Liver Humanized Chimeric PXB-Mouse® as a Model to Predict Human Renal Toxicity by Aldehyde Oxidase Dependent Metabolism of SGX523

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Introduction

Aldehyde oxidase (AO) is a cytosolic molybdenum-containing hydroxylase that oxidizes a variety of azaheterocycles and has gained the attention of drug discovery programs due to profound species differences in activity and challenges predicting clearance. In addition, metabolites generated by AO are poorly predicted from preclinical studies in rodents and canines, complicating safety assessment programs. SGX523, a selective drug candidate for MET receptor tyrosine kinase, was halted in the clinic due to acute renal failure.[1] The observed clinical findings were unexpected due to SGX523 demonstrating an acceptable safety profile in preclinical toxicology studies in rat and dog. Metabolite profiling of plasma from human patients identified a key AO-mediated circulating metabolite (M11), which was not observed in rats and dogs. Retrospective toxicology studies in monkey (a species with high AO-activity) provided evidence of intratubular crystals of M11 and associated inflammatory changes in kidney tissue, consistent with crystallization of this poorly soluble metabolite in the kidney.[2] As an alternative to utilizing non-human primates, the humanized PXB-mouse® (Phoenix Bio) has been developed as a predictive model of human metabolism. In effort to assess the suitability of this humanized mouse model for recreating the renal toxicity observed by SGX523, PXB-mice were exposed to SGX523 by single or repeat oral dose daily for up to seven days at 100 mg/kg, followed by analyses of blood chemistry, renal histopathology, and SGX523 and M11 exposure. In addition to measuring effects on serum creatinine (CREAT) and blood urea nitrogen (BUN) as markers of kidney injury, we have included urinary kidney injury molecule-1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL) as more sensitive markers of tubular injury. Upon dosing PXB-mice with SGX-523, elevated levels of serum creatinine, BUN, urinary KIM-1 and NGAL were observed with increasing dose. This correlated with high M11 metabolite/SGX523 ratios in the urine and kidney tissue, in addition to higher circulating exposure of M11. Microscopically, obstructive/retrograde nephropathy was characterized by renal tubular dilatation in a linear distribution and multifocal birefringent amorphous crystals in tubules. This work showcases the utility of KIM-1 and NGAL for early risk assessment of kidney injury for drug candidates, in addition to the utility of a humanized liver mouse model to assess safety of specific human metabolites as an alternative to standard preclinical toxicology species, such as non-human primates.

Figure 1. SGX523 Metabolism by Aldehyde Oxidase

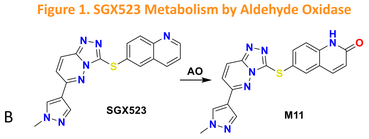


Table 2. Representative Hepatic AO Expression by Species

Species	Level of AO Activity*
Human	+++
Monkey	+++
Dog	-
Rat	+
Mouse	+
PXB Mouse	*+++#*

*Basit et al. DMD.2022, 50,197-203

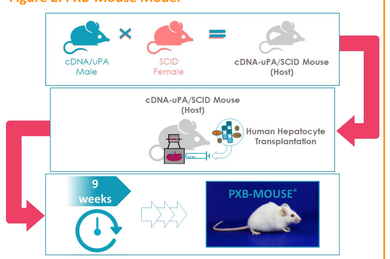
#Implied categorization, AO expression not determined

Table 1. Predicted Phys Chem Properties of SGX523 and M11

Property	SGX523	M11
Chemical Formula	C18H13N7S	C18H13N7OS
MW	359.41	375.41
LogP	3.0/2.74	2.7/1.96
Quinoline pKa	4.2	**N/A**
TPSA	73.81	93.78
Solubility (µg/mL, in water)	2.5-5.4	**0.13-0.56**

Refs: https://www.simulations-plus.com/resource/silicomodeling-can-predict-unforeseen-renal-failure-caused-sgx523-c-met-kinase-inhibitor/; https://www.molinspiration.com/cgi/properties ; and Diamond, S., et al, Drug Metab. And Disp., 2010, vol38 (8), p1277

Figure 2. PXB-Mouse Model



Methods

In Life Study Design: Dose range finding (DRF) studies were performed at 10, 30, and 60 mg/kg/day with humanized liver PXB-mice (KMT Hepatech – part of the PhoenixBio Group, 28-46 weeks of age) to determine the optimal dosing regimen to produce moderate toxicity with renal findings. Once determined, eight male PXB-mice (19-22 weeks of age, replacement indices = 82-100%) and eight male CB17/Icr-Prkdcscid/IcrIcoCrl (Charles River Laboratories, 19-21 weeks of age) were administered 100 mg/kg SGX-523 once daily for 7 days via oral gavage. Body weights and clinical observations were recorded daily; gross findings were documented at the time of sacrifice on Day 8.

Pharmacokinetic analysis: Blood, urine, and kidney tissues were collected according to Table XXX. Blood was processed to plasma. Kidney was homogenized in 70% IPA (1:3 tissue:solvent ratio by volume). The resulting suspension were extracted with ACN containing internal standard and supernatants analyzed via LC-HRMS with a Sciex 7600 Zeno-Tof Mass Spectrometer. Bioanalysis was performed on SGX523 and M11 (AO Metabolite). The data was additionally analyzed for other major metabolites (MS and MSMS).

Metabolite Analysis: Samples prepared for bioanalysis of high dose groups were reanalyzed to determine the metabolite profile of select samples. Samples were analyzed analyzed via LC-HRMS with a Thermo Exploris Orbitram 120 Mass Spectrometer. Data was searched manually for known metabolites and processed via MassMetasite software.

Clinical Pathology and Urinary Biomarkers: Whole blood (processed to serum) and urine samples were collected prior to dose administration for baseline data. Serum samples were collected at necropsy on Day 8 approximately 24 hours following the Day 7 dose administration for blood urea nitrogen (BUN) and creatinine analysis performed using a Randox Daytona instrument clinical chemistry analyzer. Urine was collected overnight on Day 7 for BUN, creatinine, NGAL, and KIM-1 analysis. Concentrations of KIM-1 and NGAL were determined using commercially available ELISA kits (R&D Systems: NGAL cat# MLCN20, KIM-1 cat# MKM100) analyzed on a SpectraMax384 plate reader. Human (PXB-mice) and mouse albumin (SCID-mice) were measured prior to initiation and following completion of dosing to monitor the degree of humanization and whether SGX523 treatment impacted the health of the human hepatocytes (human albumin was stable during study, *data not shown here*).

Necropsy and Pathology At a limited necropsy, liver and kidneys were examined, and gross abnormalities were recorded. Right kidneys were fixed in 10% neutral buffered formalin, trimmed, processed routinely, embedded in paraffin, sectioned and stained with hematoxylin and eosin (HE). Left kidneys were embedded in optimal cutting temperature (OCT compound), frozen in liquid nitrogen and stored at -80°C. Following sectioning, frozen sections were stained with HE. Liver observations not reported here.

Table 3. In Life Study Design

Study Phase	Animals	Dose Level (mg/kg)	Days	Sample Collection	Time Collections (Hours Postdose)		
					Plasma	Urine	Kidney
1	**PXB**-Mice	10	1	Day 1	0.5, 1, 2, 4, 8	24	N/A
2	**PXB**-Mice	30	4	Day 1 & 4	0.5, 2, 8	24	Terminal
3	**PXB**-Mice	60	4	Day 4	0.5, 2, 8, 24	24	Terminal
4	**PXB**-Mice	100	7	Day 7	1, 4, 8, 24	24	Terminal
	SCID-Mice	100	7	Day 7	1, 4, 8, 24	24	Terminal

Results

Table 4. Concentration of SGX523 and M11-AO Metabolite in Plasma, Urine, and Kidney of PXB-Mice and SCID Mice.

Analyte	Dose Level (mg/kg/day)	Strain	Day	Plasma AUC_{0-8}	Plasma AUC_{0-24}	Urine (24 hr)	Kidney (24 hr)
				(hr*ng/mL)	(hr*ng/mL)	ng/mL	ng/g
SGX523	10	PXB	1	9510 ± 2760	N/A	23.2 ± 26.8	N/A
	30	PXB	1	32100 ± 5960	N/A	94.7 ± 45.4	N/A
	30	PXB	4	34000 ± 12100	N/A	343 ± 339	770 ± 550
	60	PXB	4	52200 ± 20300	93200 ± 45400	569 ± 465	2550 ± 1160
	100	PXB	7	49800 ± 17600	64900 ± 22600	370 ± 287	1560 ± 1350
	100	*SCID*	7	27100 ± 6450	30500 ± 8420	18.3 ± 28.4	BLQ
M11 (AO)*	10	PXB	1	991 ± 96	N/A	1660 ± 1090	N/A
	30	PXB	1	2410 ± 448	N/A	4560 ± 2690	N/A
	30	PXB	4	2420 ± 480	N/A	6350 ± 5460	52700 ± 50300
	60	PXB	4	3320 ± 1510	3320 ± 1510	5040 ± 5260	71100 ± 45700
	100	PXB	7	2000 ± 669	2900 ± 799	958 ± 473	54000 ± 75800
	100	*SCID*	7	497 ± 160	534 ± 186	372 ± 234	BLQ

*Quantitation of M11 AO Metabolite was performed using SGX523 standard curve (LC-MS ionization efficiency assumed to be similar). Identity and retention time of M11 was confirmed via generation of the AO metabolite via in vitro incubation of SGX523 in cynomolgus liver S9. Quantitation of M11 in samples likely underestimates total metabolite concentrations in plasma, urine, and kidney.

Figure 3. Day 7 Plasma Concentration Over Time of SGX523 and M11 AO Metabolite in PXB-mice and SCID-mice dosed at 100mg/kg/day for 7 days

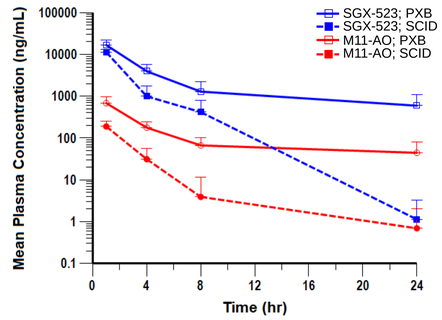


Figure 5. Fold Change in Renal Injury Markers at SGX-523 at 100 mg/kg for 7 Days PXB vs SCID

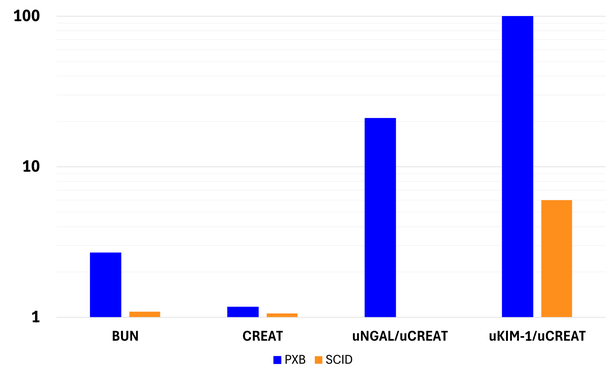
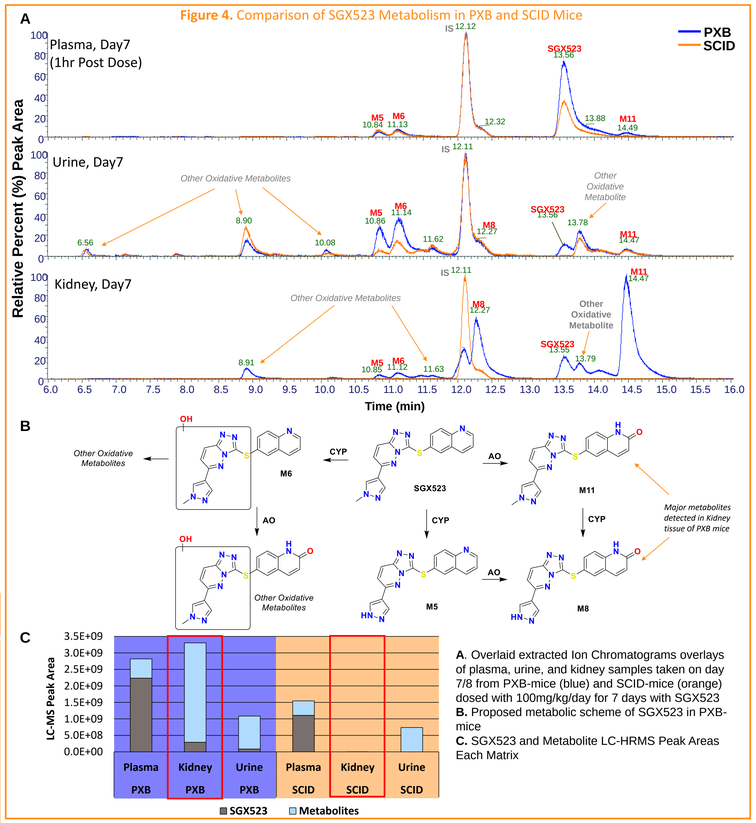


Figure 4. Comparison of SGX523 Metabolism in PXB and SCID Mice

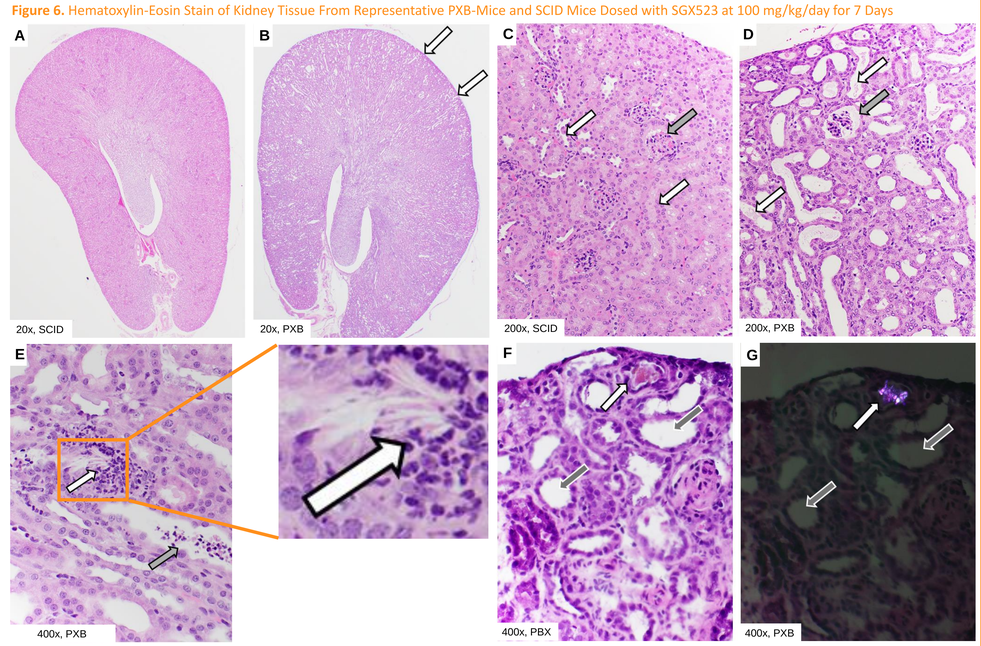


A. Overlaid extracted Ion Chromatograms overlays of plasma, urine, and kidney samples taken on day 7/8 from PXB-mice (blue) and SCID-mice (orange) dosed with 100mg/kg/day for 7 days with SGX523

B. Proposed metabolic scheme of SGX523 in PXB-mice

C. SGX523 and Metabolite LC-HRMS Peak Areas Each Matrix

Figure 6. Hematoxylin-Eosin Stain of Kidney Tissue From Representative PXB-Mice and SCID Mice Dosed with SGX523 at 100 mg/kg/day for 7 Days



A.SCID mouse 5M002 ("normal" kidney): Formalin Fixed Paraffin-Embedded (FFPE), 20x

B.PXB-mouse 4M003: Tubular dilatation in a linear pattern (white arrows). FFPE, 20x

C.SCID mouse 5M002: normal tubules (white arrows) and normal glomerulus (grey arrow). FFPE, 200x.

D.PXB-mouse 4M003: dilated tubules containing wispy eosinophilic material (white arrows). Distended glomerulus the urinary space containing wispy eosinophilic material (grey arrow). FFPE, 200x.

E.PXB-mouse 4M002: Left: The "ghost" of needle-shaped crystals surrounded by a mixed inflammatory infiltrate containing neutrophils (white arrow). A dilated tubule contains a cast of cellular debris and inflammatory cells (grey arrow). FFPE, original magnification 400x. Right: Enlarged image.

F.PXB-mouse 4M004: A tubule contains amorphous yellow-brown crystals (white arrow). Surrounding tubules are empty and dilated (grey arrow). Frozen section, 400x.

G.PXB-mouse 4M004: A tubule contains amorphous birefringent crystals (white arrow) whereas surrounding tubules are empty and dilated (grey arrow). Frozen section, polarized, 400x

Summary of Conclusions

- Repeat PO gavage dosing with 100 mg/kg SGX523 to PXB and SCID was well-tolerated in SCID mice but was not well-tolerated in PXB mice based on:
  - The early mortality of two (of 8) PXB mice on Day 7; all SCID mice survived until the scheduled sacrifice.
  - Marked weight loss (20%) in PXB mice with maintenance (2% gain) of body weight in SCID mice
  - Observations of unkempt appearance in some PXB mice
- The level of AO metabolism in the PXB-mouse was more representative of human metabolism than the control (SCID) mice
- Observed dose proportional increases in plasma and renal exposure of SGX523 and M11 (and other metabolites) in the PXB-mice
- In PXB mice, microscopic findings of tubular dilatation, casts, inflammatory infiltrates and tubular basophilia (not shown) were observed and correlated to increases in serum BUN, CREAT, and urinary NGAL and KIM-1.
- Intralesional crystals in sections examined were consistent with high renal concentrations of M11.
- These observations were collectively indicative of renal injury and impaired renal function in PXB mice dosed with SGX-523, not observed in similarly dosed SCID-mice.
- Comparison of *in vivo* metabolites reported with *in vitro* incubations using liver homogenates of collected tissues from unused PXB-mice and SCID-mice planned (not dosed).

References

1. Infante, J.R. *Invest New Drugs* (2013) 31:363–369
2. Lolkema, M.P. et al. *Clin Cancer Res*. 2015 May 15; 21(10): 2297–2304
3. Basit et al. *Drug. Metab. & Disp.*2022, 50,197-203
4. Diamond, S., et al, *Drug Metab. And Disp*., 2010, vol38 (8), p1277
5. Physcial chemical properties calculated from:https://www.simulations-plus.com/resource/silicomodeling-can-predict-unforeseen-renal-failure-caused-sgx523-c-met-kinase-inhibitor/ and https://www.molinspiration.com/cgi/properties

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