Accurate estimation of human drug exposure variability for mixed CYP3A/2D6 substrates by the use of $f_{m,CYP}$ determined in human hepatocytes

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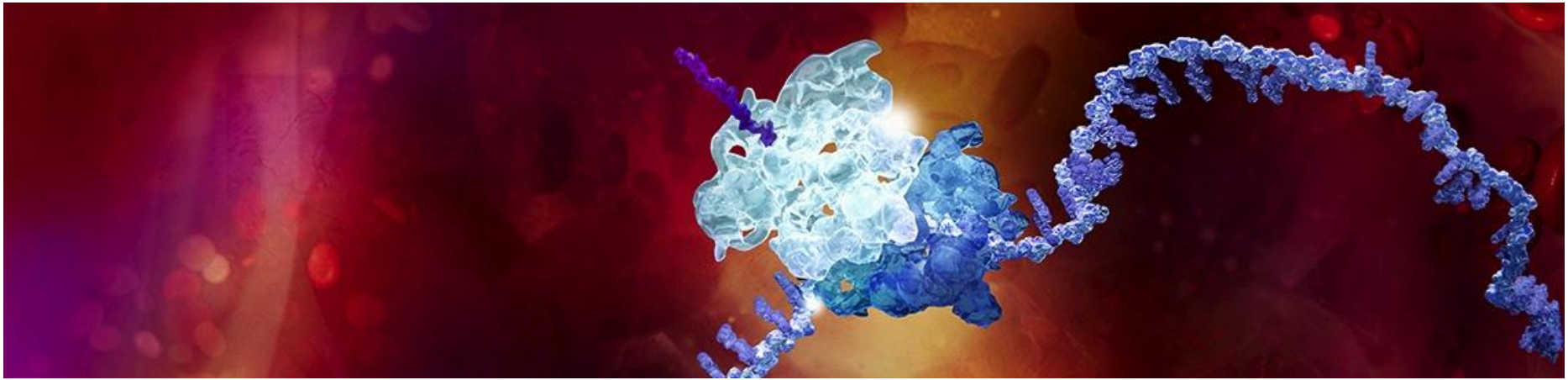


Aim: To evaluate

Which system is most accurate for prediction of variability in human compound exposure: Human recombinant CYP enzymes (hrCYP) or human hepatocytes.

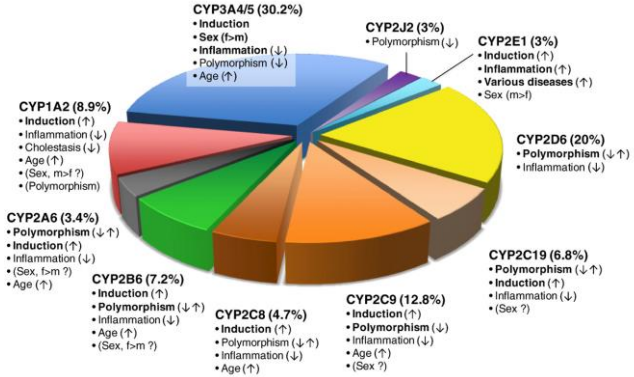
Estimated $f_{m,CYP}$ values were used to predict:

1. AUC increase of mixed CYP3A/2D6 drug substrates following co-administration of potent and selective CYP3A-inhibitors

2. AUC differences of mixed CYP3A/2D6 substrates between CYP2D6 poor and extensive metabolizers

Metabolism by CYP3A and CYP2D6 – why bother?

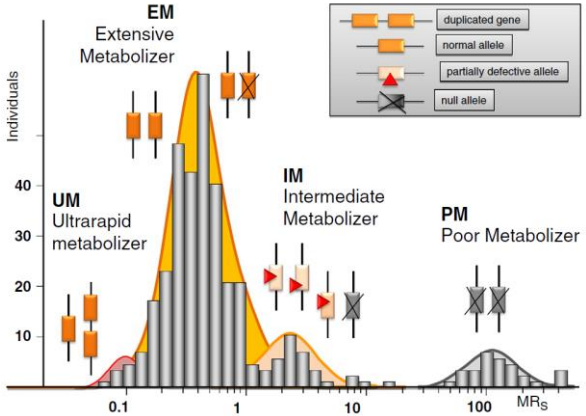
Fraction of clinically used drugs metabolized by P450 isoforms



Polypharmacy

https://dangersofpolypharmacy.wordpress.com/

Sparteine oxidation phenotype and genotype distribution

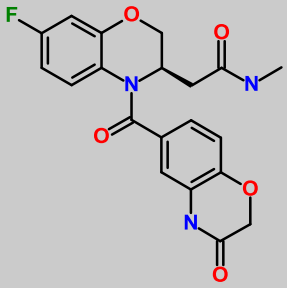
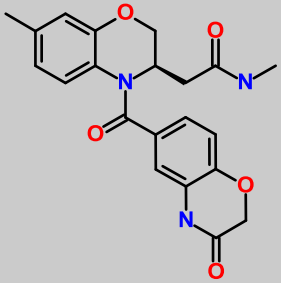
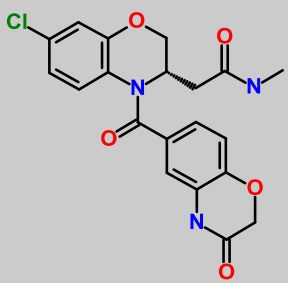
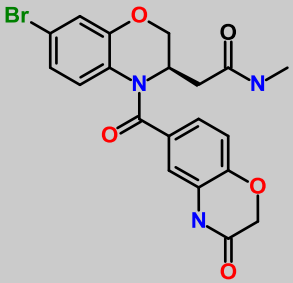


Zanger et.al. Pharmacology & Therapeutics, 2013

For drugs that are cleared mainly by CYP3A or CYP2D6, large variations in exposures are expected in the patient population

Lead Optimization of CYP3A-mediated metabolism

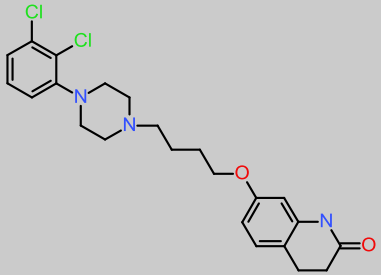
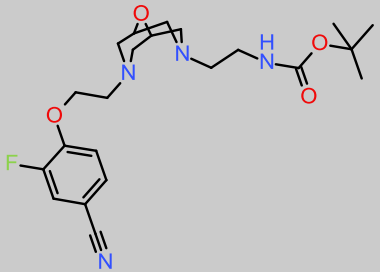
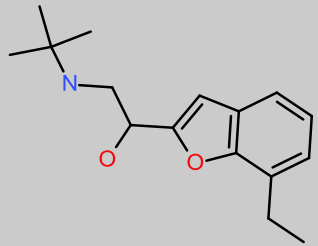
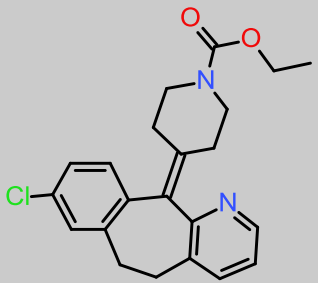
Different results on fmCYP3A in a compound series of mixed CYP3A/2D6 substrates when assessed in hrP450 and human hepatocytes, respectively.

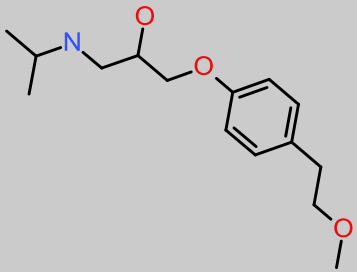
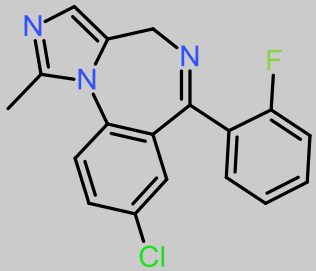
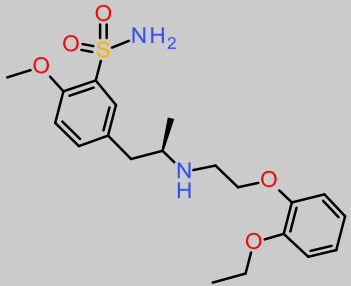
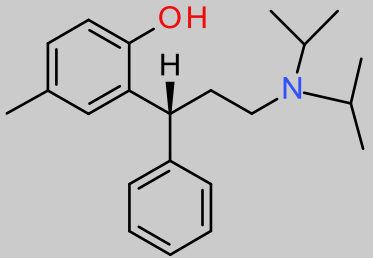
AZD9977	AZ2	AZ3	AZ4
			
hrP450: 100% CYP3A Hepatocytes: 93% CYP3A	hrP450: 93% Hepatocytes: 58%	hrP450: 95% Hepatocytes: 56%	hrP450: 92% Hepatocytes: 48%

Red if >90% CYP3A, Green if <90% CYP3A

- The lead compound AZD9977 (eventually selected for clinical development) was identified as a CYP3A substrate.
- Lead optimization intitiated to introduce additional metabolic pathways.
- When assed in hrP450, no compound with less than 90% CYP3A-mediated metabolism was identified.
- When assesed in human hepatocytes, a much wider range in CYP3A-dependent metabolism was captured.

CYP3A and/or CYP2D6 substrates with well described human in vivo data used for model evaluation

Aripiprazol (ARI)	AZD1305	Bufuralol (BUF)	Loratadine (LOR)
			

Metoprolol (MET)	Midazolam (MID)	Tamsulosin (TAM)	Tolterodine (TOL)
			

In vitro methods to assess fm_{CYP}

hrP450:

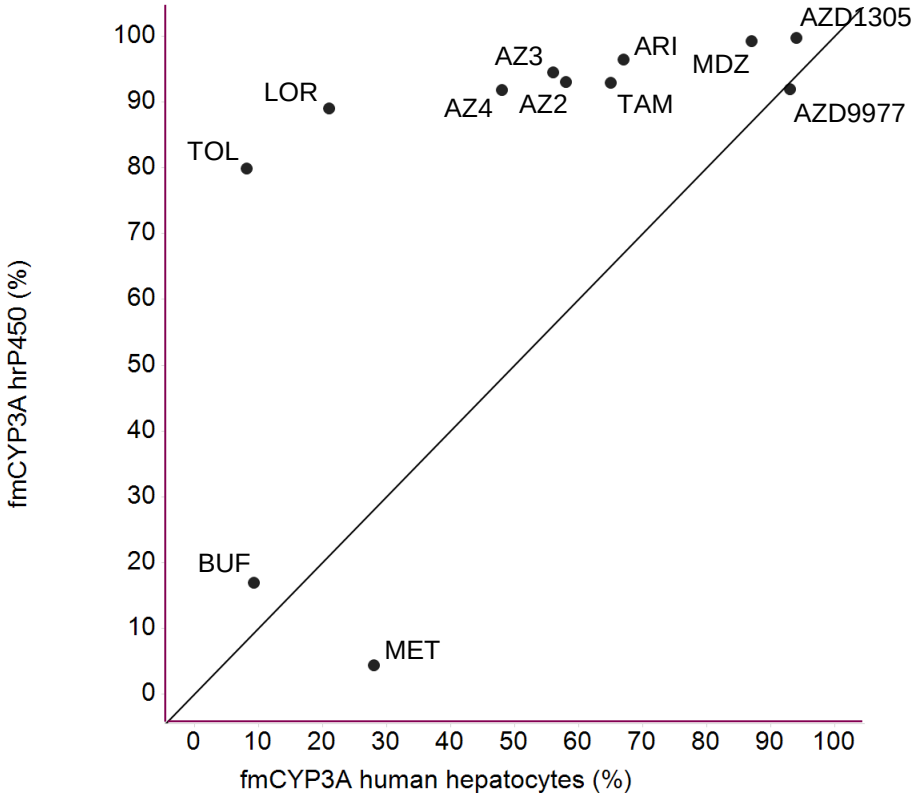
- Compounds incubated individually in a panel of 10 P450s, including CYP3A and CYP2D6.
- CL_{int} estimated in each P450 by monitoring the rate of compound depletion.
- $F_{m,CYP}$ calculated from CL_{int}-values in combination with hepatic abundance of each P450 and ISEFs* determined in-house.

Human hepatocytes:

- Compounds incubated in human hepatocytes +/- the CYP3A-inhibitor ketoconazole and the CYP2D6-inhibitor quinidine, respectively.
- CL_{int} estimated by monitoring rate of compound depletion +/- inhibitor.
- $F_{m,CYP}$ calculated from the difference in CL_{int}-values estimated +/- inhibitor.

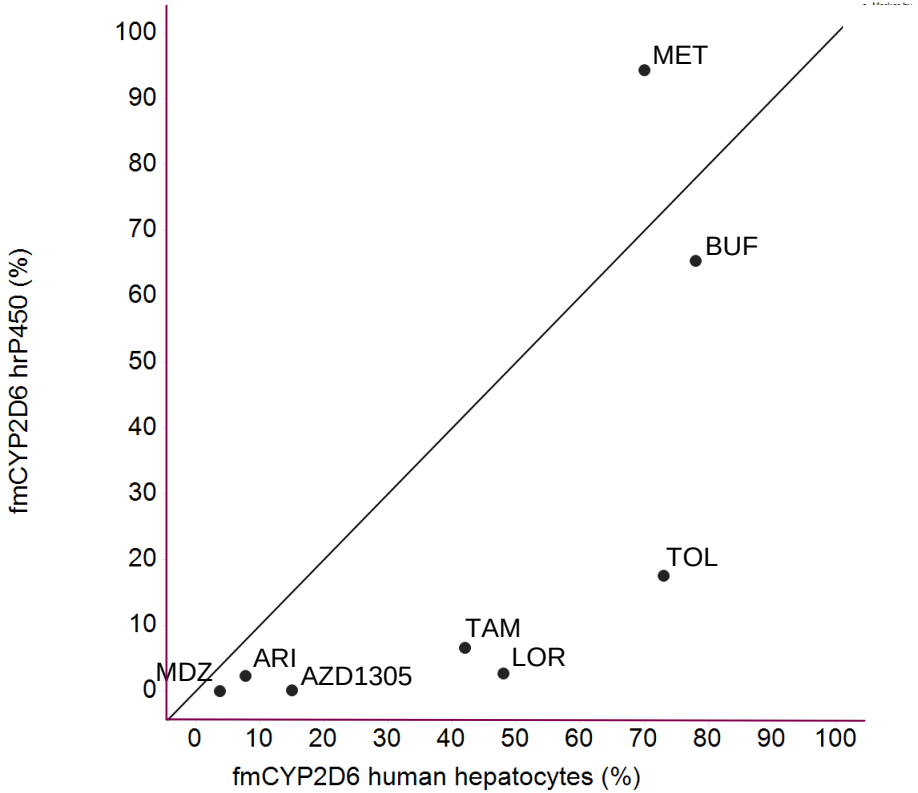
*Intersystem extrapolation factors

Estimated $f_{m,CYP3A}$ from hrP450 versus human hepatocytes



- In general, larger $f_{m,CYP3A}$ estimated from hrP450 compared to hepatocytes
- Metoprolol is an outlier with smaller $f_{m,CYP3A}$ estimated in hrP450 compared to hepatocytes
- For all compounds, except bufuralol and metoprolol, fm_{CYP3A} was predicted to $\geq$80% when estimated in hrP450
- Much larger dynamic range for mixed CYP3A/2D6 substrates in $f_{m,CYP3A}$ estimated in hepatocytes compared to hrP450

Estimated $f_{m,CYP2D6}$ from hrP450 versus human hepatocytes



- In general, smaller fm_{CYP2D6} estimated in hrP450 compared to hepatocytes
- Metoprolol is an outlier with larger $f_{m,CYP2D6}$ estimated in hrP450 compared to hepatocytes
- For all compounds, except bufuralol and metoprolol, $f_{m,CYP2D6}$ was predicted to <20% when estimated in hrP450
- Much larger dynamic range for mixed CYP3A/2D6 substrates in $f_{m,CYP2D6}$ estimated in hepatocytes compared to hrP450

Static equation used for prediction of CYP3A DDI

Predicted AUC-ratio in presence/absence of inhibitor:

$$\frac{AUC_i}{AUC} = \frac{f_{g,i,}}{f_g} \times \frac{1}{\frac{\sum f_m \times f_{m,CYP}}{1+\sum \frac{I_u}{K_{i,u}}} + 1 - \sum f_m \times f_{m,CYP}}$$

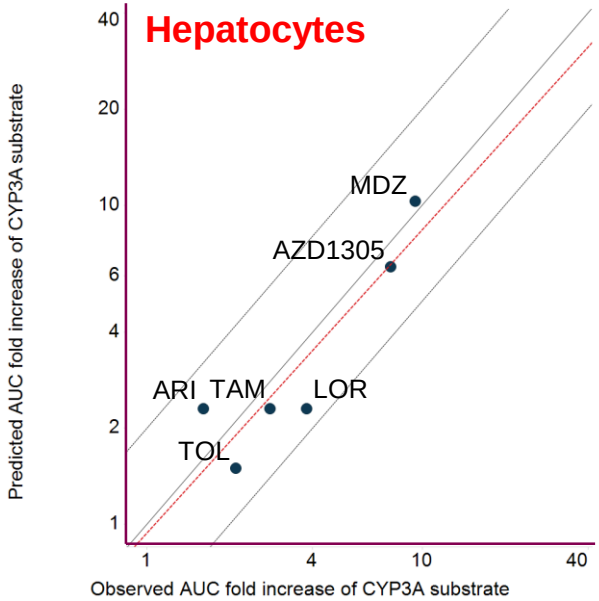
Predicted maximum unbound hepatic inlet concentration of ketoconazole:

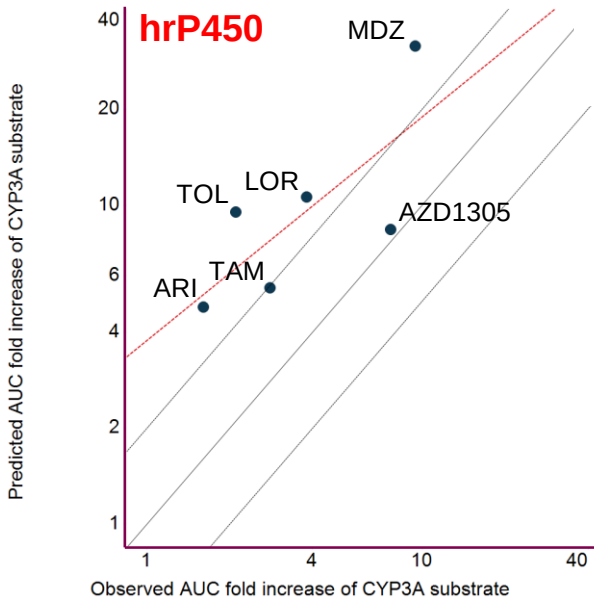
$$I_u = I_{in,u} = I_{ss} \times f_u + \frac{k_a \times f_a \times f_g \times D \times f_u}{Q_{pv}}$$

Assumptions:

- The fraction escaping gut metabolism (fg) is all due to CYP3A metabolism in the gut
- Complete inhibition av CYP3A in the gut by ketoconazole
- No difference in gut metabolism between CYP2D6 EM and PM

AUC-fold increase following oral co-administration of ketoconazole is well predicted from $f_{m,CYP3A}$ estimated in hepatocytes, but not hrP450

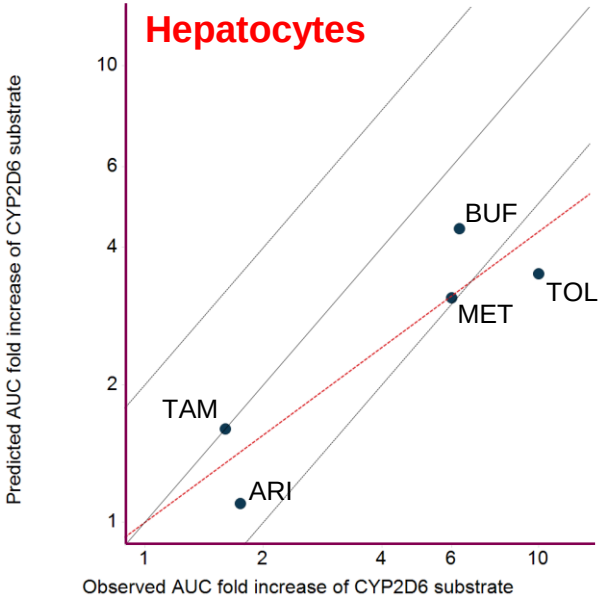


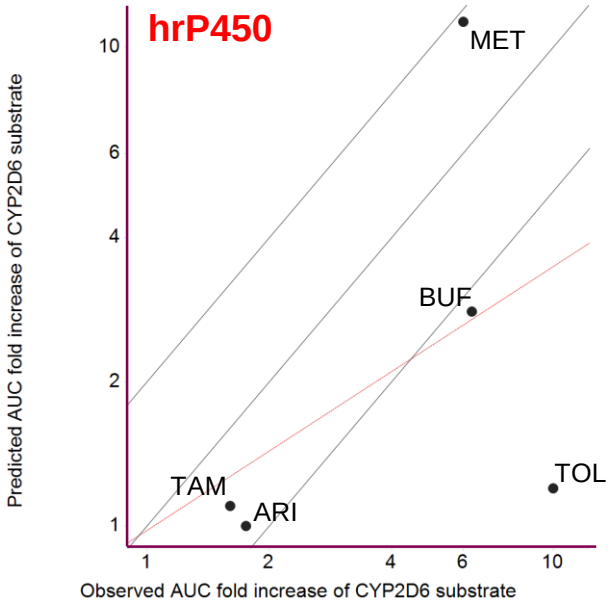


- r^2: 0.83 (correlation)
- RMSE: 0.98 (precision)
- ME: -0.4 (accuracy)

- r^2: 0.55 (correlation)
- RMSE: 9.8 (precision)
- ME: 6.5 (accuracy)

AUC-fold increase in CYP2D6 PMs is reasonably well predicted from $f_{m,CYP2D6}$ estimated in hepatocytes, but not hrP450





- r^2: 0.82 (correlation)
- RMSE: 3.1 (precision)
- ME: -1.5 (accuracy)

- r^2: 0.20 (correlation)
- RMSE: 4.6 (precision)
- ME: -0.85 (accuracy)

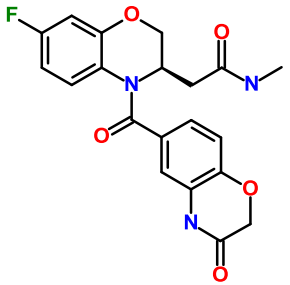
Why are human hepatocytes a preferred in vitro-system over hrP450s

- Depending on the substrates selected for estimation of ISEFs, the $f_{m,CYP}$ values could vary substantially[1].
- P450 function is complex and their enzymatic activities are influenced by interactions with redox partners, allosteric mediators and with other P450 isoforms. Several of these interactions are interrupted in a simple system as hrP450[2].
- Hepatocytes represent a more complete '*in vivo*-like' *in vitro* system compared with hrP450s where the risk of incorrect estimation of $f_{m,CYP}$ is alleviated.
- Hepatocytes also represent a more complete *in vitro* system with regards to drug metabolizing enzymes expressing non-P450 phase I as well as phase II enzymes.

[1]Siu YA, Lai WG (2017) Drug Metab Dispos 45: 183–189.
[2]Kandel SE, Lampe JN (2014) Chem Res Toxicol 27: 1474–1486.

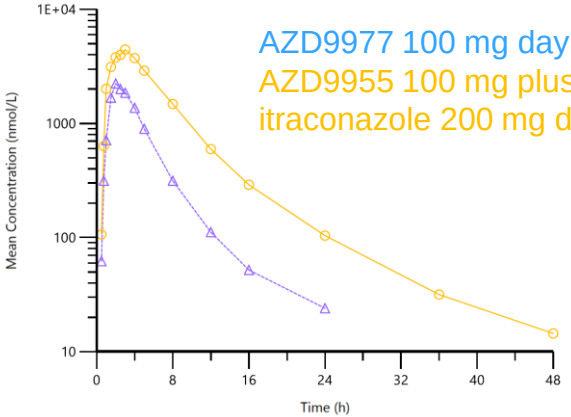
AZD9977 is in clinical development for protection of Heart Failure and Chronic Kidney Disease

- Mineralocorticoid receptor (MR) antagonists like eplerenone and spironolactone are known to improve outcomes in heart failure but mechanism based hyperkalemia risk limits their use.
- AZD9977 is a novel MR modulator currently in clinical development exerting similar organ protection as the MR antagonists, but with minimal urinary electrolyte effects demonstrated in preclinical studies and therefore carries less risk of hyperkalemia.
- Proof of Mechanism has been demonstrated for AZD9977 in healthy volunteers.
- AZD9977 is eliminated by P450-mediated metabolism (CYP3A) and renal excretion.
- AZD9977 is safe and well tolerated at doses from 5 to 1200 mg in healthy volunteers.
- Ongoing patient study with AZD9977 to demonstrate differentiated electrolyte effects versus spironolactone.

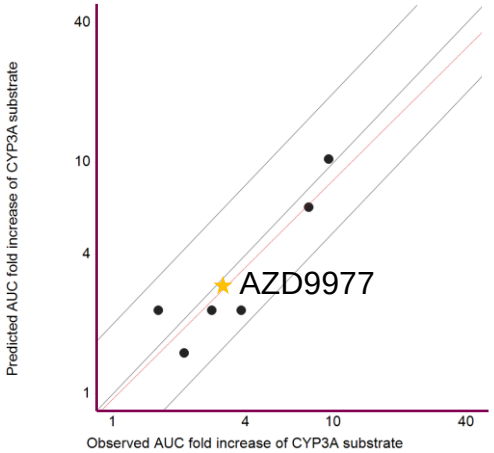


Br. J. Clin. Pharmacol. 2018, 84, 1486–1493
J. Med. Chem. 2019, 62, 1385−1406

Exposure of AZD9977 in healthy volunteers when administered alone and with itraconazole



DDI-risk of AZD9977 is accurately predicted from $f_{m,cyp}$ estimated in human hepatocytes



- 14 healthy volunteers
- Daily administration of 200 mg itraconazole from day 4 and onwards
- Only 3.1-fold increase in AUC AZD9977 despite >90% CYP3A of total metabolism
- Extrahepatic (renal) route of elimination makes AZD9977 less sensitive to strong CYP3A inhibitors
- AZD9977 can be classified as a moderate sensitive substrate of CYP3A

$F_{m,CYP}$ estimated in human hepatocytes is preferred over hrP450 to predict exposure variability of CYP3A/2D6 substrates

- CYP3A/2D6 substrates can be accurately rank-ordered for risk of CYP3A victim DDI and PM/EM exposure-ratio by using $f_{m,CYP}$-values estimated in human hepatocytes.
- There is an excellent accuracy and precision in predicted CYP3A victim DDI-risk when using $f_{m,CYP3A}$ from human hepatocytes.
- PM/EM exposure ratios are somewhat underpredicted for compounds mainly cleared by CYP2D6 when $f_{m,CYP2D6}$ estimated in human hepatocytes is used.

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Confidentiality Notice