Utility of a Humanized Liver Chimeric Mouse Model to Investigate Acute Obstructive Nephropathy by an Insoluble Metabolite

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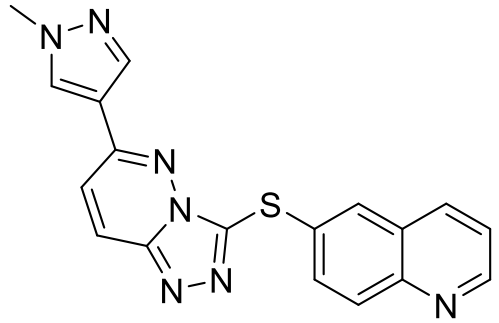
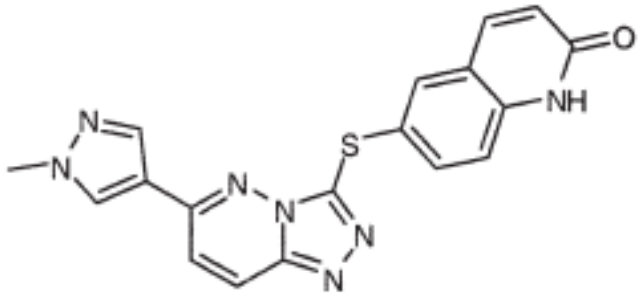
DVDMDG 2026

SGX523 – A Promising Anti-Cancer Kinase Inhibitor

- SGX523 targets mesenchymal-epithelial transition factor (c-MET), a receptor tyrosine **kinase,** implicated in tumor growth
- Microsomal metabolite profile of SGX523 was similar in rat, dog, monkey, and human, and thus, toxicology studies were conducted in rat and dog ------> **SAFETY DATA SUPPORTED MOVING FORWARD**
- In all 6 patients receiving doses ≥80 mg of SGX523, **acute renal failure** observed (Not observed in rat/dog tox studies)
- Retrospective survey of human plasma uncovered **M11**, identified as 2-quinolinone-SGX523; not observed *in vitro* or in rat and dog tox studies



SGX523



M11, m/z 376 (2-quinolinone-SGX523)

Diamond et al. (2010) *Drug Metab Dispos*. 38; 1277-85

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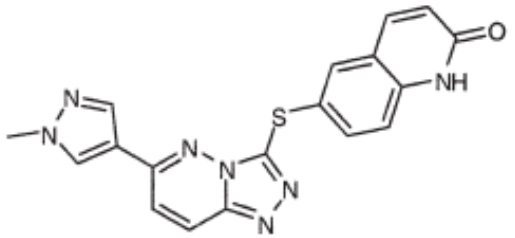
SGX523 Retrospective *In Vitro* Analysis

- Retrospective *in vitro* Metabolite ID in S9 fraction showed **M11** formed by Cyno monkey
- **M11** levels in urine were 70-fold greater than SGX523 and **solubility** was found to be ~3% of SGX523 in urine
- Observed toxicity thus attributed to accumulation and crystallization of poorly soluble metabolite **M11** in kidney



M11, m/z 376 (2-quinolinone-SGX523)

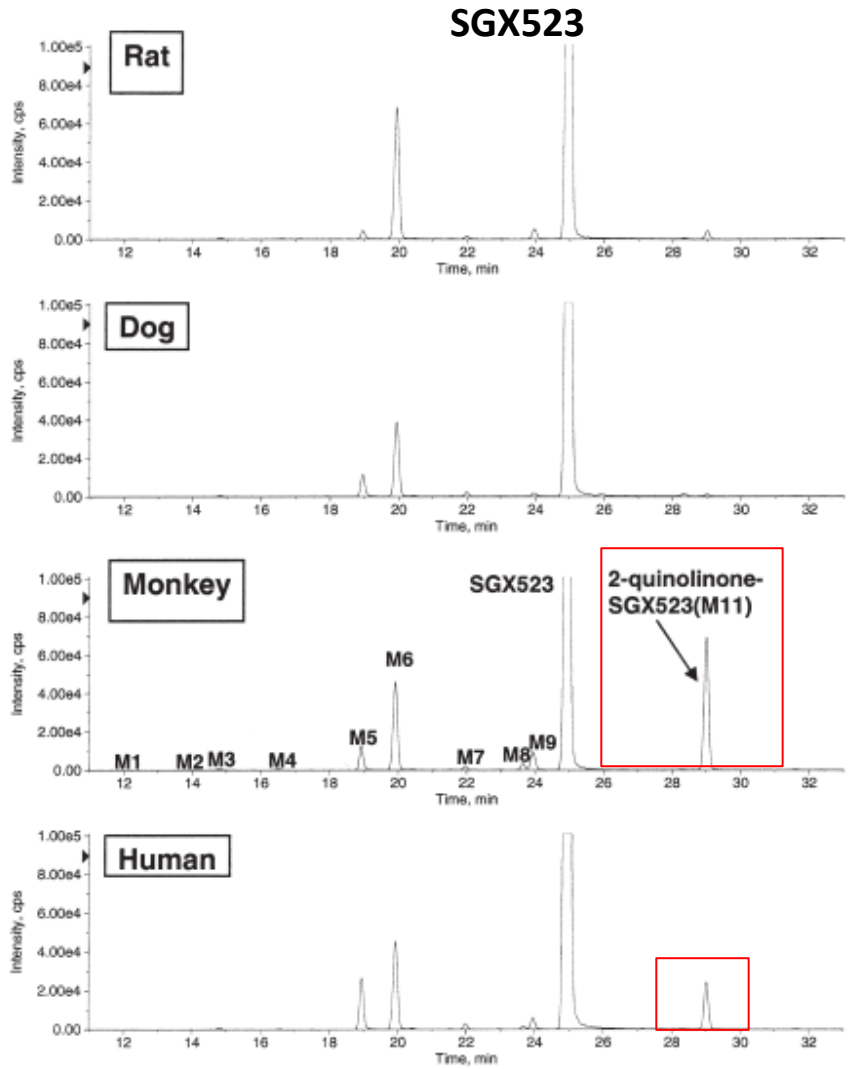


FIG. 1. The in vitro metabolism of SGX523 by hepatic S-9 fractions from rats, dogs, monkeys, and humans in the presence of NADPH. SGX523 (5 μM) was incubated at 37°C with S-9 fractions from various species (1 mg of protein/ml) and potassium phosphate buffer (50 mM, pH 7.4) with NADPH (1.0 mM). Incubations were terminated with organic, centrifuged, and the resulting supernatants analyzed by LC/MS.

Diamond et al. (2010) *Drug Metab Dispos*. 38; 1277-85

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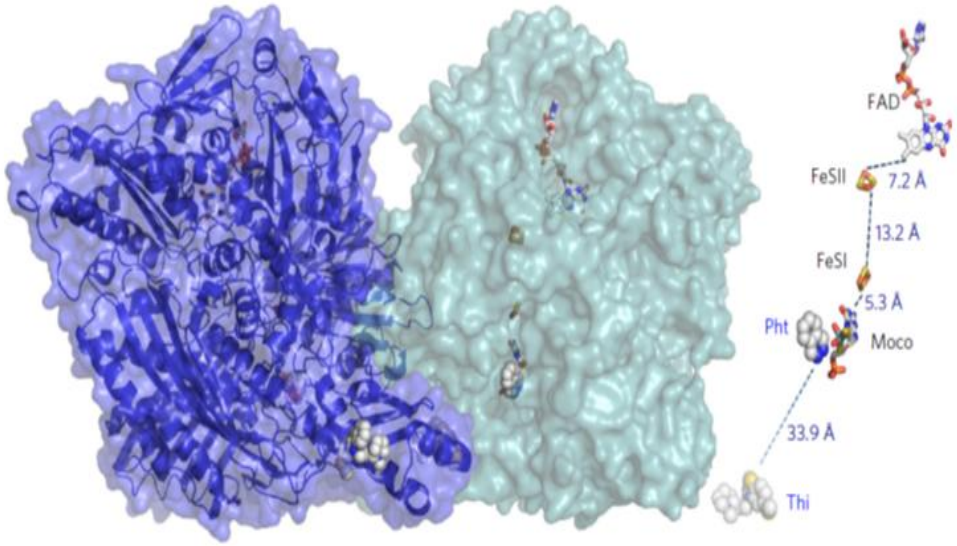
Aldehyde Oxidase (AO)

- Aldehyde oxidase (AO) is a **cytosolic** enzyme that belongs to the family of structurally related molybdoflavoproteins
- Predominantly expressed in liver, lung and kidney
- Characterized by **broad substrate specificity**
- Several reviews on the role of AO in drug metabolism have been published in the past ~15 years indicative of its **influence in metabolism**
- Impact on drug development has been severe in some cases
- **Marked species difference in expression of AO makes it is difficult to predict PK properties in human with high confidence**



Active as homodimer ~ 150 kDa
Coelho et al. (2015) *Nat Chem Biol* 11(10); 779-83

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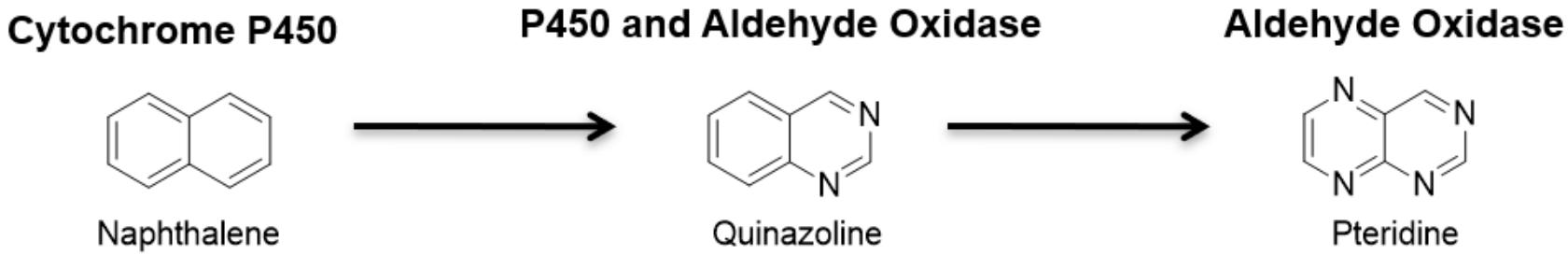
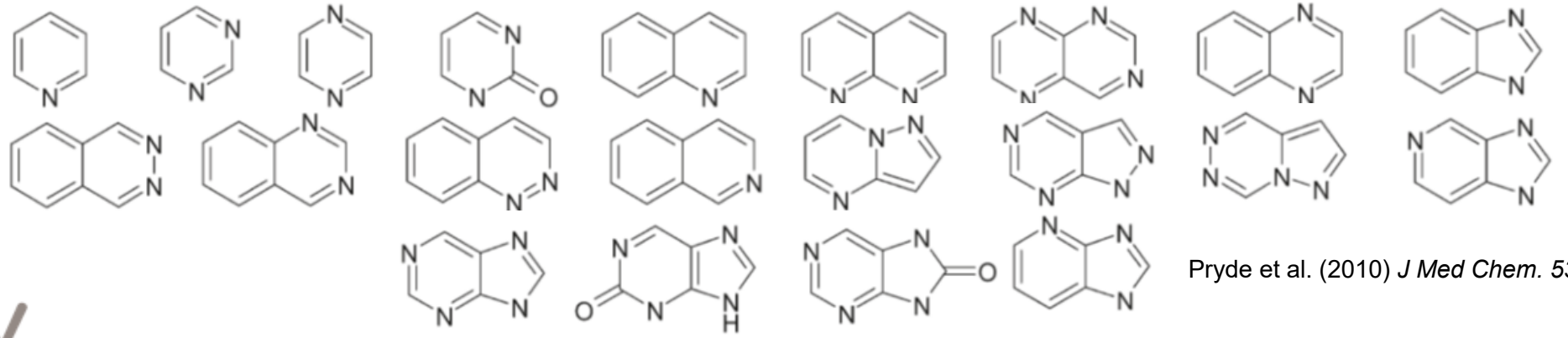
Avoiding P450 Metabolism Presents New Challenges

- As medicinal chemists introduce nitrogens into aromatic ring systems to circumvent P450-mediated metabolism or optimize pharmacological target potency (**e.g. kinase targets**), the potential for AO-mediated oxidation increases



Common substructures susceptible to AO-mediated metabolism



Pryde et al. (2010) *J Med Chem. 53*; 8441-8460

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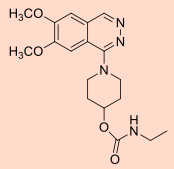
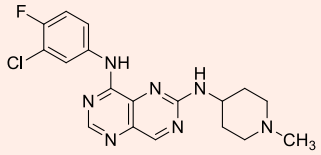
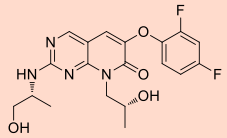
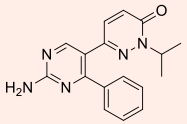
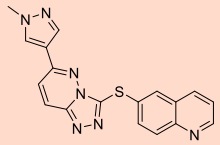
Clinical Implications of AO Metabolism

What About Safety?

Drug	Structure	Preclinical DMPK Data	Clinical Outcome	Year
Carbazeran		Dog PK (62-74% F)	<5% F	1984
BIBX1382		Liver Microsomes (stable) mouse, rat and dog PK (50-100% F)	5% F	2002
RO1		Rat PK (6.2 hr half-life) Dog and Monkey PK (4 hr half-life)	0.7 hr half-life *(6 hrs projected by allometric scaling)	2010
FK3453		Liver Microsomes (stable) Rat PK (31-55% F) Dog PK (71-93% F)	Low oral exposure	2011
SGX523		**Tox species (Rat and Dog)**	**Acute Renal Toxicity in Human**	**2010**

- Notice any common themes in the species selected?

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AOX1 and Species Differences

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.

- AO expression in animal species varies considerably, from no active gene (dog) to expression of one (human and some primates) up to 4 isoenzymes (rodents)
- Results of drug disposition studies in rat/dog should not be extrapolated to humans
- Cyno Monkey potentially good surrogate model for human

Species	Sequence Similarity
Human	100%
Chimpanzee	99%
Rhesus Monkey	96%
Cyno Monkey	96%

UniProtKB Website

Garattini and Terao (2012) *Expert Opin Drug Metab Toxicol* 8(4); 487-503

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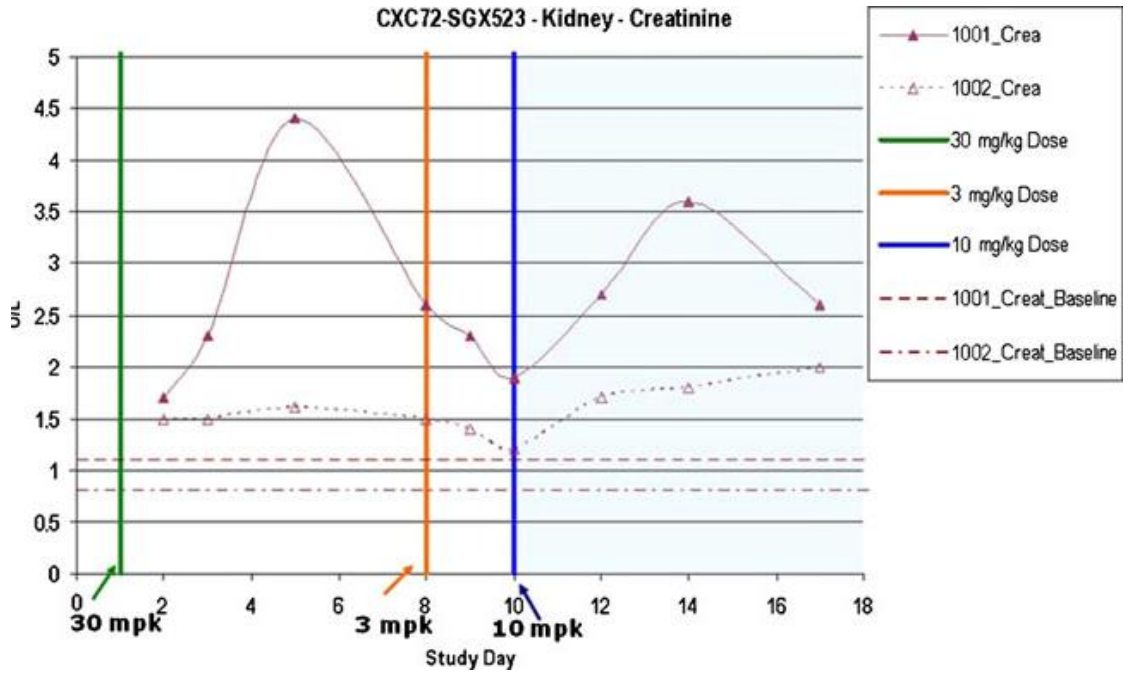
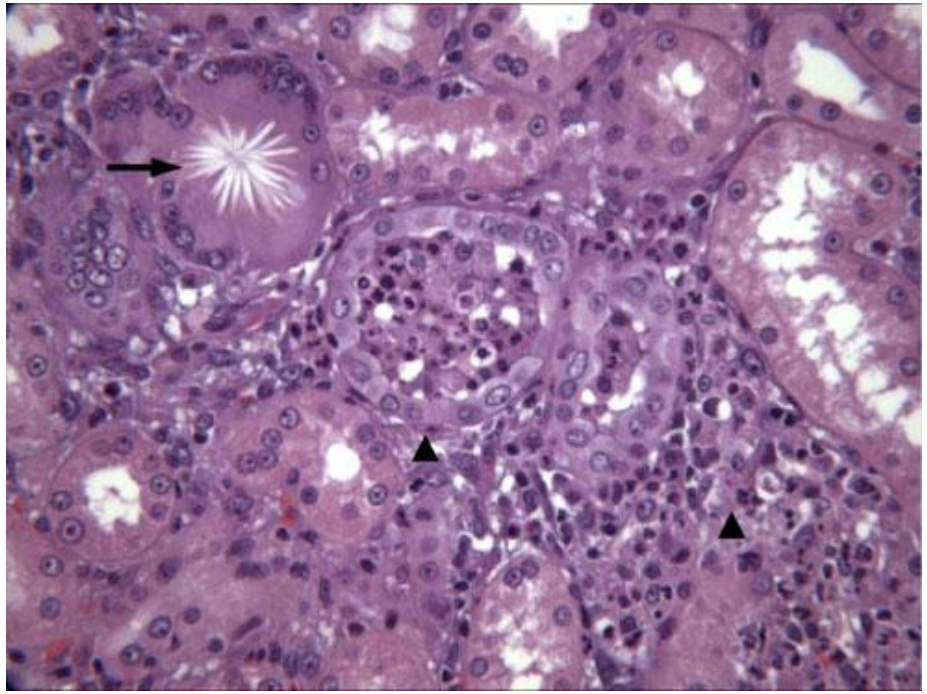
SGX523 – Retrospective Study in Monkey





→ = crystalline structure within a multinucleated giant cell

▲ = tubulointerstitial nephritis

- Renal effects of SGX523 reproduced in cynomolgus monkeys

Infante et al. (2013) *Invest New Drugs*. 31; 363-369

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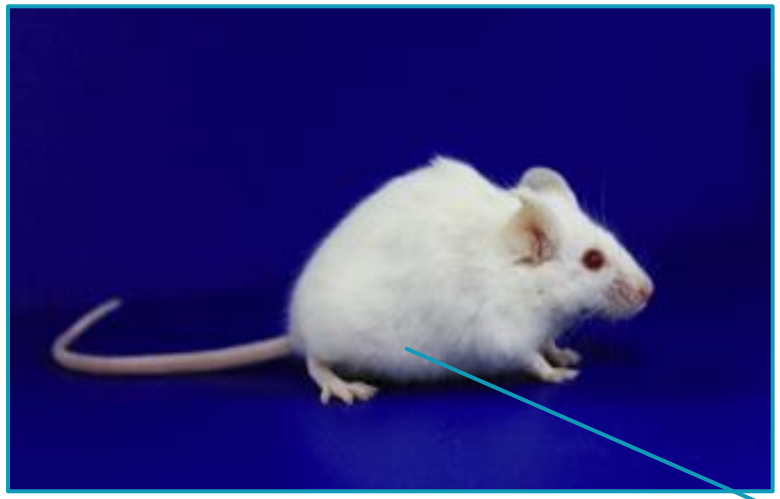
What is a Chimeric Liver-Humanized PXB-Mouse® ?



PhoenixBio

Key Features:

- Up to 95% Human hepatocyte engraftment
- Histologically normal liver constitution
- Human specific metabolism and excretion pathways
- Expression of human genes, mRNA and proteins

cDNA-uPA/SCID
Liver weight: 0.7 – 1 g

Transplantation

PXB-mouse®
Liver weight: 2 – 2.5 g (RI: 98 %)

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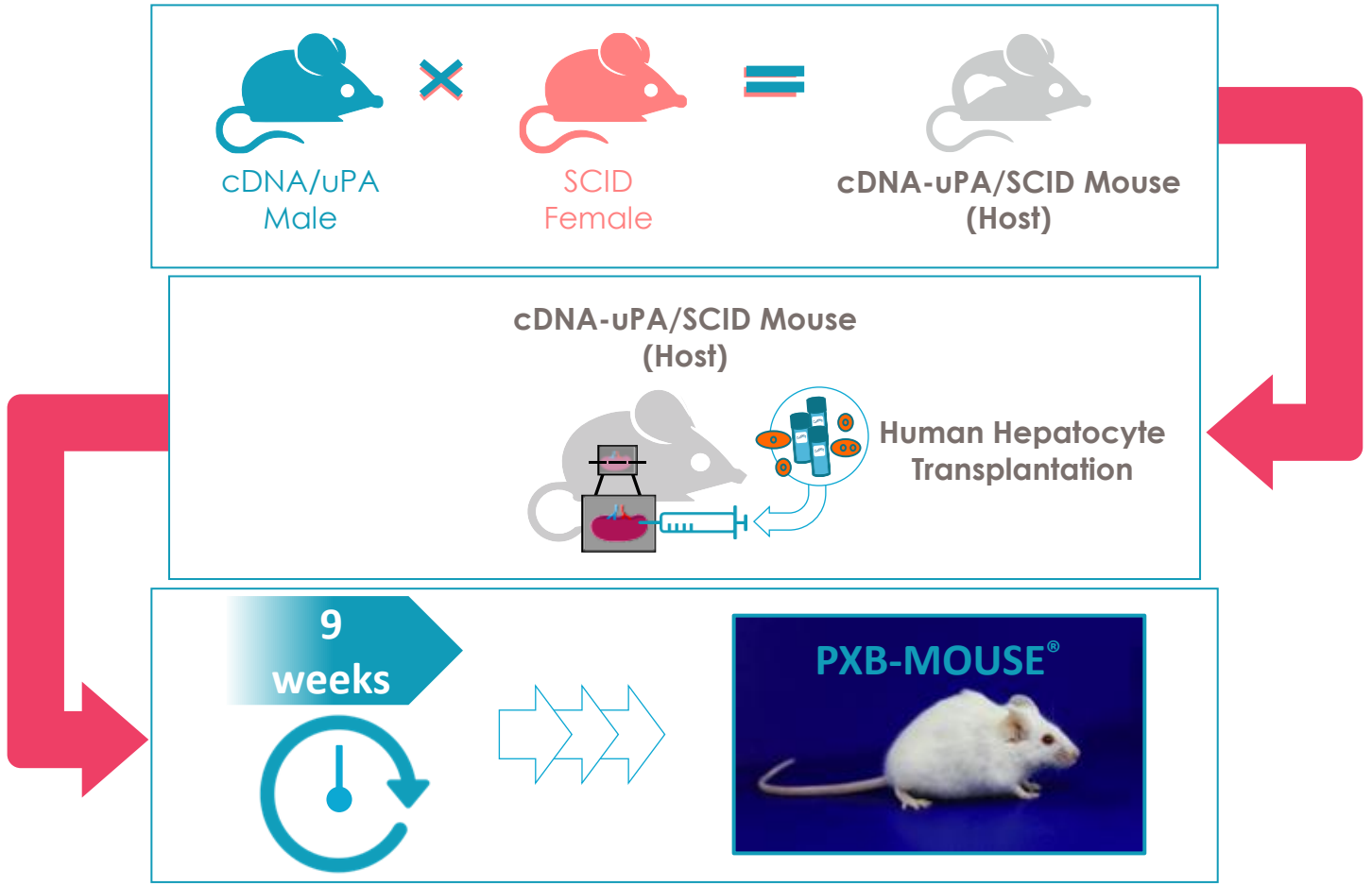
How to Produce a PXB-Mouse®

1. Breeding of Host Animals
   - > cDNA-uPA$^{+/-}$/SCID mice (urokinase-type plasminogen activator cDNA/severe combined immunodeficiency mice)
2. Transplantation of Human Hepatocytes
   - > Using commercially available cryopreserved hepatocytes
   - > Inject 10^5 to 10^6 cells per mouse into the spleen
   - > Age of host animals: 3 weeks
3. Rearing and Humanization
   - > Rearing: ~9 weeks after transplantation
   - > Quality Control: Blood or serum Human Albumin (huAlb) levels



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Exploratory PXB-Mouse® Study Design

Group 1: PXB-Mice
(N=8, 19-22 weeks of age)

Group 2: SCID-Mice
(N=8, 19-21 weeks of age)

100 mg/kg SGX523
Oral Gavage
QD for 7 Days

Day 1 → Day 7

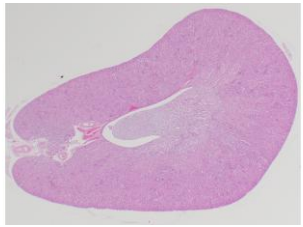
Body Weights and
Clinical Observations
Daily

Blood collections
1, 4, 8, 24 hours

Urine collections
Over 24 hours

Kidney Tissue Collected at termination of study for exposure and histopathology

Hatteras CCS2000
Chiller System/Metabolic Cage



Renal Injury Biomarkers
(NGAL and KIM-1)
ELISA

Bioanalysis
Sciex 7600 Zeno-ToF

Metabolite ID
Thermo Exploris 120

Clinical Pathology (BUN, Creatinine)
Randox Daytona Analyzer

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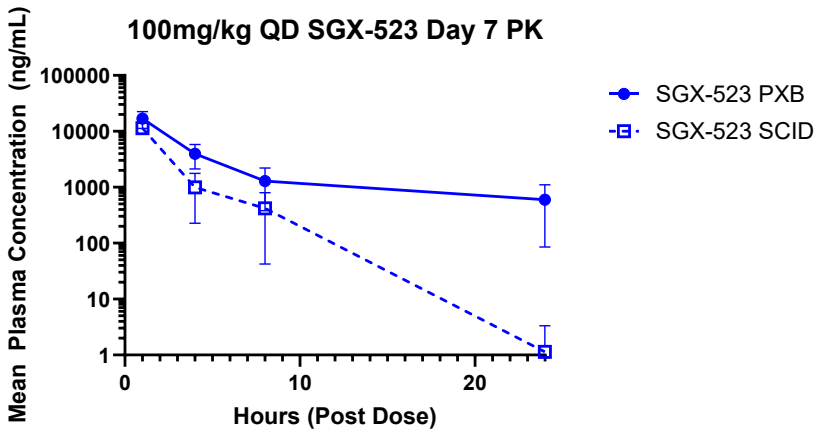
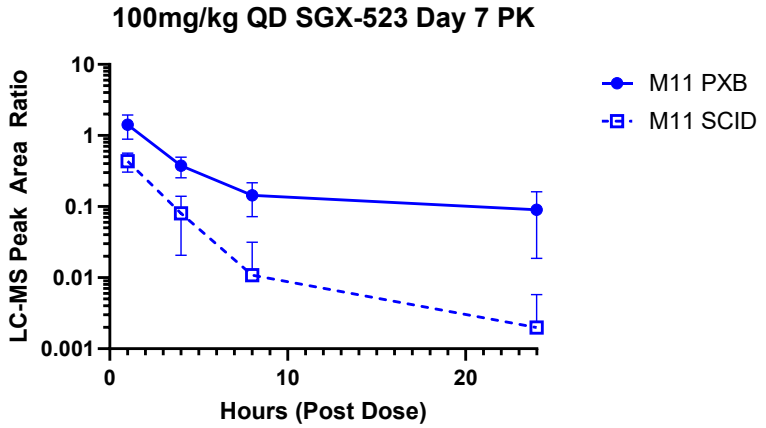
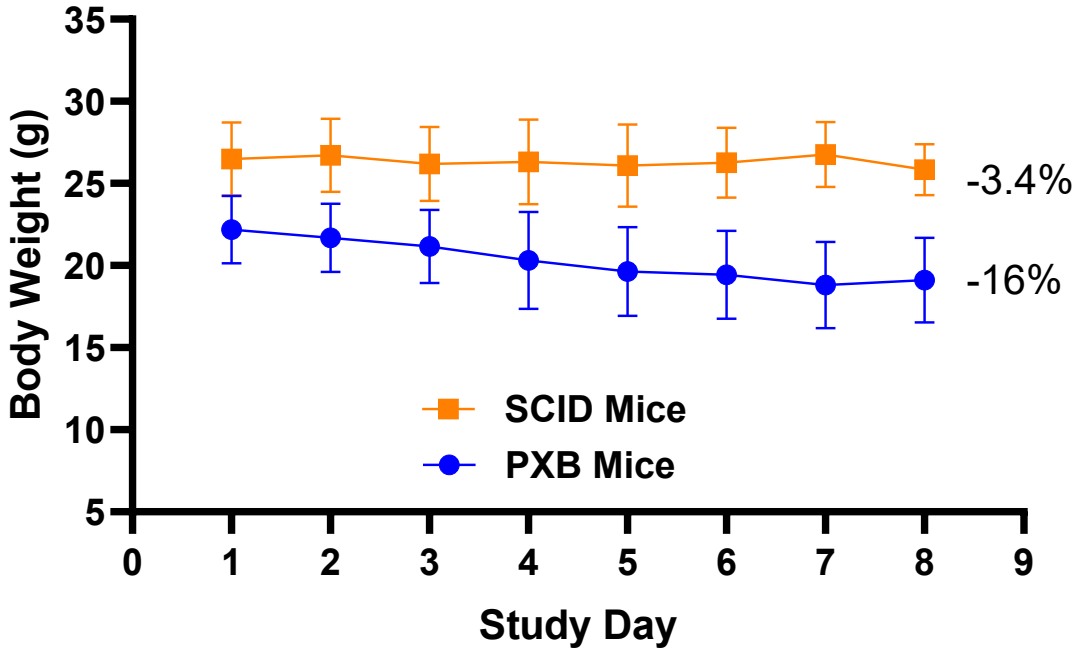
Relative Exposure of SGX523







- **M11** exposure ~5-fold higher in PXB mice compared to SCID mice
- Body weight loss more prominent in PXB mice compared to SCID mice

- PXB mouse 4M002 sacrificed moribund on Day 7 (27% body weight loss) and PXB mouse 4M007 sacrificed unscheduled Day 7 (20% body weight loss)

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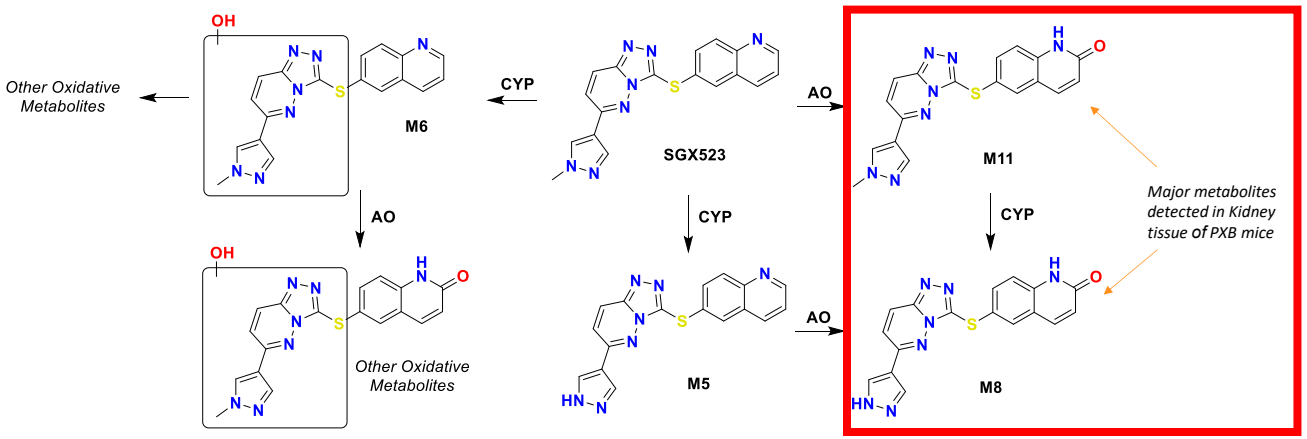
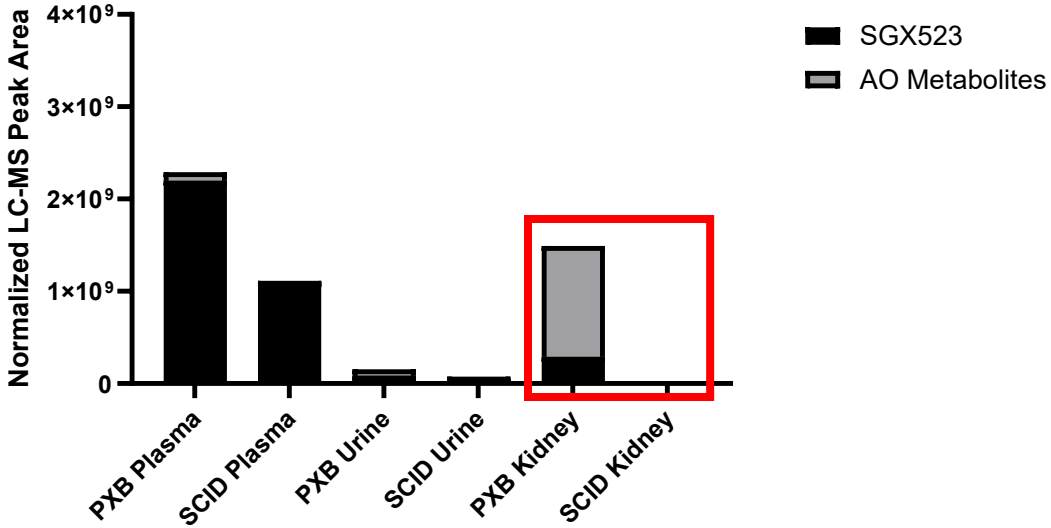
Relative Exposure of Metabolites



SGX523 and AO Metabolite Relative Abundance In AUC Pooled Plasma, Urine, and Kidney Tissue



- "AO Metabolite" refers to all confirmed aldehyde oxidase mediated metabolite signals detected (**M11** and **M8**)

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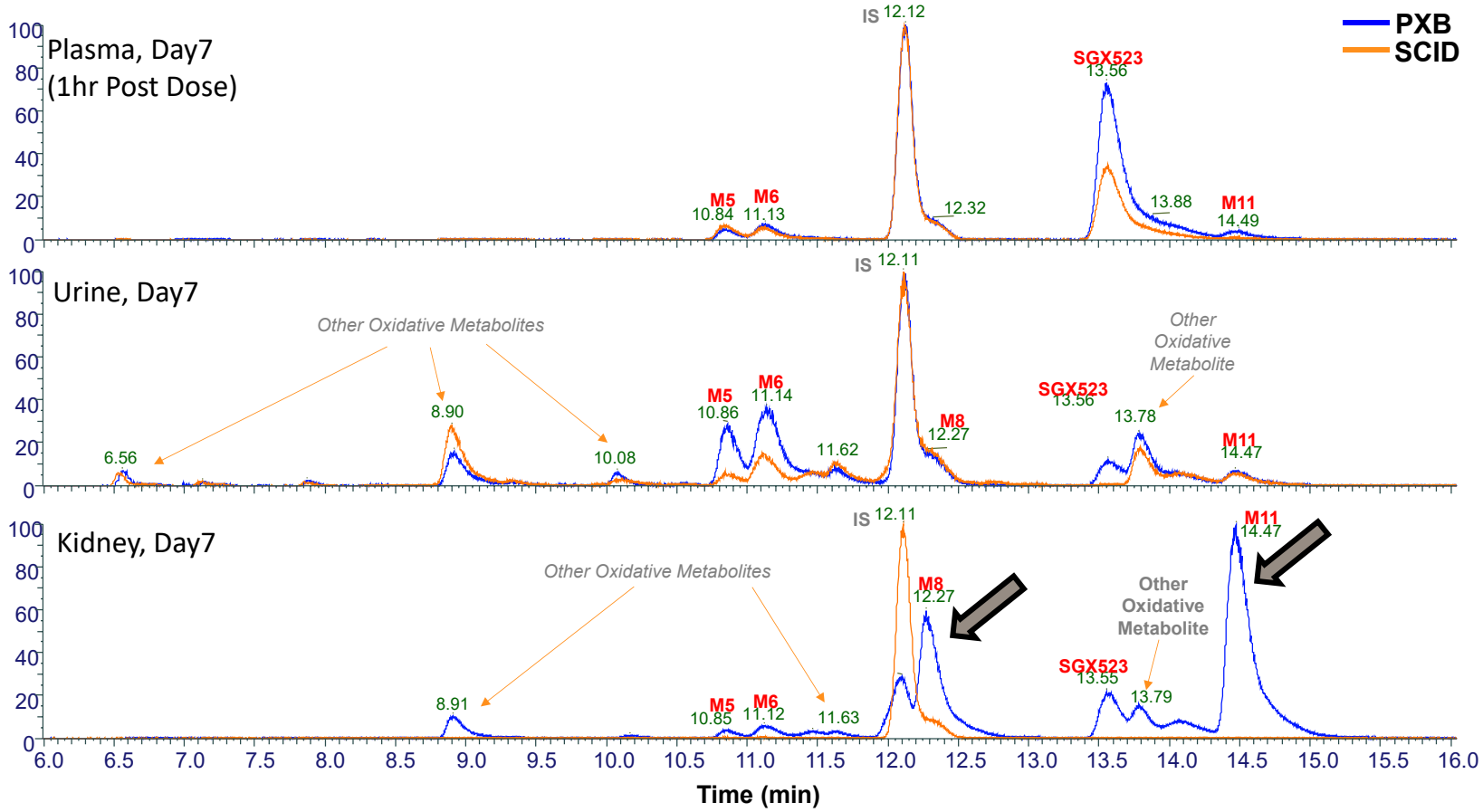
SGX523 Metabolite Profile

- Several oxidative metabolites of SGX523 observed
- Apparent abundance of **M11** and **M8** were not distinctly different in plasma and urine
- **M11** and **M8** are profoundly more abundant in kidney tissue from PXB-Mice as compared to SCID Mice



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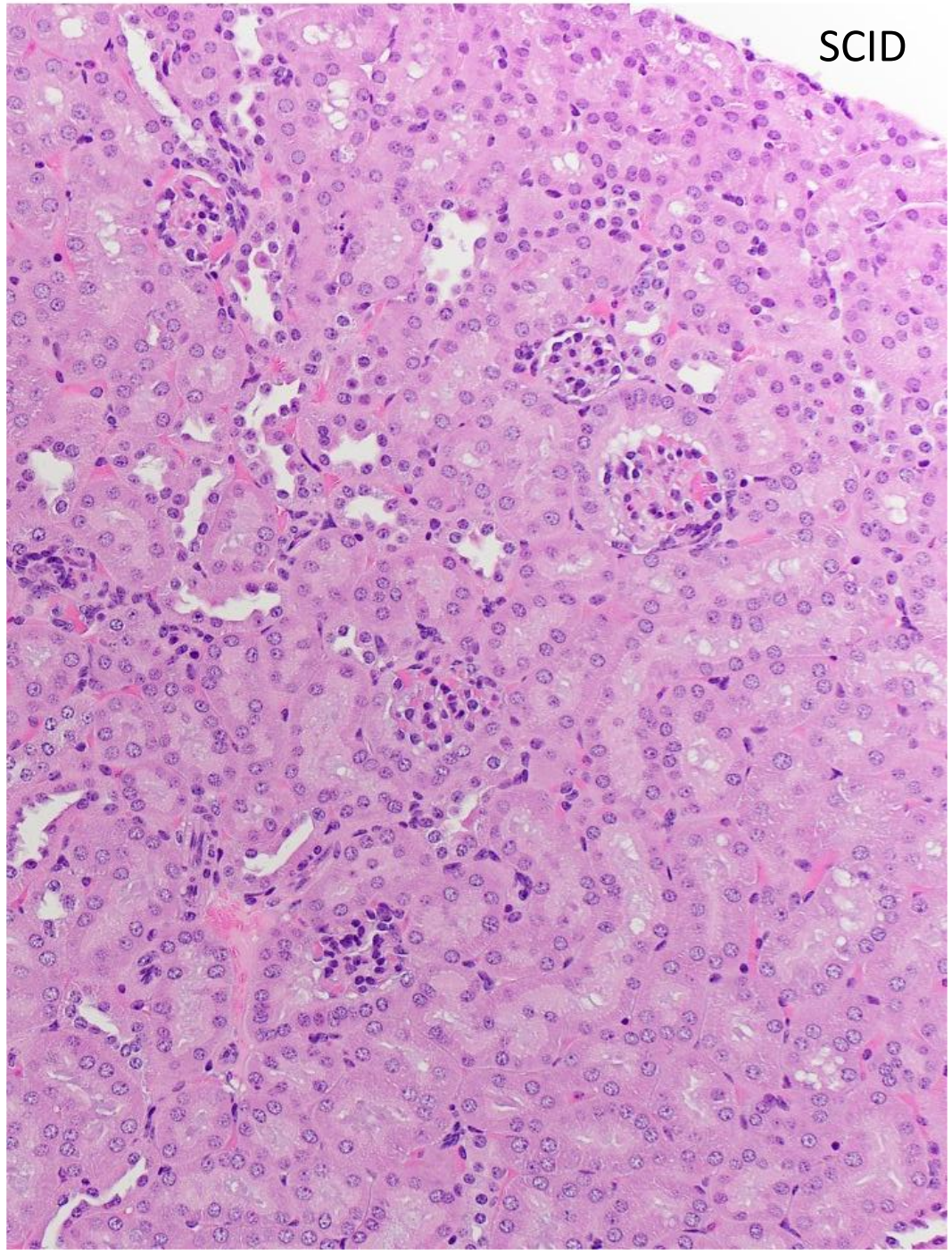
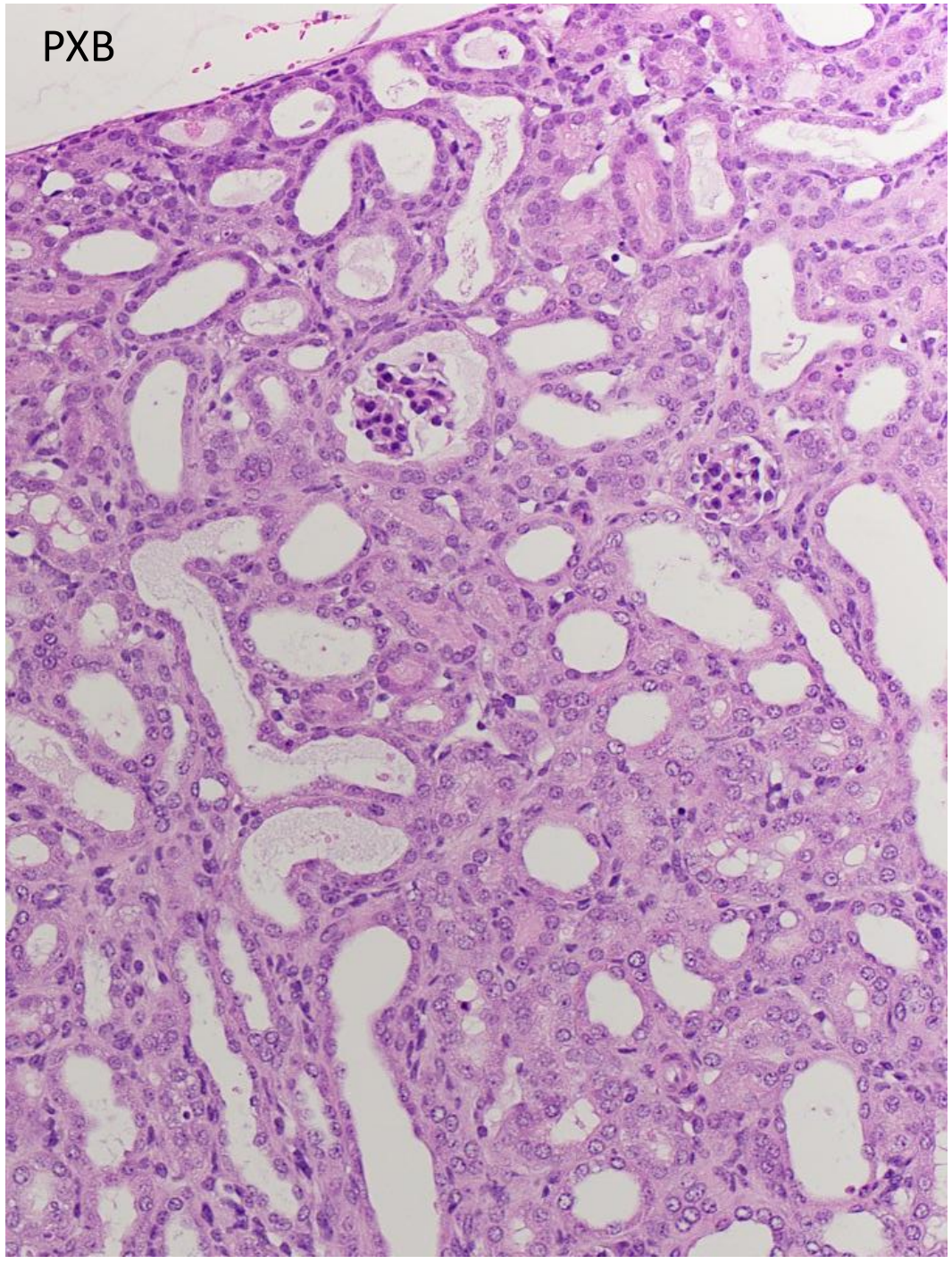

SCID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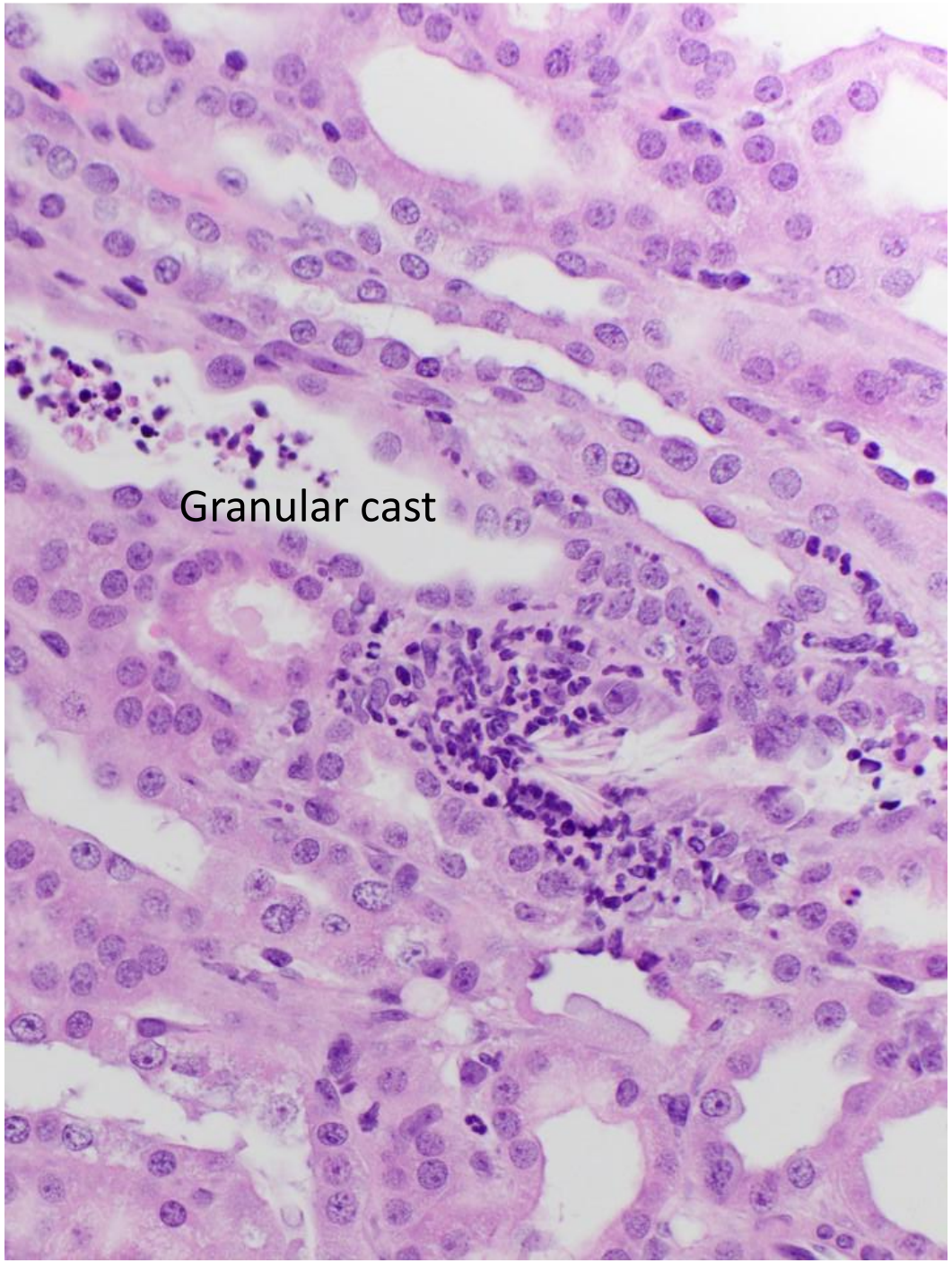
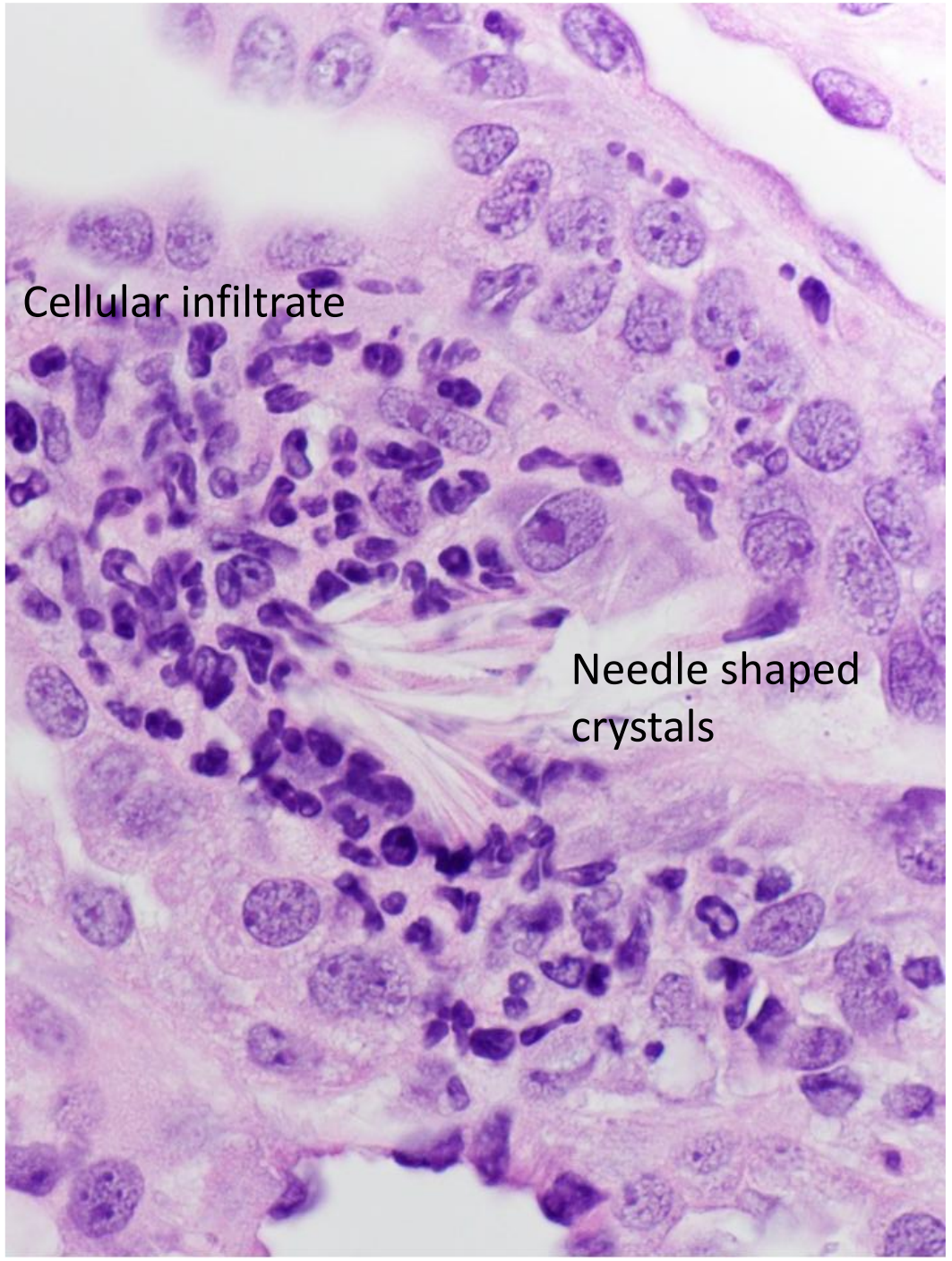
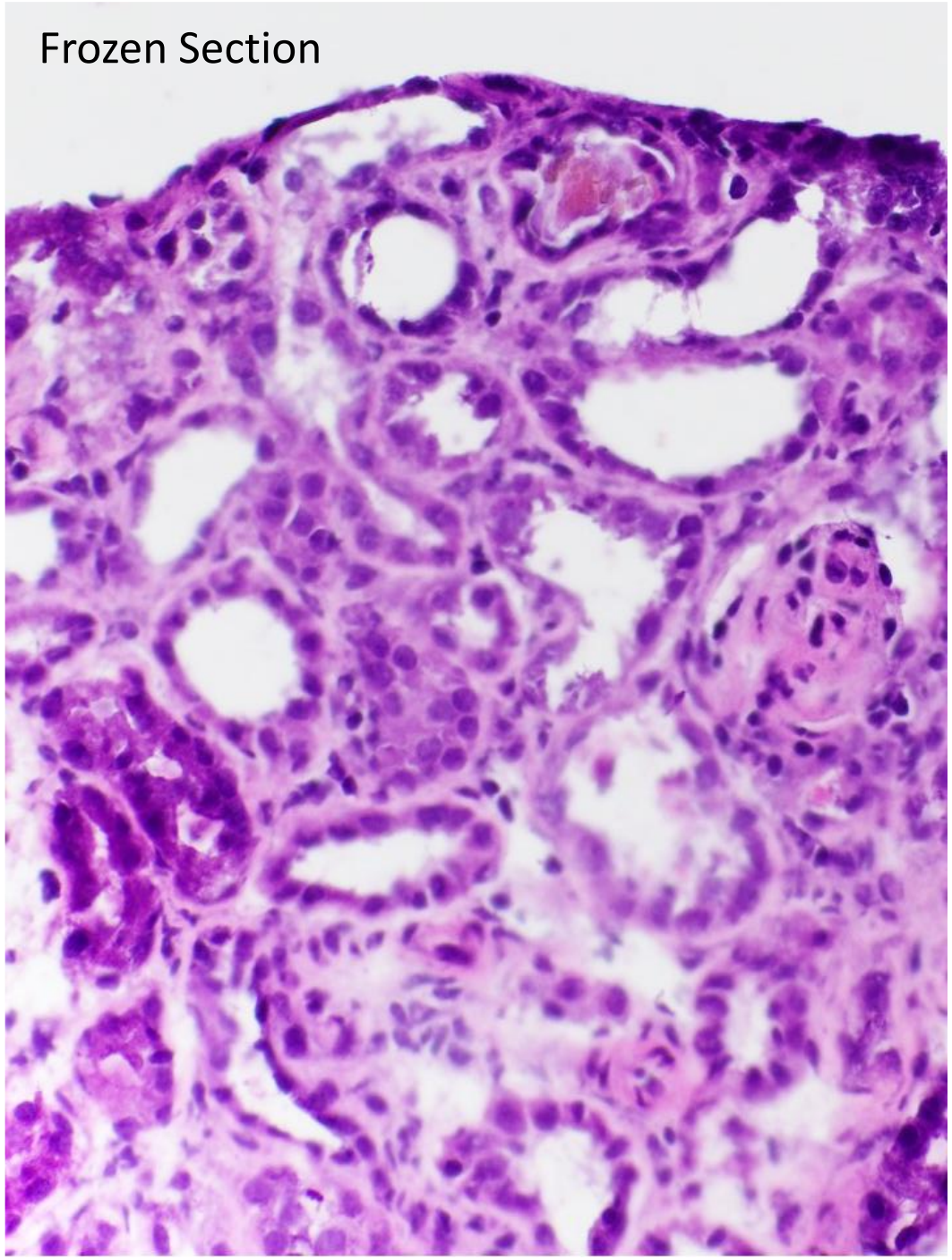
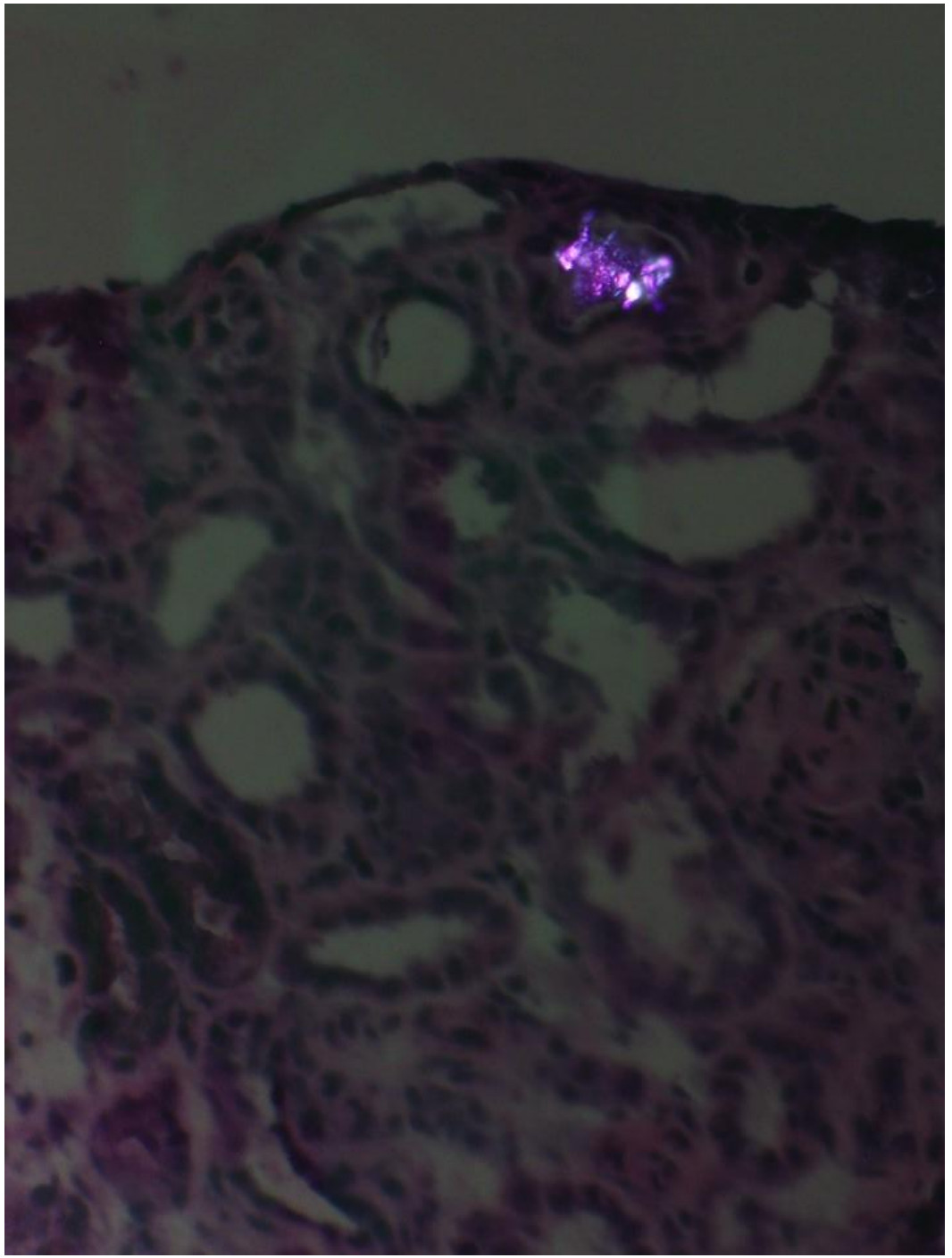
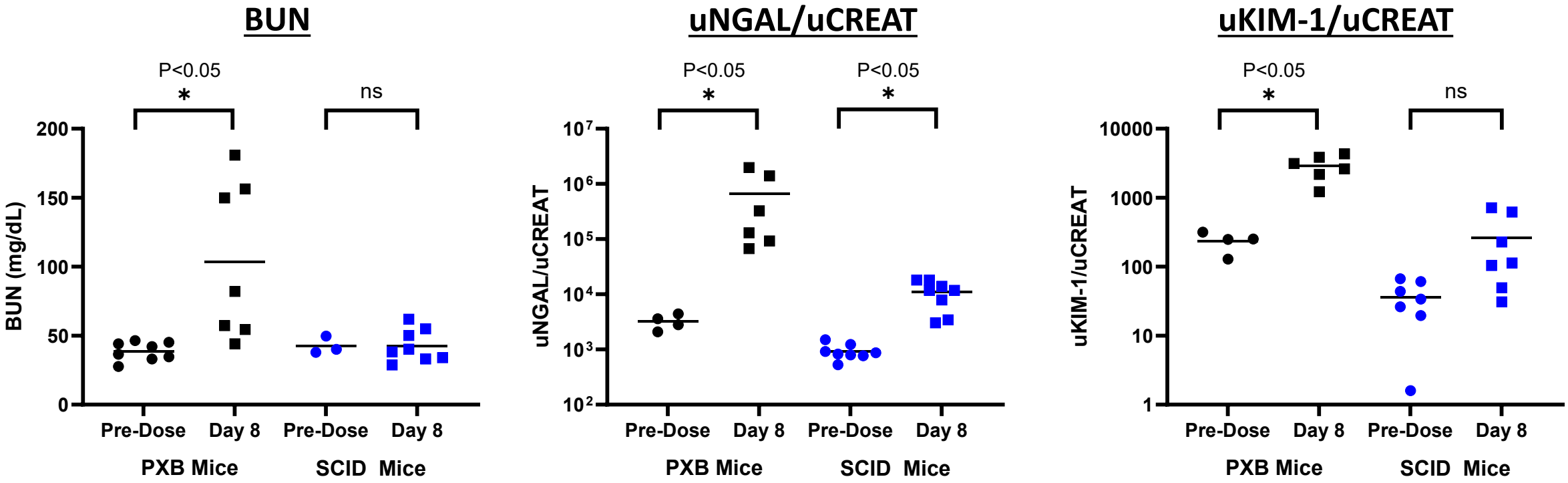

Granular cast


Cellular infiltrate
Needle shaped crystals


Frozen Section



Renal Injury Biomarkers



- Weight of evidence suggests that the renal effects caused by AO metabolite(s) of SGX523 were able to be recreated in the humanized liver PXB-Mouse®

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Conclusions

- Aldehyde oxidase (AO) is an important drug-metabolizing enzyme to be aware of, especially for **heterocycle-containing drugs**
- DMPK project leaders for **Kinase discovery programs** should pay close attention to not just metabolites, but also mechanisms of metabolism early in the program
- Selecting the right *in vitro* metabolism system (e.g. **cytosol, S9 fraction or hepatocytes**) is critical to not overlook key metabolic pathways such as AO
- Important to integrate metabolism information with species comparisons to inform toxicology strategy, specifically species selection -----> **DMPK and Toxicology closely connected**!
- **Chimeric Liver-Humanized PXB-Mouse®** can be a viable alternative model for not just understanding PK, but also investigative safety studies associated with human-specific AO-derived metabolites

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Acknowledgements

- PhoenixBio for providing the Chimeric Humanized Liver PXB-Mice

Inotiv Project Team:

- Kelly Long (MS, DABT) – Study Design and Planning
- Erin Touchette (MS, LATg) – In Vivo Study Director
- Josh Cooper (Ph.D.) – LC-MS/MS Bioanalysis
- Langston Jones (B.S.) – Pharmacokinetic Modeling
- Kevin Johnson (Ph.D.) – Metabolite Identification
- David Thompson (Ph.D.), Toni Steiner, Heather Sexton (MS) – Renal Biomarker Analysis
- Adam Aulbach (DVM, DACVP, DABT) – Clinical Chemistry/Pathology
- Judit Markovits (DVM, Ph.D., DACVP, Fellow IATP) and Chris Bollinger (MS) – Histopathology

Thank You!

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