Evaluation of Chimeric Mice with Humanized Liver to Predict Human Intrinsic Clearance of Drug Molecules at Preclinical Phase

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Drug Metabolism and Preclinical Safety

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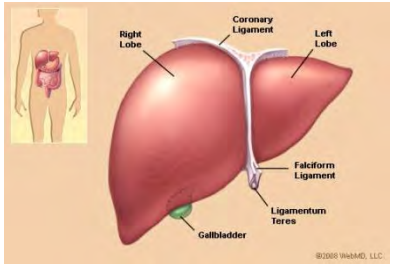
Why Hepatic Clearance ?

- Liver expresses highest levels of drug metabolizing enzymes
- Most marketed drugs (~70%) are metabolized by liver



Liver

↓

Intrinsic Clearance (Cl_{int}) - - - - - → Drug-drug Interactions, Biotransformation, Biliary Excretion

↓

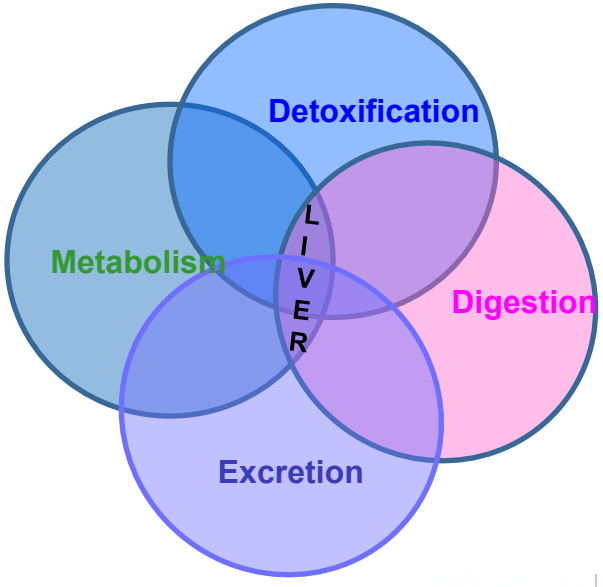
$Clearance_{plasma}$ (Cl_{plasma})

↓

Pharmacokinetics (PK)

↓

Human Dose
(Safety & Efficacy)



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Model Systems To Predict Human Cl

- In vitro systems: ⟶ IVIVC

  -Subcellular fractions (microsomes, S9)

  -Intact cells (fresh & cryopreserved hepatocytes)

  Inaccurate scaling due to non-physiological in vitro conditions

  Normal liver levels of metabolic activity over a wide range of functions absent

  NCEs have to be primarily eliminated via liver

- In vivo systems:

  - ***Allometry*** (or equivalent extrapolation)

    Limited Predictability due to species difference in metabolism/transport

  - ***Single gene-targeted Transgenic Mice***

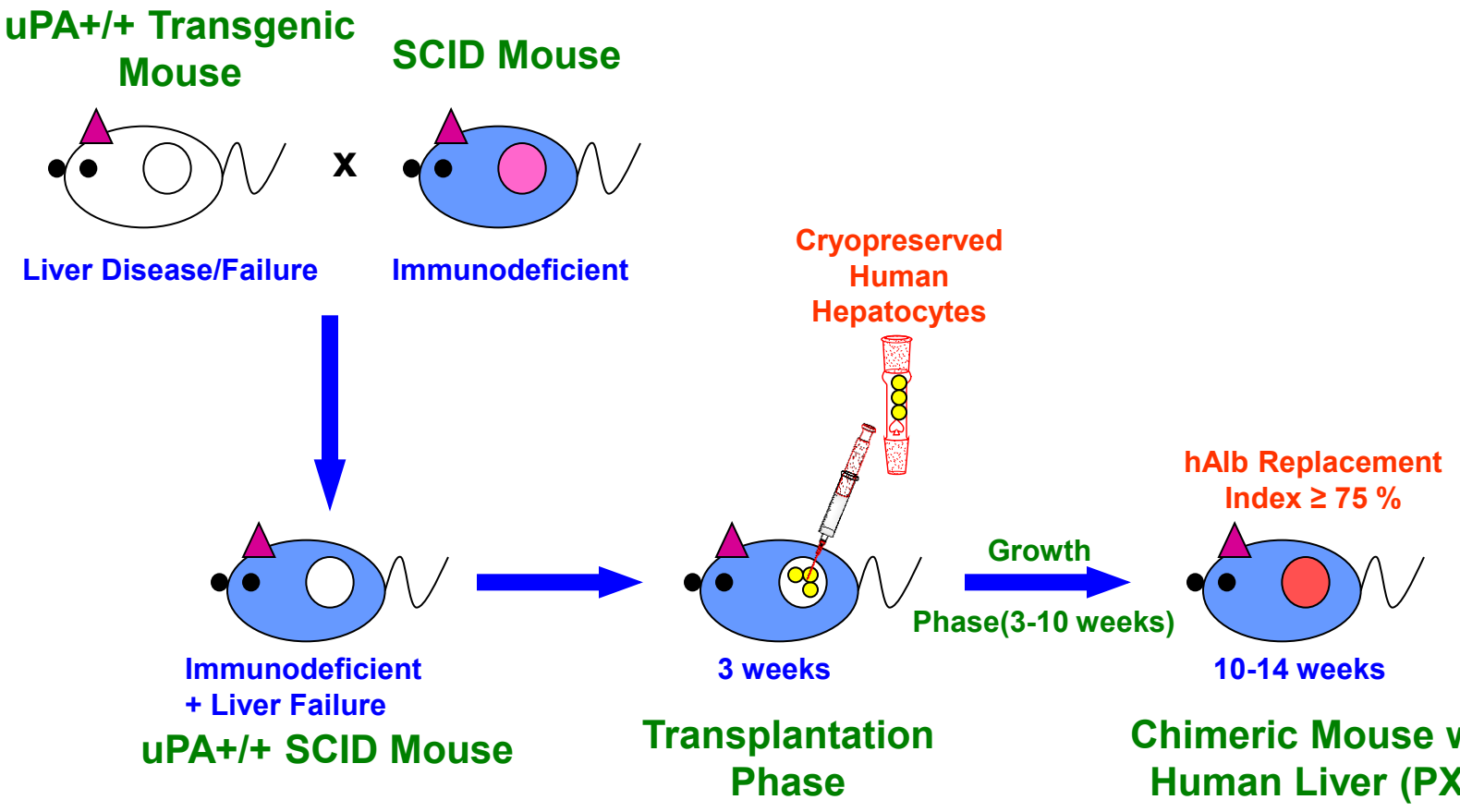
    Complex gene transcription and translation

    Inability to assess entire range of human DME and transporters

Current Challenge: Accurately predict Human Cl

Can chimeric mice with humanized liver be useful to predict human Cl??

Chimeric Mice With Humanized Liver



- hAlb ↑ in host blood with h-hepatocyte replacement
- mALT ↓ in host blood with h-hepatocyte replacement
- Sustained engrafment occurs in $Tg^{+/+}_{Alb\text{-}uPA}$ but not in $Tg^{+/-}_{Alb\text{-}uPA}$

Tateno et.al, Am.J.Pathology, 2004, 165(3), 901
Tateno et.al, Toxicology, 2008, 246, 9
Tateno et.al, PPAR Research, 2009, 1

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Replacement Index Of Humanized Liver

$$\text{Replacement Index (RI)} = \frac{\text{Area occupied by hCK8/18-positive hepatocytes}}{\text{Entire area examined in the immunohistochemical stain}} \times 100$$

- RI correlates well with serum hAlb

$$\text{hAlb (mg/mL)} = 0.0413 \times e^{0.0676 \times \text{RI\%}}$$

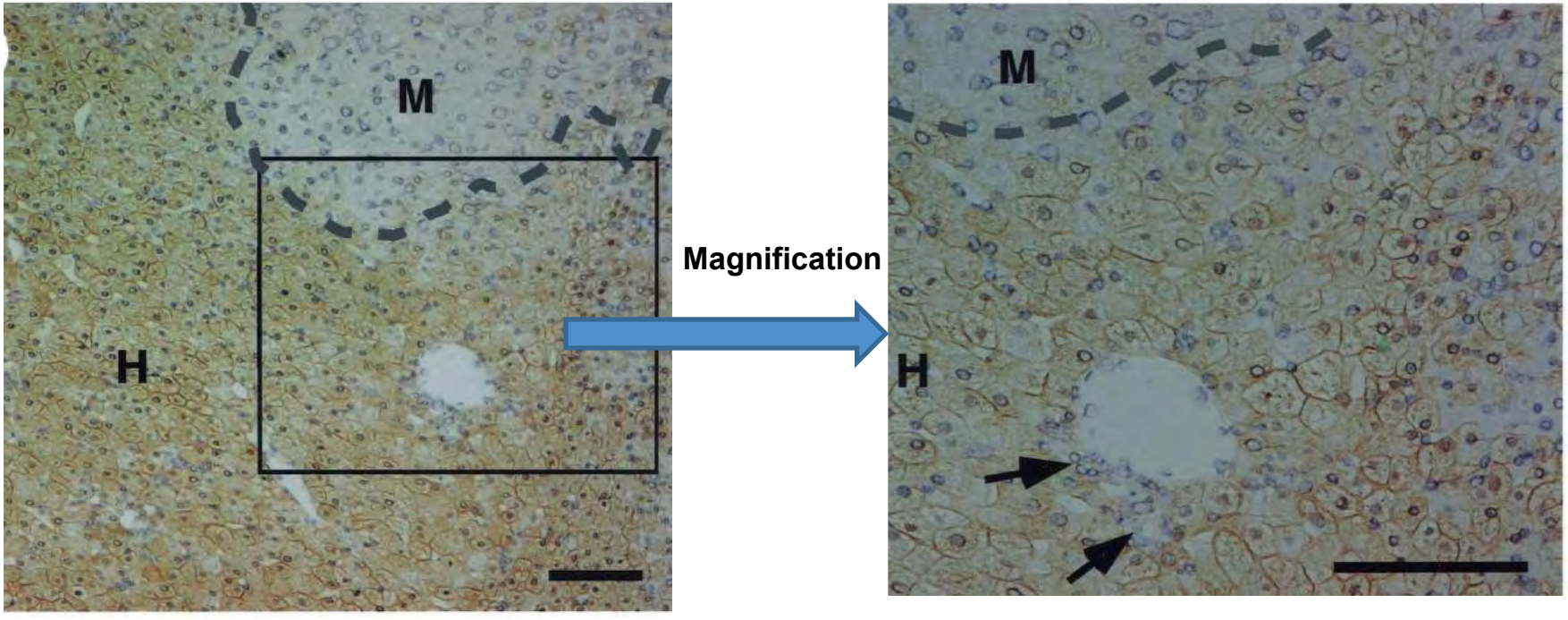
Host with > 5-6 mg/mL hAlb → RI >70%

Chimeric mouse liver with high RI → majority h-parenchymal cells + m-non-parenchymal cells + m-extra cellular matrix

- Histology shows highly organized liver architecture
- Sinusoidal structure between h-hepatocytes and m-stellate cells clearly observed
- Chimeric mice liver sections stained for liver cell markers (hepatocytes, endothelial cells, Kupffer cells, stellate cells)

Tateno et.al, Am.J.Pathology, 2004, 165(3), 901

Staining With hCK8/18



Tateno et.al, Am.J.Pathology, 2004, 165(3), 901

Confirmation Of DME And Transporters

mRNA (RT-PCR)

- 52 Ph I drug metabolizing enzymes
  - 20 CYPs including CYPs 1A, 2B, 2C, 2D6, 3A
  - 32 other Ph I including ADH, ALDH, MAO, FMO, CES, EPHX, PTGS
- 26 Ph II drug metabolizing enzymes
  - UGT, SULT, GST, NAT, TPMP, COMT, PNMT
- 21 Transporters
  - MDR1, MRP, OCT, OCTN, BSEP

Enzyme activity

- CYPs 2C8, 2C9, 2D6, 3A4, UGT2B7, SULTs 1A1, 1E1

- mRNA and/or protein levels, enzyme activity all correlated with h-Alb-concentration
- some enzyme activity were lower while some higher than donor
- *mRNA levels not reflective of enzyme activity*
- chimeric mice maintained genotype and phenotype of donor

Katoh et.al, DMD, 2004, 32(12), 1402
Katoh et.al, DMD, 2005, 33(9), 1333
Nishimura et.al, Xenobiotica, 2005, 35(9), 877

Chimeric Mice With Humanized Liver

- **h-specific metabolism (CYP2D6, 2C9, ALD)**

  Katoh et.al, Xenobiotica, 2005, 35(9), 863
  Emoto et.al, Xenobiotica, 2008, 38(3), 239
  Inoue et.al, DMD, 2008, 36(12), 2429
  Ohta et.al, DMD, 2008, 36(7), 1202

- **h-specific CYP inhibition (2D6, 3A4)**

  Kakuni et.al, PhoenixBio SOT, 2009
  Katoh et.al, J.Pharm.Sci, 2007, 96(2), 428

- **h-specific CYP induction (1A1/2, 3A4)**

  Katoh et.al, Xenobiotica, 2005, 35(9), 863
  Emoto et.al, Xenobiotica, 2008, 38(3), 239

- **h-specific metabolic profile**

  Yamazaki et.al, CRT, 2010, 23, 152
  Foster et. al AZ/PhoenixBio ISSX Meeting 2010 Turkey
  Sanjeev Kumar- APA Meeting 2009 Boston

- **validation of human excretory pathway**

  Okumura et.al, Tox Sci., 2007, 97(2), 533

Goal Of Current Study

Can this model be used for human Cl_{int} prediction?

Whole body clearance prediction was not attempted (physiological and anatomical differences between human and mouse)

- Test compounds selection
- $Cl_{in\ vivo}$ measurement in chimeric mice
- Convert $Cl_{in\ vivo}$ to $Cl_{intrinsic}$
- Obtain $Cl_{intrinsic}$ of test compounds from literature reported
- Comparison of $Cl_{intrinsic}$ obtained in chimeric mice vs reported
- Summary

Selection Of Compounds

- Investigative drugs selected for this study → Cl is known to be hepatic metabolism
- Majority CYP3A4 substrates
- Investigate rank ordering of Cl_{int} → range of Cl of investigative drugs

Substrate	DME	Cl % Qh in Human	Cl % Qh in Mice
Antipyrine	CYP3A4	4	25
Midazolam	CYP3A4	33	46
Verapamil	CYP3A4/2C9	75	~100
Zidovudine	UGT2B7	~100	33

Test Compound	Mice Strain	Dose Level (mg/kg)
Antipyrine	PXB	5
	uPA$^{+/+}$/SCID	
	SCID	
Midazolam	PXB	1
	uPA$^{+/+}$/SCID	
	SCID	
Zidovudine	PXB	5
	uPA$^{+/+}$/SCID	
	SCID	
Verapamil	PXB	1
	uPA$^{+/+}$/SCID	
	SCID	

Vehicle: PBS

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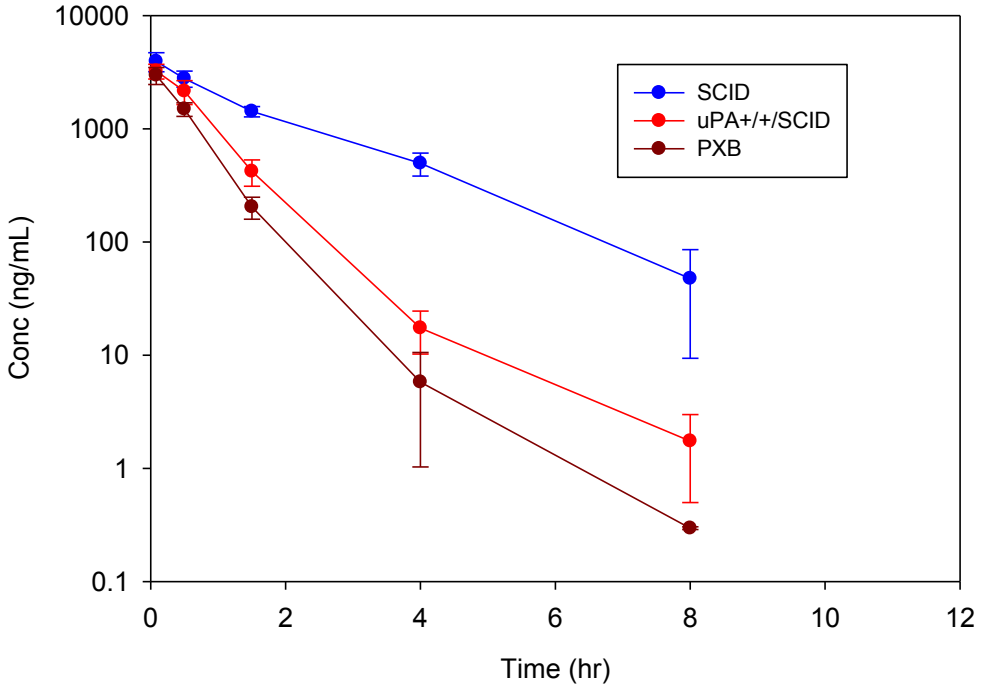
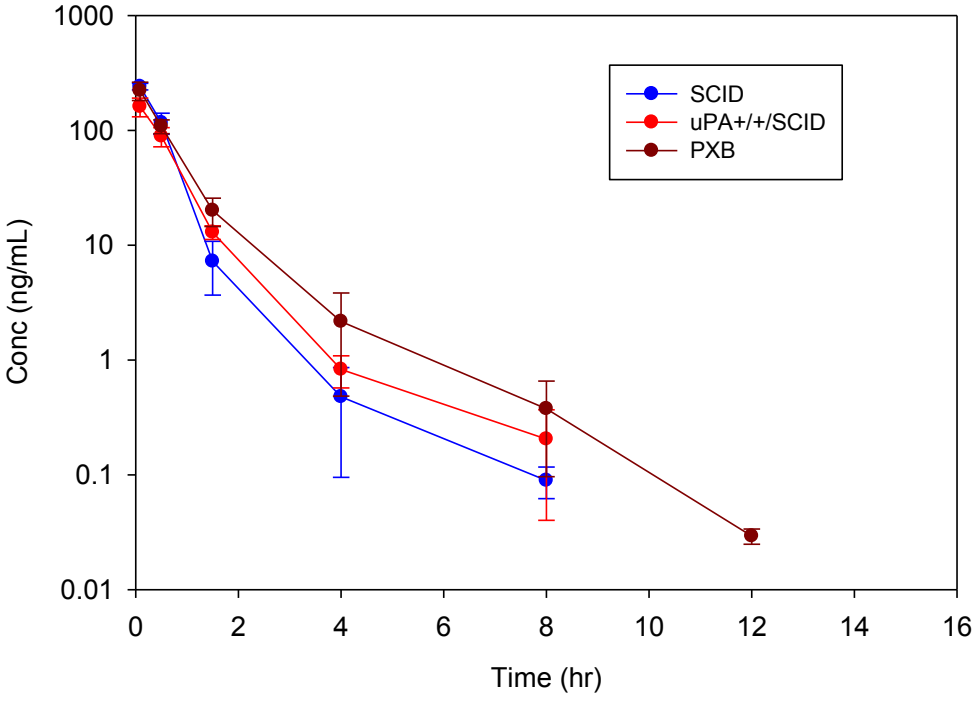
Antipyrine Mouse PK



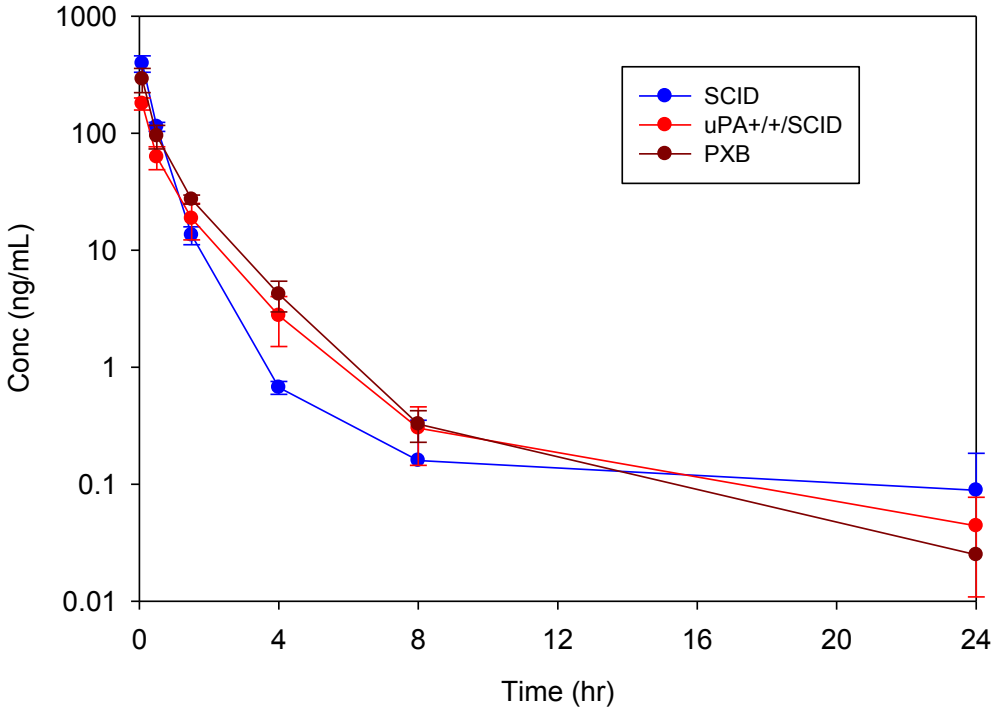
Mouse Type	$t_{1/2}$ (hr)		$AUC_{0\text{-last}}$ (hr*ng/mL)		Cl (mL/min/kg)		V_{dss} (L/kg)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
PXB	0.5	0.2	2313	245	36	4	1.1	0.1
uPA+/+/SCID	1.2	0.4	3271	512	26	4	1.0	0.1
SCID	1.2	0.3	7323	915	11	2	1.2	0.1

Midazolam Mouse PK



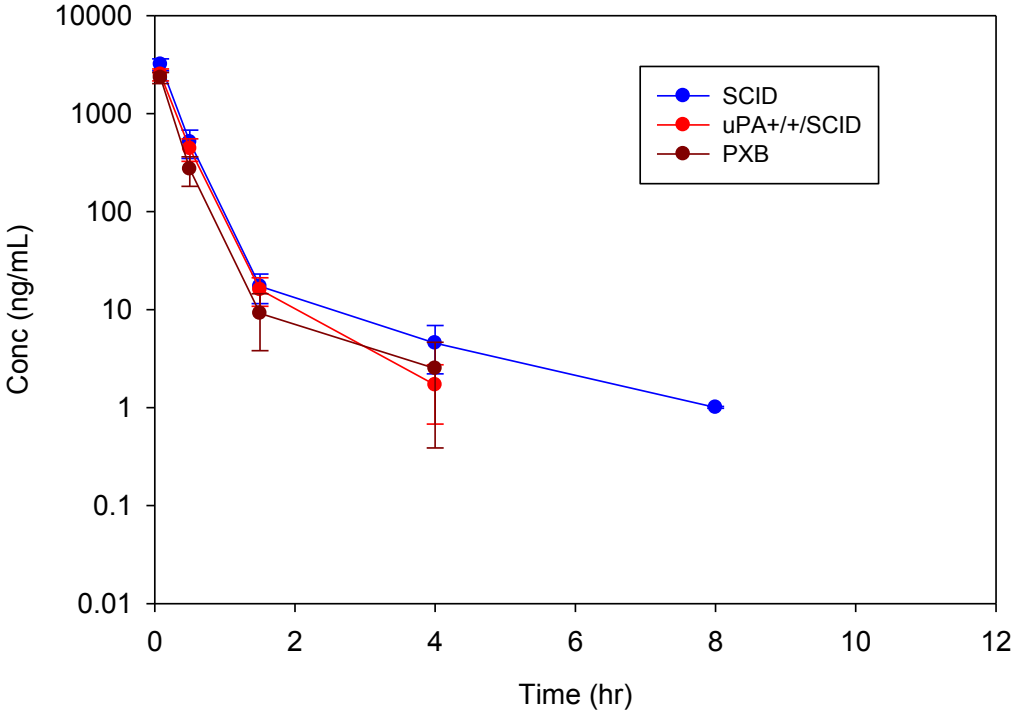
Mouse Type	$t_{1/2}$ (hr)		$AUC_{0\text{-last}}$ (hr*ng/mL)		Cl (mL/min/kg)		V_{dss} (L/kg)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
PXB	5.6	2.7	189	21	89	9	4.7	1.1
uPA+/+/SCID	2.0	1.1	137	18	123	15	4.9	0.3
SCID	2.4	1.6	169	27	101	17	2.7	0.1

Verapamil PK



Mouse Type	$t_{1/2}$ (hr)		$AUC_{0\text{-last}}$ (hr*ng/mL)		Cl (mL/min/kg)		V_{dss} (L/kg)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
PXB	1.8	1.6	217	31	78	11	3.9	0.7
uPA+/+/SCID	5.9	6.8	142	30	120	23	8.0	3.1
SCID	11.1	11.1	228	25	73	8	3.2	1.2

Zidovudine PK



Mouse Type	$t_{1/2}$ (hr)		$AUC_{0\text{-last}}$ (hr*ng/mL)		Cl (mL/min/kg)		V_{dss} (L/kg)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
PXB	1.3	0.4	1391	248	61	9	1.0	0.2
uPA+/+/SCID	1.2	1.1	1123	163	75	11	1.3	0.6
SCID	0.4	0.3	939	155	91	18	1.0	0.1

Summary Of Mouse PK

Compound	Mice Strain	$t_{1/2}$ (hr)		$AUC_{0\text{-last}}$ (hr*ng/mL)		Cl (mL/min/kg)		Cl	V_{dss} (L/kg)	
		Mean	SE	Mean	SE	Mean	SE	%Qh	Mean	SE
Antipyrine 5mg/kg	PXB	0.5	0.2	2313	245	**36**	4	**40**	1.1	0.1
	uPA$^{+/+}$/SCID	1.2	0.4	3271	512	**26**	4	**29**	1.0	0.1
	SCID	1.2	0.3	7323	915	**11**	2	**13**	1.2	0.1
Midazolam 1 mg/kg	PXB	5.6	2.7	189	21	**89**	9	**99**	4.7	1.1
	uPA$^{+/+}$/SCID	2.0	1.1	137	18	**123**	15	**137**	4.9	0.3
	SCID	2.4	1.6	169	27	**101**	17	**112**	2.7	0.1
Verapamil 1 mg/kg	PXB	1.8	1.6	217	31	**78**	11	**86**	3.9	0.7
	uPA$^{+/+}$/SCID	5.9	6.8	142	30	**120**	23	**134**	8.0	3.1
	SCID	11.1	11.1	228	25	**73**	8	**81**	3.2	1.2
Zidovudine 5 mg/kg	PXB	1.3	0.4	1391	248	**61**	9	**68**	1.0	0.2
	uPA$^{+/+}$/SCID	1.2	1.1	1123	163	**75**	11	**84**	1.3	0.6
	SCID	0.4	0.3	939	155	**91**	18	**101**	1.0	0.1

High Cl observed in uPA$^{+/+}$/SCID mice

Cl_{int} In Mice Microsomes

Compound	Mice Strain	In Vitro Cl_{hep} (Microsomes) % Qh	In Vivo Cl (Plasma) % Qh
Antipyrine	SCID	23	12
	uPA$^{+/+}$/SCID	<27.0	29
	PXB	<27.0	40
Midazolam	SCID	96	112
	uPA$^{+/+}$/SCID	96	136
	PXB	94	100
Verapamil	SCID	85	81
	uPA$^{+/+}$/SCID	87	133
	PXB	83	87

Good IVIVC

$$Cl_{int} = \frac{0.693}{\text{in vitro } T_{1/2}} * \frac{\text{mL incubation}}{\text{mg microsomes}} * \frac{45 \text{ mg microsomes}}{\text{gm liver}} * \frac{X \text{ gm liver}}{\text{kg B.W.}}$$

$$Cl_{hep} = \frac{Q_h \, Cl_{int}}{Q_h + Cl_{int}}$$

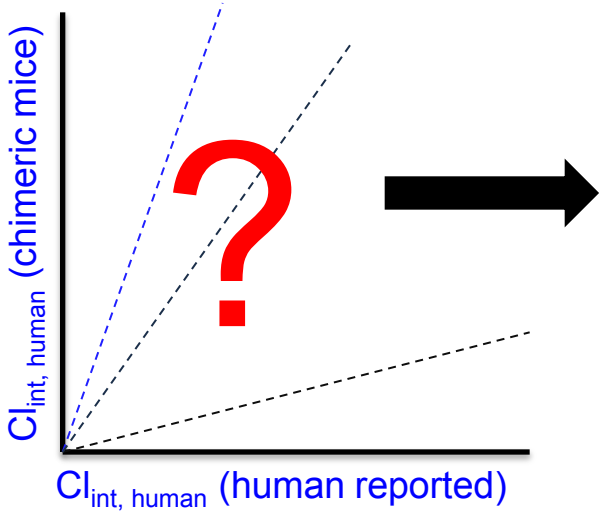
Cl_{int} = intrinsic clearance
Cl_{hep} = hepatic clearance
Q_h = hepatic blood flow

incubation volume:	0.5	mL
mg microsomes:	0.25	mg
mouse scale up factor **X**:	87.5	gm liver/kg body weight
human scale up factor **X**:	20	gm liver/kg body weight
SCID scale up factor **X**:	52	gm liver/kg body weight
uPA/SCID scale up factor **X**:	67	gm liver/kg body weight
PXB scale up factor **X**:	123	gm liver/kg body weight
mouse hepatic blood flow **Qh**:	90	mL/min/kg
human hepatic blood flow **Qh**:	20	mL/min/kg

Microsomes generously donated by PhoenixBio

Summary So Far

- All 3 mice groups show similar Cl
- High Cl observed in uPA$^{+/+}$/SCID mice
- Good *in vitro* (microsome) vs *in vivo* (plasma Cl) correlation



I. $Cl_{chimeric} \xrightarrow{\textbf{RI}}$ Corrected $Cl_{human,predicted}$

II. $Cl_{human,predicted} \rightarrow$ **$Cl_{human,predicted,intrinsic}$**

III. $Cl_{human,reported} \rightarrow$ **$Cl_{human,reported,intrinsic}$**

IV. **$Cl_{human,predicted,intrinsic}$ vs $Cl_{human,reported,intrinsic}$**

Cl_{human} Calculated From $Cl_{chimeric}$

Cl_{total} seen in PXB mice is coming from :

% human hepatocytes re-populated in host mouse liver + residual host mouse hepatocytes

$$\mathbf{Cl_{Chimeric} = RI*Cl_{human} + (1\text{-}RI) * (Cl_{upa+/+SCID})}$$

$Cl_{Chimeric}$ = Clearance in chimeric mice with human liver
RI = Replacement Index (measure of contribution of human liver)
$Cl_{upa+/+SCID}$ = Clearance in host upa+/+ SCID mice
Cl_{human}= Predicted Clearance in human liver

Antipyrine : $36.3 = 0.782*Cl_{human} + 0.218 * 26.2$
Verapamil : $77.8 = 0.82*Cl_{human} + 0.18 * 120$
Midazolam : $88.9 = 0.816*Cl_{human} + 0.184 * 123$
Zidovudine : $61.04 = 0.778*Cl_{human} + 0.222 * 75.4$

Cl_{human} calculated from $Cl_{chimeric}$

Antipyrine : $36.3 = 0.782 * Cl_{human} + 0.218 * 26.2$
Verapamil : $77.8 = 0.82 * Cl_{human} + 0.18 * 120$
Midazolam : $88.9 = 0.816 * Cl_{human} + 0.184 * 123$
Zidovudine : $61.04 = 0.778 * Cl_{human} + 0.222 * 75.4$

Compd	Mice type	$Cl_{chimeric}$ mL/min/kg	RI	$Cl_{human,corrected}$ mL/min/kg
Antipyrine	PXB	36	78.2	**39**
	uPA$^{+/+}$/SCID	26		
Verapamil	PXB	78	82	**69**
	uPA$^{+/+}$/SCID	120		
Midazolam	PXB	89	81.6	**81**
	uPA$^{+/+}$/SCID	123		
Zidovudine	PXB	61	77.8	**57**
	uPA$^{+/+}$/SCID	75		

Compd	Cl_{human} reported mL/min/kg	fu reported
Antipyrine	4	0.9
Verapamil	15	0.1
Midazolam	7	0.05
Zidovudine	20	0.8

$Cl_{human,predicted,intrinsic}$

$Cl_{human,reported,intrinsic}$

Calculation of $Cl_{int,\ human}$ (predicted from chimeric mice)

$$Cl_{hep} = \frac{Q_h * Cl_{int}}{Q_h + Cl_{int}} \Longrightarrow Cl_{int} = \frac{Q_h * Cl_{hep}}{Q_h - Cl_{hep}}$$ no protein binding

$$Cl_{hep} = \frac{Q_h * f_u * Cl_{int}}{Q_h + f_u * Cl_{int}} \Longrightarrow Cl_{int} = \frac{Q_h * Cl_{hep}}{(Q_h - Cl_{hep}) * f_u}$$ with protein binding

Well- stirred

Cl_{hep} : Plasma Cl in chimeric mice
Q_h : Chimeric mice liver blood flow ~90 mL/min/kg
Cl_{int} : Predicted Intrinsic Cl in human

$$Cl_{hep} = Q_h * \left(1 - e^{-\frac{Cl_{int}}{Q_h}}\right) \Longrightarrow Cl_{int} = -Q_h * \ln\left(1 - \frac{Cl_{hep}}{Q_h}\right)$$ no protein binding

$$Cl_{hep} = Q_h * \left(1 - e^{-\frac{f_u * Cl_{int}}{Q_h}}\right) \Longrightarrow Cl_{int} = -\frac{Q_h}{f_u} * \ln\left(1 - \frac{Cl_{hep}}{Q_h}\right)$$ with protein binding

Parallel-tube

Cl_{hep} : Plasma Cl in chimeric mice
Q_h : Chimeric mice liver blood flow ~90 mL/min/kg
Cl_{int} : Predicted Intrinsic Cl in human

Calculation of $Cl_{int,\ human}$ (predicted from literature reported Cl_{human})

$$Cl_{hep} = \frac{Q_h * Cl_{int}}{Q_h + Cl_{int}} \Longrightarrow Cl_{int} = \frac{Q_h * Cl_{hep}}{Q_h - Cl_{hep}}$$ no protein binding

$$Cl_{hep} = \frac{Q_h * f_u * Cl_{int}}{Q_h + f_u * Cl_{int}} \Longrightarrow Cl_{int} = \frac{Q_h * Cl_{hep}}{(Q_h - Cl_{hep}) * f_u}$$ with protein binding

Well- stirred

Cl_{hep} : Plasma Cl in human (reported)
Q_h : Human liver blood flow ~20 mL/min/kg
Cl_{int} : Predicted Intrinsic Cl in human

$$Cl_{hep} = Q_h * \left(1 - e^{-\frac{Cl_{int}}{Q_h}}\right) \Longrightarrow Cl_{int} = -Q_h * \ln\left(1 - \frac{Cl_{hep}}{Q_h}\right)$$ no protein binding

$$Cl_{hep} = Q_h * \left(1 - e^{-\frac{f_u * Cl_{int}}{Q_h}}\right) \Longrightarrow Cl_{int} = -\frac{Q_h}{f_u} * \ln\left(1 - \frac{Cl_{hep}}{Q_h}\right)$$ with protein binding

Parallel-tube

Cl_{hep} : Plasma Cl in human (reported)
Q_h : Human liver blood flow ~20 mL/min/kg
Cl_{int} : Predicted Intrinsic Cl in human

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Predicted $Cl_{int,human}$ From Chimeric Mice

Well-Stirred model

Compound	*Without* fu Cl_{int}(Predicted from Chimeric) mL/min/kg	Cl_{int}(human reported) mL/min/kg
Antipyrine	69	5
Verapamil	296	60
Midazolam	810	11
Zidovudine	155	>1000
Compound	***With* fu** **Cl_{int}(Predicted from Chimeric)** **mL/min/kg**	**Cl_{int}(human reported)** **mL/min/kg**
Antipyrine	76	5
Verapamil	2957	600
Midazolam	16200	215
Zidovudine	194	>1000

In vivo Cl used for Cl_{int}

Compound	Cl_{human} predicted from Chimeric Mice mL/min/kg	Cl_{human} reported mL/min/kg
Antipyrine	39	4
Verapamil	69	15
Midazolam	81	7
Zidovudine	57	20

Cl_{int} of CYP3A4 substrates > reported
Cl_{int} of zidovudine < reported

Predicted $Cl_{int,human}$ from Chimeric Mice

Parallel-tube Model

Compound	*Without* fu Cl_{int}(Predicted from Chimeric) mL/min/kg	Cl_{int}(human reported) mL/min/kg
Antipyrine	51	4
Verapamil	131	28
Midazolam	207	9
Zidovudine	90	152

Compound	*With* fu Cl_{int}(Predicted from Chimeric) mL/min/kg	Cl_{int}(human reported) mL/min/kg
Antipyrine	57	4
Verapamil	1310	277
Midazolam	4145	172
Zidovudine	113	190

In vivo Cl used for Cl_{int}

Compound	Cl_{human} predicted from Chimeric Mice mL/min/kg	Cl_{human} reported mL/min/kg
Antipyrine	39	4
Verapamil	69	15
Midazolam	81	7
Zidovudine	57	20

Cl_{int} of CYP3A4 substrates > reported
Cl_{int} of zidovudine ~ 2-fold reported

Does Cl_{Blood} Improve Prediction?

Compound	Literature Reported				Observed	
	f_u	R_b	Cl_{human} mL/min/kg	$Cl_{int,human}$ mL/min/kg	$Cl_{PXB\ Mouse}$ mL/min/kg	$Cl_{int,PXB\ mouse}$ mL/min/kg
Antipyrine	0.96	1.4	4	**5**	39	**59**
Verapamil	0.1	0.77	15	**2073**	69	**159390**
Midazolam	0.05	0.55	7	**355**	81	**NA**
Zidovudine	0.8	0.86	18	**6773**	57	**270**

Well-Stirred Model

$$Cl_{int} = \frac{Q_h * (f_u/R_b) * Cl}{Q_h - (f_u/R_b) * Cl}$$

Cl : Plasma Cl in human or PXB mice

Q_h : Human liver blood flow ~20 mL/min/kg

: PXB liver blood flow ~90 mL/min/kg

Cl_{int} : Predicted Intrinsic Cl in human or PXB

f_u : Fraction unbound in plasma

R_b : Red blood cell partitioning

Cl_{int} of CYP3A4 substrates >> reported

Cl_{int} of zidovudine << reported

Does Cl_{Blood} Improve Prediction?

Compound	Literature Reported				Observed	
	f_u	R_b	Cl_{human} mL/min/kg	$Cl_{int,human}$ mL/min/kg	$Cl_{PXB\ Mouse}$ mL/min/kg	$Cl_{int,PXB\ mouse}$ mL/min/kg
Antipyrine	0.9	1.4	4	**3**	39	**41**
Verapamil	0.1	0.77	15	**360**	69	**1701**
Midazolam	0.05	0.55	7	**313**	81	**7536**
Zidovudine	0.8	0.86	18	**221**	57	**131**

Parallel-Tube Model

$$Cl_{int} = -\frac{Q_h}{f_u/R_b} * \ln\left(1-\frac{Cl}{Q_h}\right)$$

Cl : Plasma Cl in human or PXB mice

Q_h : Human liver blood flow ~20 mL/min/kg

: PXB liver blood flow ~90 mL/min/kg

Cl_{int} : Predicted Intrinsic Cl in human or PXB

f_u : Fraction unbound in plasma

Rb : Red blood cell partitioning

Cl_{int} of CYP3A4 substrates >> reported

Cl_{int} of zidovudine ~ 2-fold reported

Cl_{human} Calculated From $Cl_{chimeric}$

Due to regenerative pressure, possible that upA transgene is deleted-
Is SCID mice better control ??

Cl_{total} seen in PXB mice is coming from :

% human hepatocytes re-populated in host mouse liver + residual SCID mouse hepatocytes

$$Cl_{Chimeric} = RI * Cl_{human} + (1\text{-}RI) * (Cl_{SCID})$$

$Cl_{Chimeric}$ = Clearance in chimeric mice with human liver
RI = Replacement Index (measure of contribution of human iver
Cl_{SCID} = Clearance in SCID mice
Cl_{human}= Predicted Clearance in human liver

Antipyrine: **$36.3 = 0.78 * Cl_{human} + 0.22 * 11.4$**
Verapamil: **$77.8 = 0.82 * Cl_{human} + 0.18 * 73.1$**
Midazolam: **$88.9 = 0.82 * Cl_{human} + 0.18 * 100.7$**
Zidovudine: **$61.04 = 0.78 * Cl_{human} + 0.22 * 90.9$**

Cl_{human} calculated from $Cl_{chimeric}$

Antipyrine: **36.3 = 0.78 * Cl_{human} + 0.22 * 11.4**
Verapamil: **77.8 = 0.82 * Cl_{human} + 0.18 * 73.1**
Midazolam: **88.9 = 0.82 * Cl_{human} + 0.18 * 100.7**
Zidovudine: **61.04 = 0.78 * Cl_{human} + 0.22 * 90.9**

Compd	Mice type	$Cl_{chimeric}$ mL/min/kg	RI	$Cl_{human,corrected}$ mL/min/kg
Antipyrine	PXB SCID	36 11	78.2	**43**
Verapamil	PXB SCID	78 73	82	**79**
Midazolam	PXB SCID	89 101	81.6	**86**
Zidovudine	PXB SCID	61 91	77.8	**51**

$Cl_{human,predicted,intrinsic}$

Compd	Cl_{human} reported mL/min/kg	fu reported
Antipyrine	4	0.9
Verapamil	15	0.1
Midazolam	7	0.05
Zidovudine	20	0.8

$Cl_{human,reported,intrinsic}$

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Predicted $Cl_{int,human}$ from Chimeric Mice

Is SCID mice better control ??

Compound	*Without* fu Well-Sirred		*With* fu Well-Sirred	
	Cl_{int}(Predicted from Chimeric) mL/min/kg	Cl_{int}(human reported) mL/min/kg	Cl_{int}(Predicted from Chimeric) mL/min/kg	Cl_{int}(human reported) mL/min/kg
Antipyrine	82	5	91	5
Verapamil	633	60	6464	600
Midazolam	1935	11	>10000	215
Zidovudine	118	>1000	147	>1000
Compound	***Without* fu Parallel-Tube**		***With* fu Parallel-Tube**	
	Cl_{int}(Predicted from Chimeric) mL/min/kg	Cl_{int}(human reported) mL/min/kg	Cl_{int}(Predicted from Chimeric) mL/min/kg	Cl_{int}(human reported) mL/min/kg
Antipyrine	58	4	65	4
Verapamil	188	28	1876	277
Midazolam	280	9	5604	172
Zidovudine	75	152	94	190

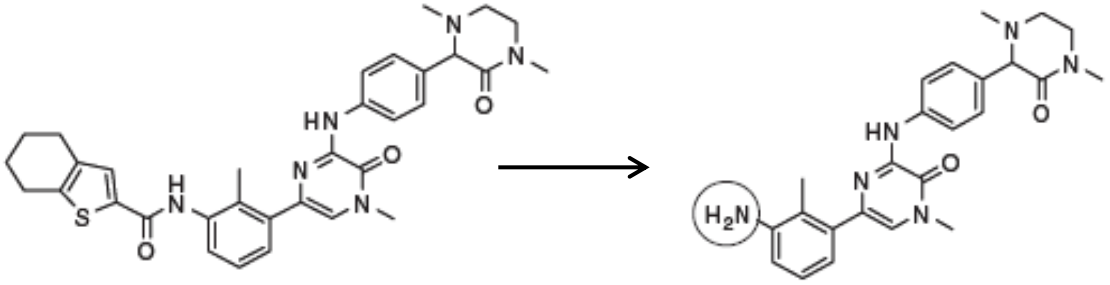
Not much different

Cl_{int} of CYP3A4 substrates >> reported
Cl_{int} of zidovudine ~ 2-fold reported

Conclusions

- CYP 3A4 substrates Cl_{int} were all significantly higher than reported
- Zidovudine Cl_{int} was predicted to be lower than reported
- Rank order of compounds was also not maintained as reported in humans
- uPA$^{+/+}$/SCID mice (liver disease + immuno-compromised) appears to highly metabolize all 3A4 substrates
- Comparison in chimeric mice from separate donor hepatocytes needs to be explored

Success of Cl Prediction with PXB Mice



GDC-0834
Potent & Selective BTK inhibitor evaluated for RA

Major human metabolite via Amide hydrolysis is inactive

Shows low Cl in all preclinical species (low metabolite formation) but high Cl in human due to extensive amide hydrolysis

Can PXB mice predict human Cl ?

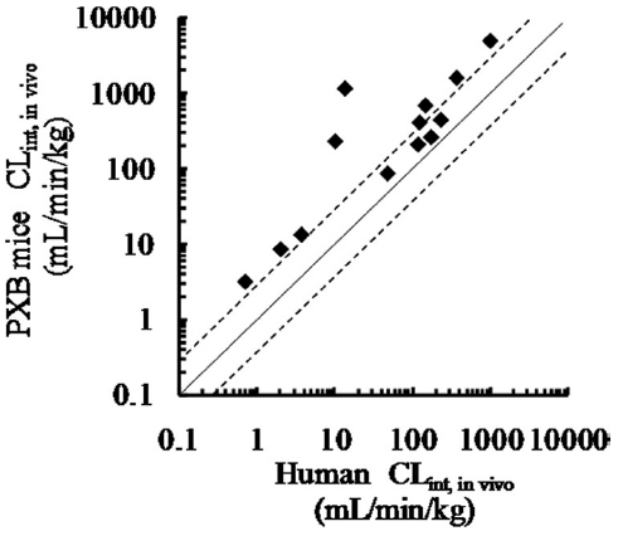
- PXB mice forms much higher levels of amide hydrolysis metabolite than SCID
- Quantitatively, significant difference in Cl between PXB mice (low Cl) vs human (high Cl)
- Cl in PXB mice was very similar to Cl in SCID mice

Quantitative prediction of human Cl not successful

Liu et.al, Drug Metab Dispos, 2011, 39; 1840-1849

Success of Cl Prediction with PXB Mice

Compound	f_u	R_b	$Cl_{human,reported}$ mL/min/kg	$Cl_{int,human}$ mL/min/kg	$Cl_{PXB,observed}$ mL/min/kg	$Cl_{int,PXB}$ mL/min/kg
S-Warfarin	0.015	0.55	0.055	**3.7**	0.2	**13.4**
S-Naproxen	0.01	0.55	0.1	**10.1**	2.2	**230.2**
Lamotrigine*	0.45	1	0.3	**0.7**	1.4	**3.2**
Dapsone	0.25	1.04	0.48	**2**	2.1	**8.6**
Ibuprofen	0.006	0.55	0.82	**147.1**	3.8	**686**
Ketoprofen	0.008	0.55	1.6	**232.2**	3.3	**442**
Sulindac*	0.069	1	3.3	**47.8**	5.6	**86.5**
Diclofenac	0.005	0.55	3.5	**1004.3**	16.4	**4905.1**
Salbutamol	0.925	0.96	7.7	**13.5**	79.9	**1148.2**
Mirtazapine	0.15	0.67	8	**123.6**	30.4	**408.7**
Zaleplon	0.4	0.99	16	**173.6**	48.1	**261.3**
Fasudil	0.51	1	18	**370.6**	81	**1588.2**
6-Deoxypenciclovir*	1	1.2	118	**118**	71.2	**209**



- 13 Investigative Drugs
- Used Well-Stirred Model with Blood Clearance
- 4 showed Cl_{int} within **3-fold**, 7 showed Cl_{int} within **3-10 fold**, 2 showed Cl_{int} **23-85 fold**
- Cl in host mice not reported

  Cl in host mice vs Cl in PXB mice comparison is important !
- ***Qualitative*** but ***not Absolute*** Rank Ordering observed

Sanoh et.al, Drug Metab Dispos, 2012, 40; 322-328

Future Considerations

- Quantitative $Cl_{int,human}$ prediction using Chimeric mice with humanized liver remains to be a challenge

- Expression of human Ph I and II DME and transporters has been confirmed
  - expression level between chimeric mice and donor not clear

- Contribution of the “leftover” 20-30% m-hepatocytes confounds data interpretation
  - prior knowledge of drug metabolism pathways necessary

- Chimeric mice contains hepatocytes from 1 individual donor
  - reflective of population??

- Chimeric liver under m-endocrine system- h-hepatocytes need GH for homeostasis

- Chimeric mice consists of h-hepatocytes in an environment of m-cells –is functional integrity of human liver maintained ?

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PhoenixBio for kind donation of microsomes

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