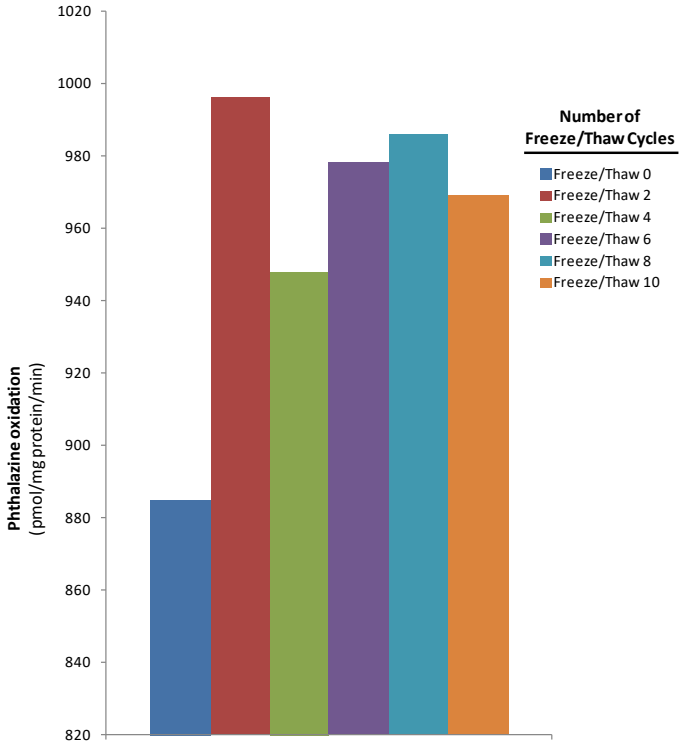
AO activity for all 3 substrates was ~2 to 3-fold higher in S9 fraction of HTK-preserved livers than UW-preserved livers; no such effect on CYPs

This is due to presence of allopurinol in UW preservation media

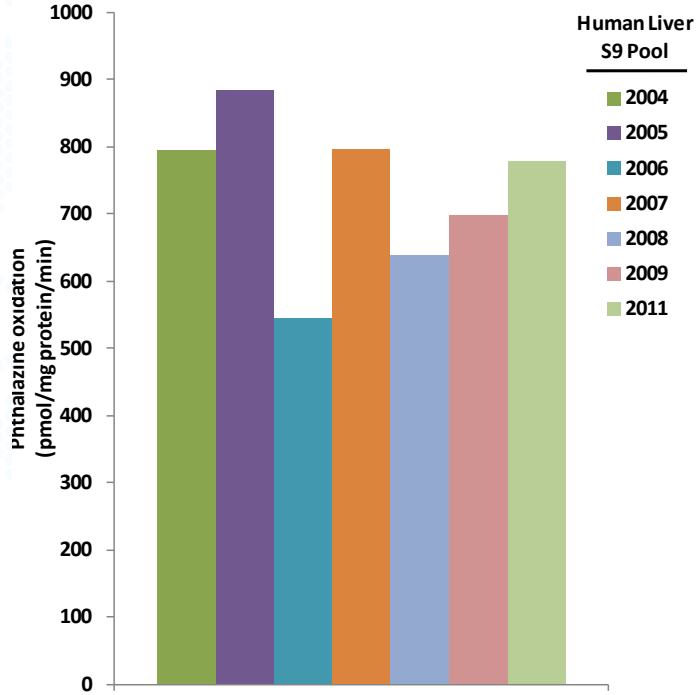
Lyon et al. 14th European ISSX meeting, Poster #P109 , 2017

Other factors suspected to be attributing to underprediction, but no effect

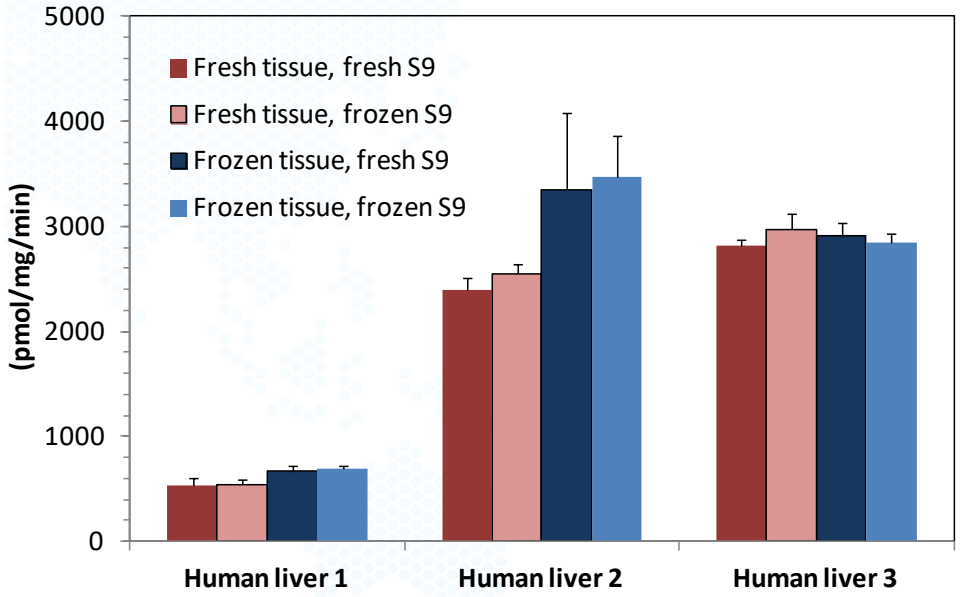
- Freeze-thaw cycles



- Long term storage



- Fresh vs. frozen tissue for cellular fraction preparation



No impact of multiple freeze-thaw cycles or long term storage

Activities are within 50% of one another regardless of the use of fresh or frozen tissue, and fresh or frozen and thawed S9

Otwell et al. ISSX, 2013, Toronto: Poster #75

Other factors suspected to be attributing to underprediction, but no effect

- No noteworthy contribution of AO activity in extrahepatic tissues

Table 3: Calculated parameters and scaled AOX clearance contributions

Tissue	K_M (μM)	V_{max} (pmol/min/mg S9)	CL_{int} (μL/min/mg S9)	$CL_{int,scaled}$ (mL/min/kg)	Total CL_{AOX} (mL/min/kg)
liver	5.2	3507	670	1721	19
kidney	5.2	6.3	1.2	0.22	0.034
lung	21	7.2	0.35	0.10	0.014
vasculature	21	12	0.58	0.071	0.011
intestine	21	0.78	0.038	0.0011	0.00017

Combined scaled AOX clearance obtained from the kidney, lung, vasculature and intestine is very low and amounted to <1% of liver.

This work suggests that AOX metabolism from extra-hepatic sources plays little role in the underprediction of activity in human.

Kozminski et al. DMD Fast Forward. Published on June 23, 2021

- Genetic polymorphism – SNPs
  - SNPs exist, but there is no strong evidence showing effect on AO clearance

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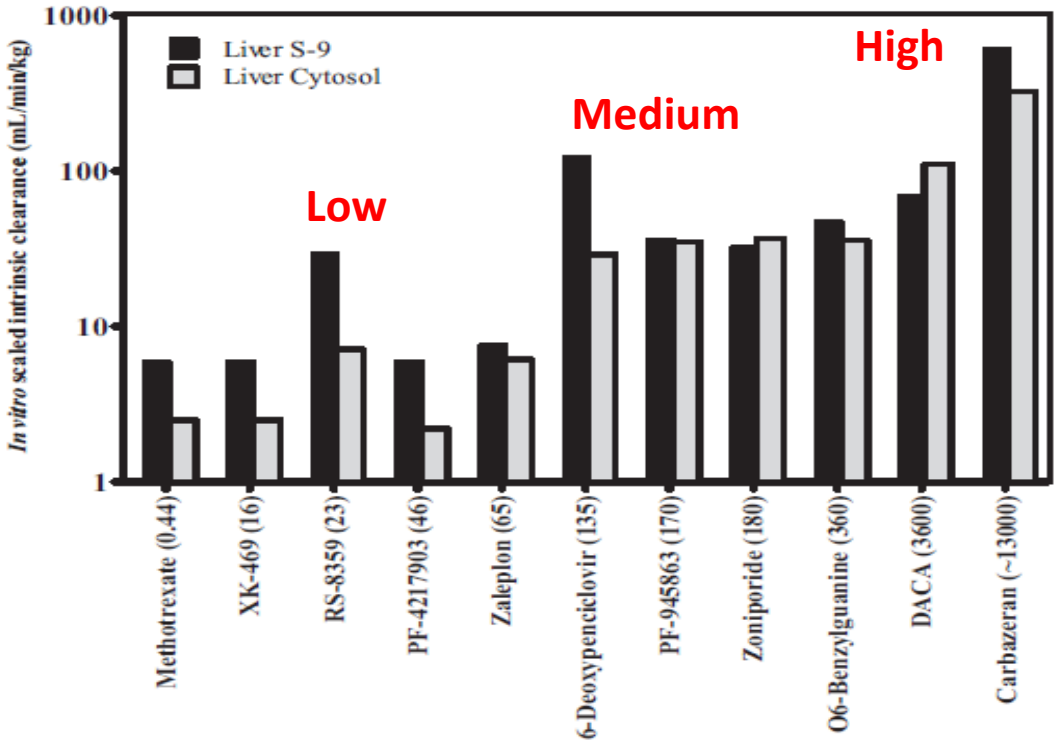
In vitro to in vivo extrapolation (IVIVE):
Yard-stick approach

- Examined 11 drugs metabolized by AO and with in vivo pharmacokinetic data
- Determined in vitro CL_{int} with pooled human cytosol and S9 and compared to the in vivo CL_{int}



Correlation allows for the qualitative scaling of a new drug (low, medium or high in vivo CL_{int})

Select compounds from this test set can be run as calibrators to determine the rank order with a new drug

Zientek et al., Drug Metab Dispos 38:1322, 2010

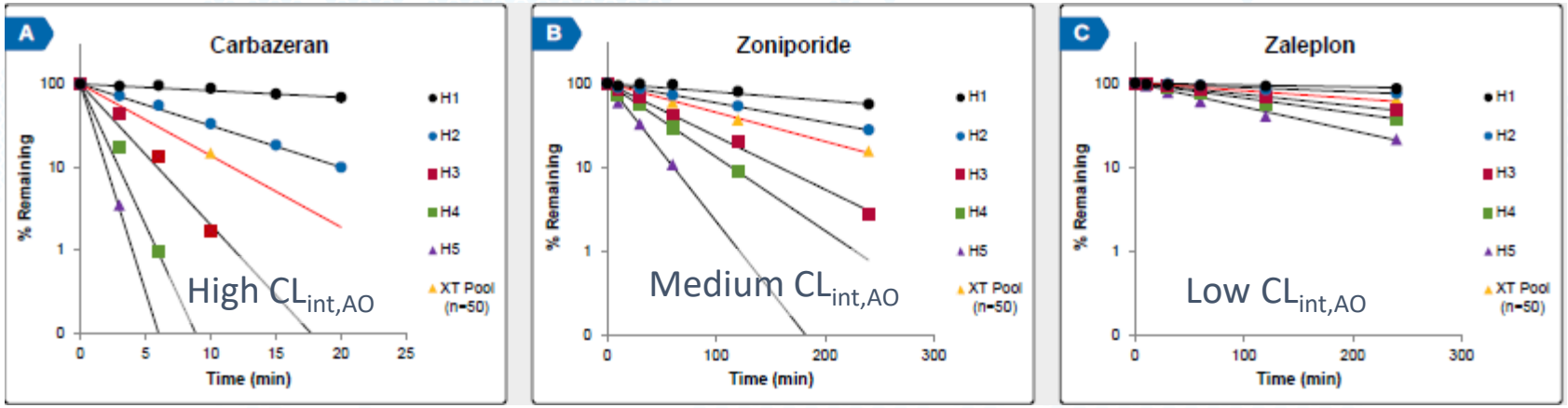
The impact of low vs. high AO activity lots on IVIVE

Question: Is the yard-stick or rank order approach dependent on the absolute AO activity in S9 or cytosol?

- XT examined the Zientek et al. (2010) test set compounds with diverse in vitro $CL_{int,AO}$ (high, medium, low)
  - Used the rank order approach
  - Used S9 and cytosol isolated from 5 human individuals and pooled products (cytosol, n = 50; S9, n = 200) spanning a 10-fold difference in AO activity
  - Determined half-lives of AO substrates and compared the rank order to the AO activity of each lot (determined by phthalazine)

Yerino et al., ISSX meeting, Poster 331, 2013

Clearance of AO substrates in human cytosol



		Half-life (minutes)					
Substrate (1 µM)	$CL_{int, AO}$ (in vitro)	H1	H2	H3	H4	H5	XT Pool (n=50)
Carbazeran	High	36	6	1.7	0.9	0.6	4
Deoxypenciclovir	Med	>240	96	25	22	12	71
Zoniporide	Med	>240	133	47	35	19	89
Benzylguanine	Med	>240	144	56	38	21	113
Zaleplon	Low	>240	>240	218	163	108	>240
Methotrexate	Low	>240	>240	>240	>240	>240	>240
Phthalazine		382	711	1524	2417	3148	1286

Rank order approach worked in every case except lowest activity individual (H1)

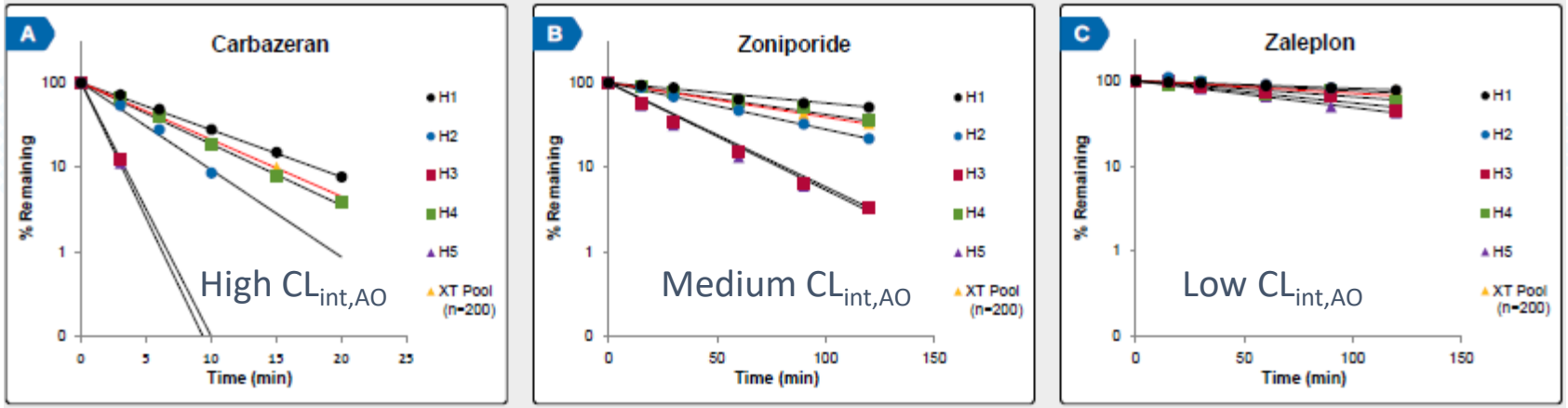
Suggests that for cytosol a threshold value of AO activity may need to be established to utilize the rank order approach

ISSX, 2013, Toronto: Yerino et al.: poster #331

Threshold value

Pooled cytosol reflected median activity

Clearance of AO substrates in human S9



		Half-life (minutes)					
Substrate (1 µM)	$CL_{int, AO}$ (in vitro)	H1	H2	H3	H4	H5	XT Pool (n=200)
Carbazeran	High	6	3	1	4	1	5
Deoxypenciclovir	Med	72	38	15	63	12	57
Zoniporide	Med	104	54	25	84	21	74
Benzylguanine	Med	101	58	25	132	22	86
Zaleplon	Low	>120	>120	105	>120	86	>120
Methotrexate	Low	>240	>240	>240	>240	>240	>240
Phthalazine		256	554	1080	1266	1946	609

Rank order approach worked in every case (including lowest activity individual)

Suggests that AO activity in H1 is above the threshold necessary for rank order

ISSX, 2013, Toronto: Yerino et al.: poster #331

Above threshold value

Pooled S9 reflected median activity

Other approaches proposed for IVIVE

- Crouch and colleagues (2018) proposed an in vitro allometric scaling approach. Guinea pigs, minipigs, and monkeys were more successful in predicting human AO-mediated clearance than data obtained from rodents. Thus, species selection for further pharmacokinetic (PK) testing and for allometric scaling of human clearance should be based on species that have comparable predicted extraction ratios to humans.

- A laboratory-specific scaling factor using known AO substrates should be employed in AO-based IVIVE approaches (De Sousa Mendes et al. 2020).

- Humanized mice – It has good correlation, but AO activity depends on donor human hepatocytes.

Calculation of fm_{CYP} vs. fm_{AO}

How do I know if my drug is predominantly metabolized by AO?

- Traditionally for CYPs, the use of chemical inhibitors is used with microsomes, S9 or hepatocytes to determine fm_{CYP}
  - In many cases, specific CYP isoform inhibitors are used to determine specific CYP contribution (e.g. fm_{CYP3A4})
  - A general CYP inhibitor may also be used to determine total fm_{CYP} (e.g. 1-ABT)
- A similar approach may be used to determine fm_{AO} by using a specific AO inhibitor

Hydralazine may be a good candidate to determine fm_{AO}

Strelevitz et al., Drug Metab Dispos 40:1441-1448, 2012

Calculation of fm_{CYP} vs. fm_{AO}

TABLE 3

Metabolic pathway, apparent intrinsic clearance, and $f_{m(AO)}$ identified for 10 selected compounds and compared in two human hepatocyte batches

Metabolic pathways were confirmed using biotransformation. Human hepatocytes were suspended at 1.5 million cells/ml. Termination time points = 0, 5, 15, 30, 60, 120, and 240 min; n = 3/time point. $Cl_{int, app}$ values were calculated from averaged $AUC_{0-\infty}$ extrapolated data. Batch 1: pooled lots AGR, FKM, EHI, TDH, and ZFB; batch 2: lot RTH.

Drug	Metabolic Pathways	$Cl_{int, app}$						$f_{m(AO)}$	
		Batch 1			Batch 2				
		No Hydralazine	25 μM Hydralazine	50 μM Hydralazine	No Hydralazine	25 μM Hydralazine	50 μM Hydralazine	Batch 1[a]	Batch 2[b]
		$\mu l \cdot min^{-1} \cdot 10^6\ cells^{-1}$							
O^6-Benzylguanine	AO	23.8	N.C.	N.C.	21.0	7.50	3.65	N.C.	0.83
PF-0945863	AO, N-demethylation	23.9	N.C.	N.C.	24.8	11.5	9.61	N.C.	0.61
Zaleplon	AO, N-deethylation	11.5	3.47	3.08	10.9	6.95	4.82	0.70	0.56
Zoniporide	AO, hydrolysis	22.0	7.97	5.08	18.3	12.9	8.16	0.64	0.55
DACA	AO, N-demethylation	55.5	25.9	21.2	51.7	22.6	12.6	0.53	0.76
Carbazeran	AO, glucuronidation	73.9	37.8	27.2	67.2	37.1	30.1	0.49	0.55
Propranolol	Hydroxylation, glucuronidation	305	198	179	294	245	236	0.35	0.20
Midazolam	Hydroxylation	34.3	31.7	32.5	43.5	45.9	41.3	0.08	0.05
Naloxone	Hydroxylation, glucuronidation	56.9	52.9	54.5	48.3	57.4	56.9	0.07	N.D.
Dextromethorphan	N-Demethylation[c]	—	—	—	30.9	29.0	18.7	—	0.39

N.C., $Cl_{int, app}$ in the presence of hydralazine could not be calculated because the slope of the ln[C] versus time curve was not statistically different from zero, which precluded a reliable measurement; N.D., not determined; —, compound not run in assay.

[a] $f_{m(AO)}$ was determined using 25 μM hydralazine data.

[b] $f_{m(AO)}$ was determined using 50 μM hydralazine data.

[c] Data from Gorski et al. (1994).

The contribution of CYP vs. AO metabolism can be identified with chemical inhibitors (1-ABT and hydralazine)

Strelevitz et al., Drug Metab Dispos 40:1441-1448, 2012

Calculation of fm_{CYP} vs. fm_{AO}

Fractional metabolism CYP vs. AO characterized with

1-ABT (1 mM) and hydralazine (25 µM) in pooled hepatocytes

Substrate	Metabolic pathway	CL_{int} (µL/10^6 cells/min) Control	25 µM Hydralazine	1 mM ABT	fm AO (XT hepatosure)
Midazolam, 1 µM	CYP, UGT	15.38	15.35	2.67	0.00
Dextromethorphan, 1 µM	CYP	16.18	0.92	0.08	0.94
Naloxone, 1 µM	UGT, CYP	39.35	39.18	31.34	0.00
Propranolol, 0.1 µM	CYP	15.53	6.32	2.95	0.59
Carbazeran, 1 µM	AO > UGT	36.49	14.35	34.68	0.61
Benzylguanine, 1 µM	AO	7.53	2.79	10.29	0.63
Zaleplon, 1 µM	AO > CYP	2.20	0.23	1.78	0.89
Zoniporide, 1 µM	AO > hydrolysis	6.65	0.66	6.76	0.90

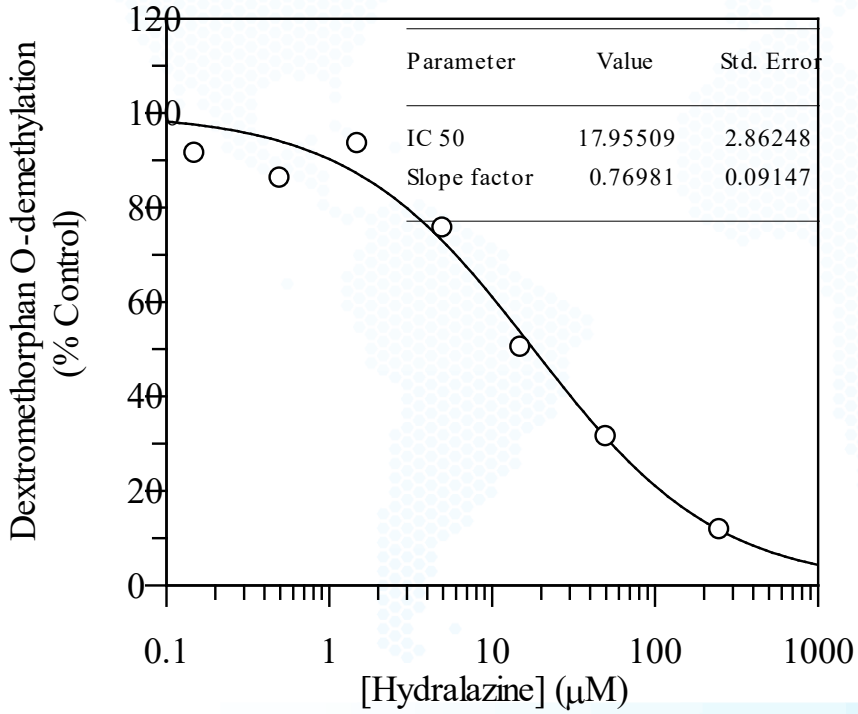
Confirmed that the fm_{AO} can be determined by chemical inhibition with hydralazine

Apparent contribution of AO for CYP2D6 substrates – **Why?**

Calculation of fm_{CYP} vs. fm_{AO}

Fractional metabolism CYP vs. AO: Hydralazine is a CYP2D6 inhibitor in hepatocytes

	CYP1A2	CYP2B6	CYP2C8	CYP2C9	CYP2C19	CYP2D6	CYP3A4
IC50 (µM)	**>250**	**215**	**>250**	**>250**	**>250**	**18**	**235**



Hydralazine may be useful to identify drugs as AO substrates that ***are not substrates of CYP2D6***

For substrates of CYP2D6, additional controls may be necessary (such as a specific CYP2D6 inhibitor or substrate)

Chemical inhibition approach with hydralazine can also be used with **S9**

Precaution when using hydralazine for fm_{AO}

- Hydralazine's potential to inhibit CYP1A2, 2B6 ,2D6 and 3A has been shown (Yang et al. 2019).

Table 1
Selectivity of Hydralazine at 4 Concentrations Against CYP Enzymes in Suspension Human Hepatocytes With 30 min Preincubation

Enzymes	Substrate	% Inhibition ± Standard Deviation			
		25 μM	50 μM	100 μM	150 μM
CYP1A2	phenacetin	38 ± 13	56 ± 22	67 ± 15	74 ± 11
CYP2B6	bupropion	15 ± 14	22 ± 14	33 ± 14	36 ± 12
CYP2C8	paclitaxel	5 ± 6	3 ± 4	7 ± 4	6 ± 8
CYP2C9	diclofenac	7 ± 7	9 ± 7	13 ± 4	10 ± 6
CYP2C19	S-mephenytoin	2 ± 5	5 ± 4	7 ± 7	8 ± 7
CYP2D6	dextromethorphan	14 ± 12	31 ± 5	49 ± 11	65 ± 9
CYP3A	midazolam	11 ± 9	25 ± 4	28 ± 14	37 ± 4
AO	zaleplon	71 ± 7	85 ± 4	94 ± 2	93 ± 1

Yang et al. J Pharm Sci 108:1627-1630, 2019

Poll question #3

Summary

- Underprediction of clearance of AO substrates has led to clinical failure of several drug candidates
- Several reasons attribute to poor prediction including marked species differences, interindividual variability, substrate specificity, poor correlation between AO protein expression and activity, etc.
- In vitro strategies such as yard-stick approach to decipher the relative clearance of the AO substrate drug
- AO metabolism and fm_{AO} knowledge crucial for further decision making

References

- Dhuria et al. Drug Metabolism Reviews 53:2, 188-206, 2021
- Beedham C., Xenobiotica 50:34-50, 2020
- Dalvie D. and Di L. Pharmacology & Therapeutics 201:137–180, 2019
- Manevski et al. J Med Chem 62, 10955–10994, 2019
- Terao et al. JBC Reviews 295:5377-5389, 2020
- Sanoh et al. Drug Metab Pharm 30:52-63, 2015
- Pryde et al. Bioorganic & Medicinal Chemistry Letters 22:2856–2860, 2012
- Pryde et al. J Med Chem, 53, 8441–8460, 2010

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Thank you!

Questions or Comments?