CHINOOK THERAPEUTICS

1st Annual Rare & Genetic Kidney Disease Drug Development

Discovery & Characterization of CHK-336: A Liver-Targeted Small Molecule Inhibitor of Lactate Dehydrogenase A (LDHA) for the Treatment of Primary Hyperoxaluria (PH)

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Chief Scientific Officer
December 1, 2021

Outline

- Introduction to primary hyperoxaluria types 1, 2 and 3 (PH1-3)
- LDHA target biology
  - The final step in hepatic oxalate synthesis
  - Potential therapeutic target to treat all forms of PH
- Discovery & characterization of CHK-336, a liver-targeted small molecule inhibitor of LDHA
  - Inhibition of LDHA enzyme activity
  - Liver targeted distribution profile
  - In vivo profile in models of PH

Primary Hyperoxaluria (PH)

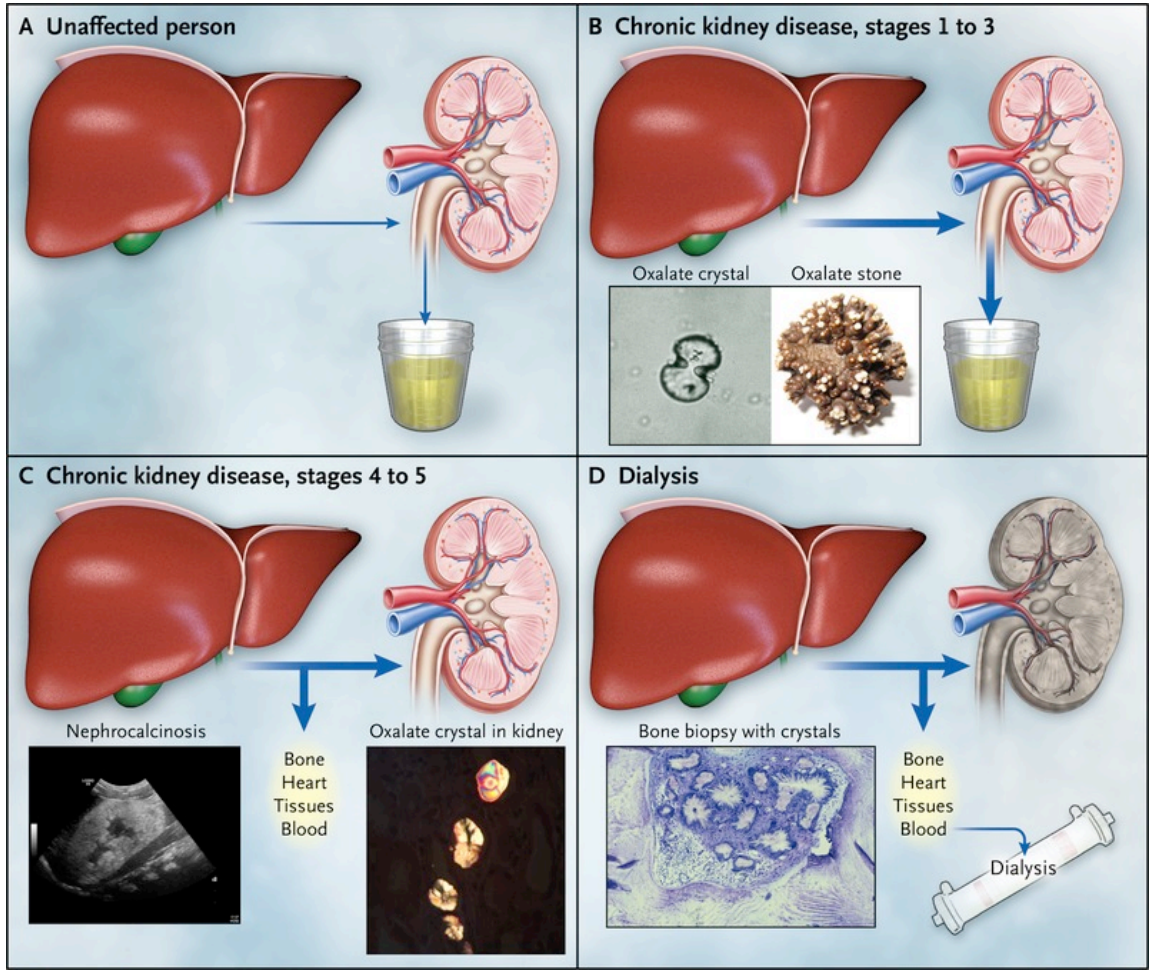
Rare and severe genetic disorder of oxalate overproduction

PH1-3 are a group of autosomal recessive disorders involving excess hepatic oxalate production

Pathogenesis of PH

- Impaired hepatic **glyoxylate detoxification**, resulting in **excess oxalate**
- Calcium **oxalate crystals** precipitate in the **kidneys**, leading to **kidney stones**
- **Tubular toxicity**, **nephrocalcinosis**, **obstruction**, **superimposed infection**
- Decline in **kidney function** results in systemic **oxalosis**
- Calcium **oxalate crystals** deposit in **the bone, heart** and other **tissues**
- Progressive **CKD** to **ESKD**
- **Dialysis** awaiting **dual liver / kidney transplant**

PH is a group of devastating genetic diseases that can result in ESKD in young patients



Cochat,2013, N Engl J Med 369;7

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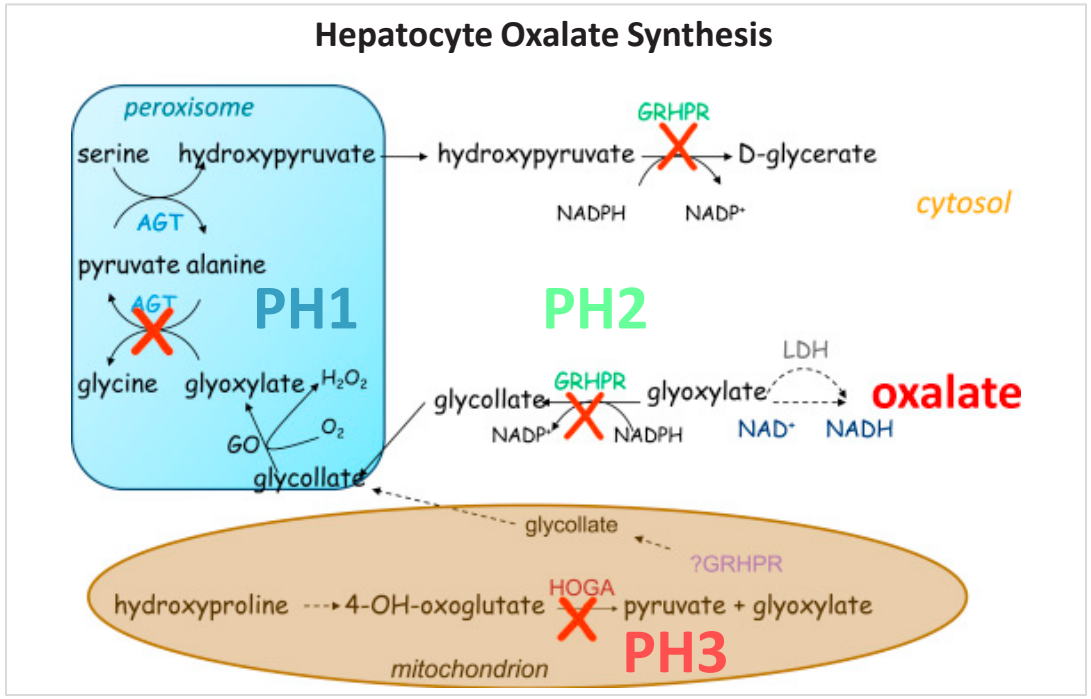
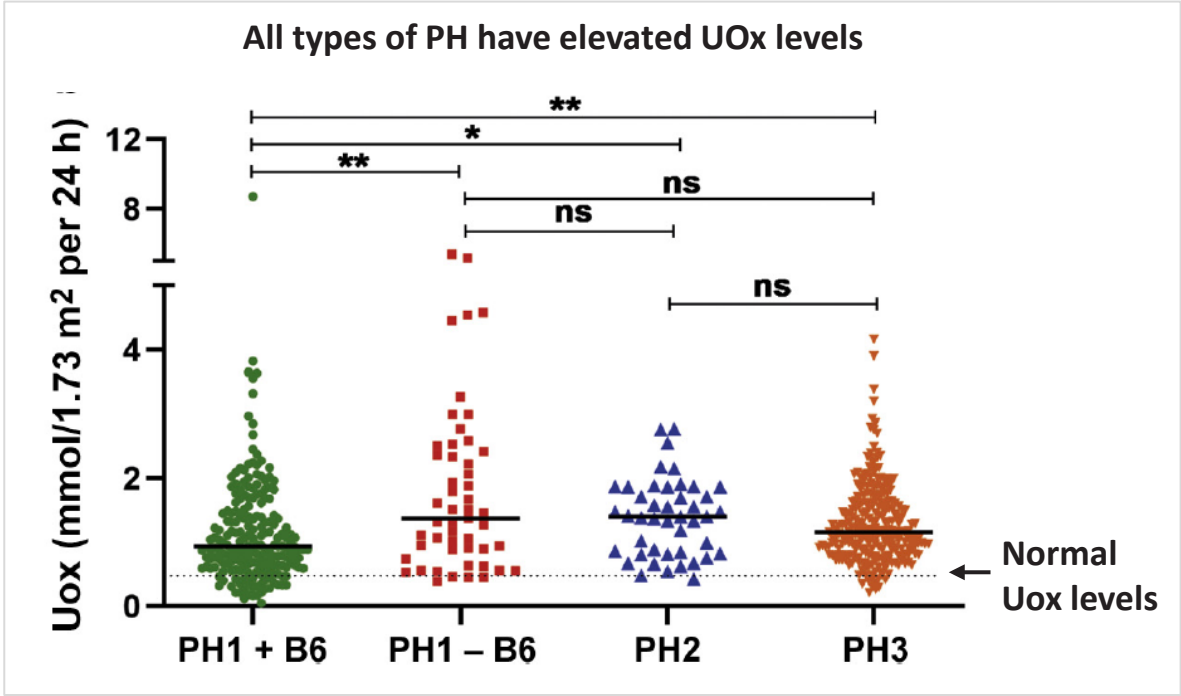
PH1-3 Driven by Mutations in Oxalate Metabolism Pathway Enzymes





- Combined prevalence of PH1-3 is estimated to be 1 in 58,000 as determined by whole exome sequencing
  - PH1 is the most commonly diagnosed (~80%), followed by PH2 and PH3, which are likely highly underdiagnosed
- PH1 is the most severe form (median age of ESKD is 23y), but approximately 50% of PH2/PH3 patients develop CKD and up to 25% progress to ESKD
- A definitive diagnosis requires genetic testing

Martin-Higueras et al Kidney Int 2021; 100: 621-635

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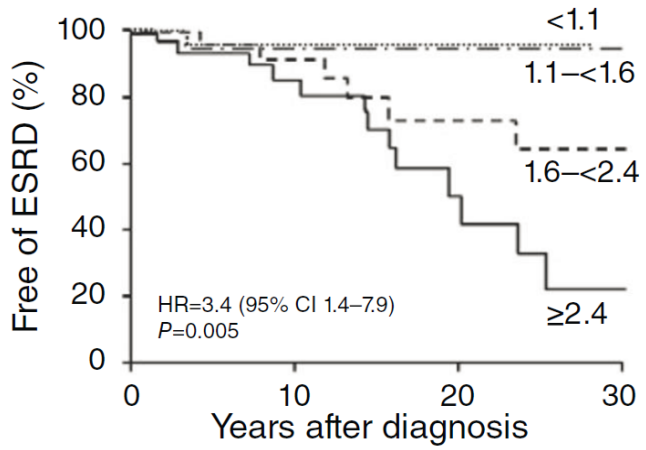
Clinical Outcomes and Management of PH

Clinical Outcomes

PH1

ESKD Risk by UOx



Less data is available for PH2 and PH3

Clinical Management

PH1

Supportive Measures

Hyper-hydration, Diuretics, Urinary alkalinization (K citrate), Dietary counseling (Na, Ox, Ca)

Drug Therapy

Pyridoxine
Lumasiran/OXLUMO (GO siRNA)

Dialysis

Kidney/Liver Transplantation

PH2 & PH3

Supportive Measures

Hyper-hydration, Diuretics, Urinary alkalinization (K citrate), Dietary counseling (Na, Ox, Ca)

Dialysis

Kidney Transplantation

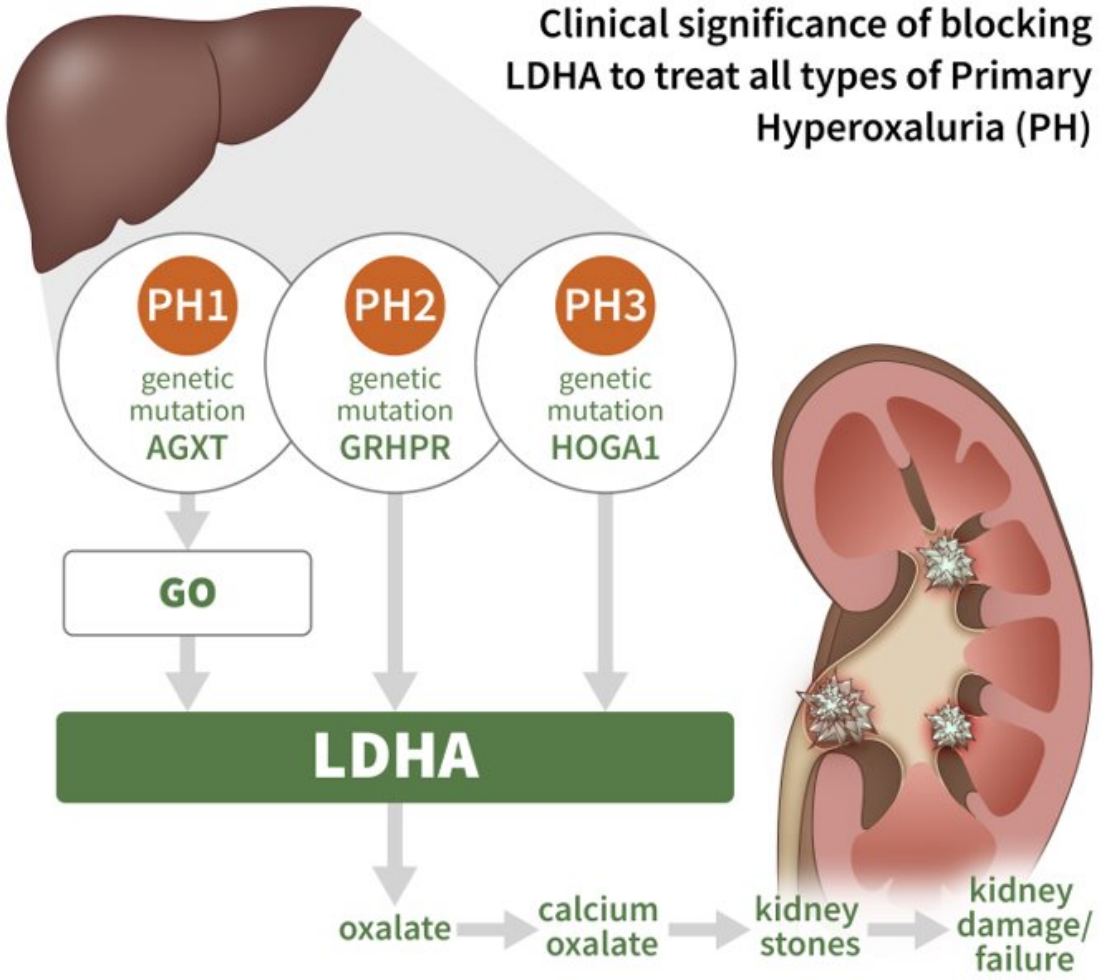
Zhao et al CJASN 2016; 11(1): 119-126, Cochat,2013, N Engl J Med 369;7

LDHA Target Biology: Potential Therapeutic Target to Treat All Forms of PH

- **Lactate dehydrogenase (LDHA) is the final and committed step in production of oxalate from glyoxylate in the liver**

- Represents a potential therapeutic target for all forms of PH, as well as other disorders arising from oxalate overproduction
- Complete loss-of-function of LDHA in humans results in exercise-induced muscle injury (heterozygotes phenotypically normal)[1]
- Liver-targeted LDHA inhibition with low systemic exposures is anticipated to avoid extra-hepatic LDHA inhibition
- Liver-targeted GalNac-LDHA siRNA has been reported to be safe, well tolerated and significantly reduce UOx in PH1 patients

Chinook designed, synthesized and characterized hundreds of LDHA inhibitors with the goal of identifying a potent and selective inhibitor with a liver-targeted tissue distribution profile for the treatment of all types of PH



1. Kanno et al Clinica Chimica Acta 1980; 108: 267-276

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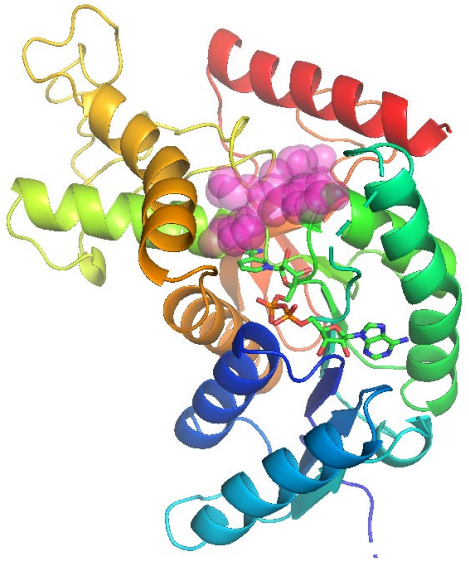
CHK-336 is a Potent LDHA Inhibitor in Enzyme and Hepatocyte Assays Across Multiple Species



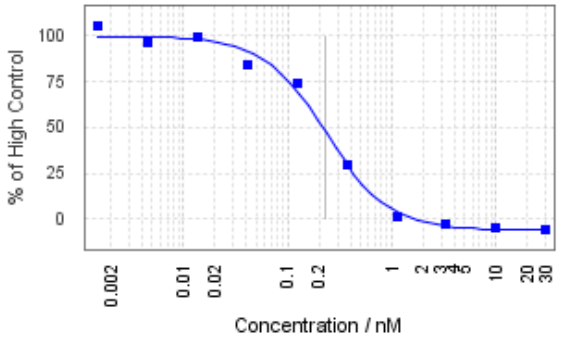
Compound design and sub-nM potency guided by structural biology and X-ray crystallography of LDHA-inhibitor complexes

W. Todd Lowther, PhD Wake Forest® School of Medicine

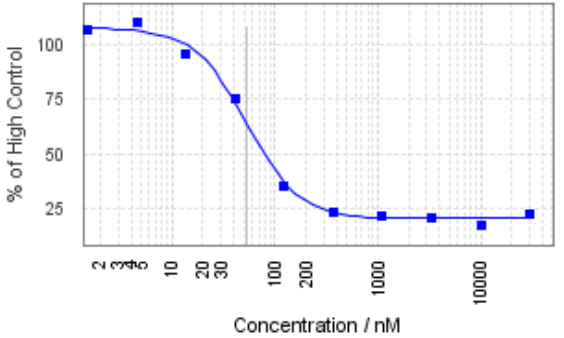
CHK-336 demonstrated high-affinity LDHA binding with a very slow off-rate (hours-days)

	ASSAY	CHK-336 IC_{50}
Enzyme	Human LDHA	**0.2 nM**
	Mouse LDHA	**0.3 nM**
Hepatocyte	Human Cryopreserved Hepatocytes	**121 nM**
	Mouse Fresh Hepatocytes	**52 nM**
	Mouse Cryopreserved Hepatocytes	**80 nM**
	Rat Cryopreserved Hepatocytes	**130 nM**
	NHP Cryopreserved Hepatocytes	**130 nM**

Human LDHA Enzyme Inhibition



Human LDHA Hepatocyte Inhibition



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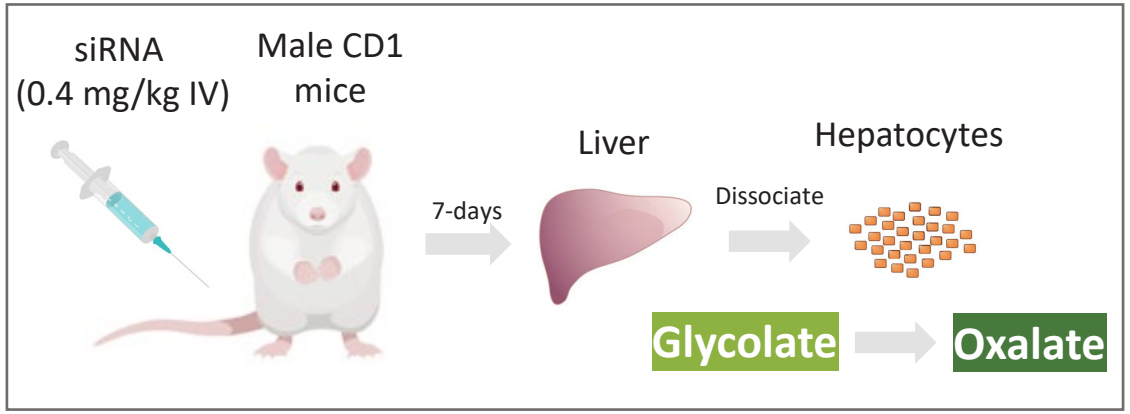
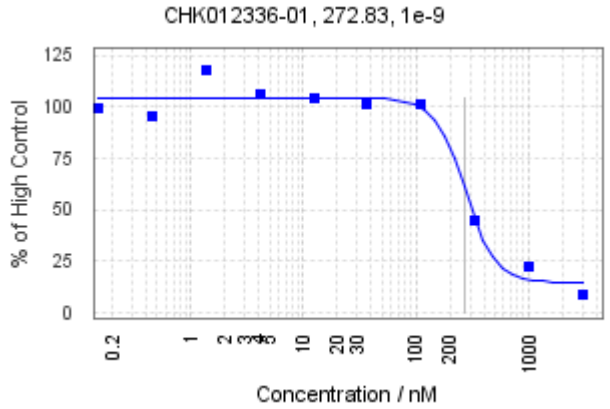
CHK-336 Blocks Oxalate Production in Hepatocytes from a PH1 Mouse Model

A PH1 mouse model was developed using *Agxt* gene knockdown by siRNA
- Encapsulated in a lipid nanoparticle (LNP)

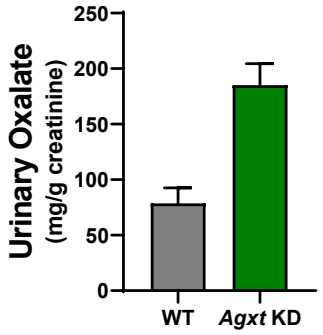
ASSAY	CHK-336 IC_{50}
Mouse *Agxt* Knockdown Hepatocytes (PH1) (Oxalate Production)	**165 nM**



Mouse PH1 Hepatocyte
Inhibition of Oxalate Production



Single dose of siRNA consistently resulted in ~90% Agxt knockdown & ~2x increase in UOx on Day 7



CHK-336 effectively blocked oxalate production in hepatocytes from a PH1 mouse model

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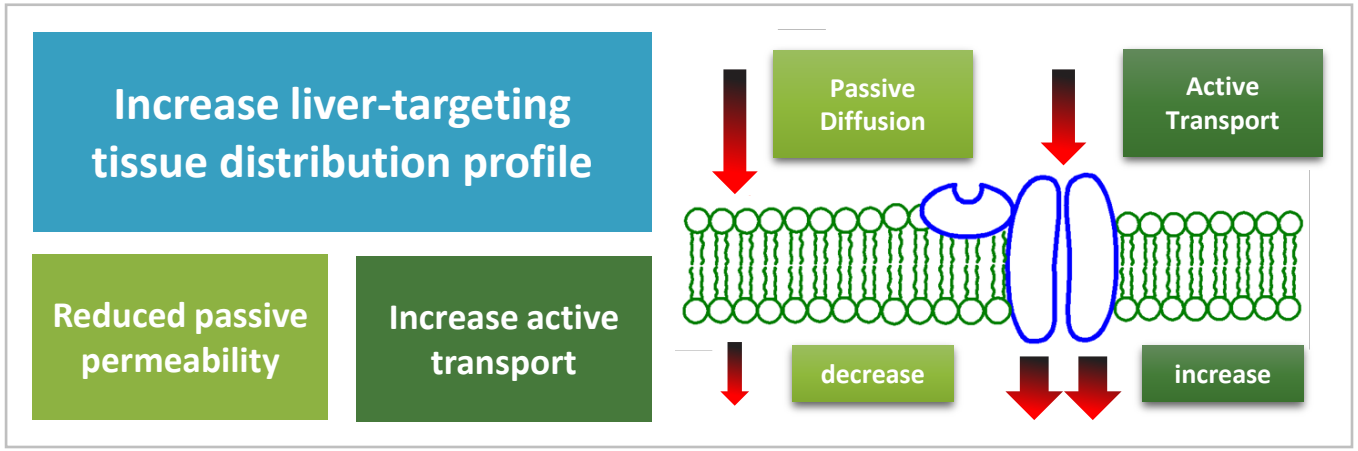
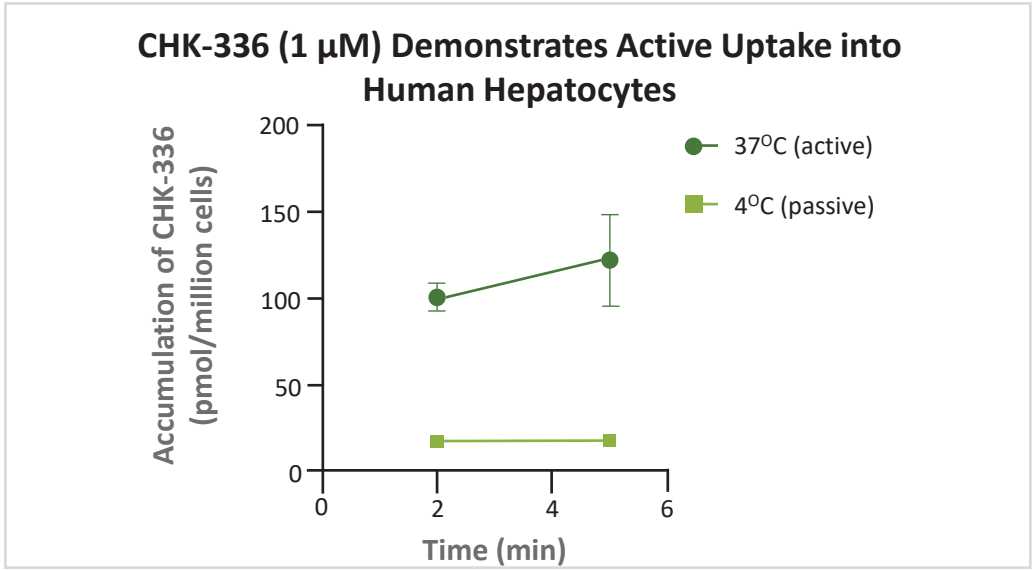
Design of a Liver-Targeted LDHA Inhibitor

- CHK-336 was engineered with a liver-targeted tissue distribution profile
  - Maximize inhibition of hepatic oxalate production
  - Avoid inhibition of extra-hepatic LDHA, including in skeletal muscle



- Strategy incorporated moieties recognized by liver-selective uptake transporters and reducing non-specific passive permeability

- CHK-336 demonstrates active uptake into human, monkey and rat hepatocytes



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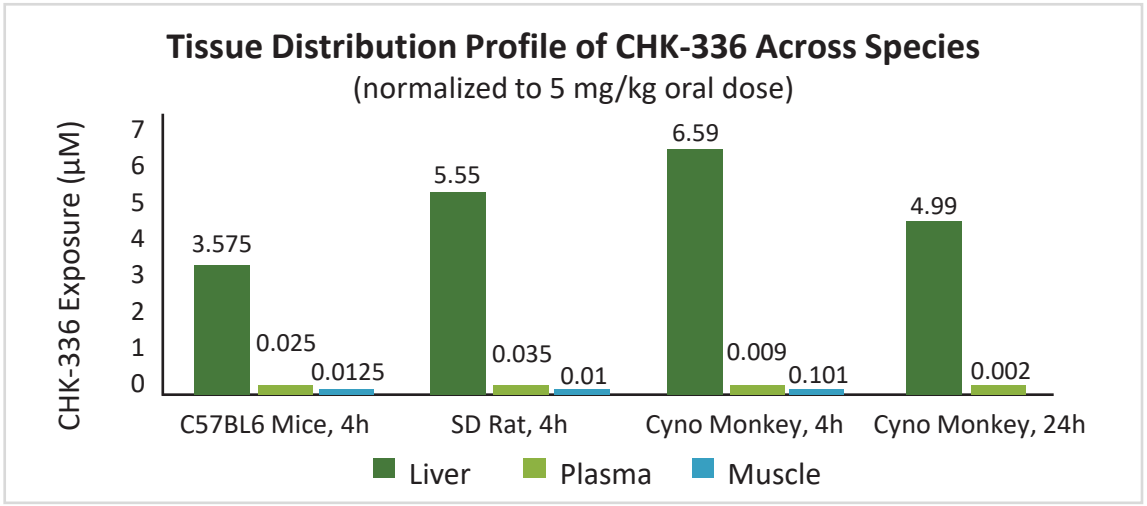
Liver Targeted Pharmacokinetic Profile of CHK-336

- CHK-336 exhibits a liver-targeted tissue distribution profile in mice, rats and monkey with high liver concentrations and low extra-hepatic tissue exposures
- Long liver half-life observed across species; driven by good metabolic stability and tight, slow-off rate binding of CHK-336 to LDHA in the liver



Human pharmacokinetic and dose predictions based on non-clinical data currently available, suggest CHK-336 has the potential to produce sustained inhibition of hepatic LDHA with low systemic exposure following a low, once-daily oral dose in humans

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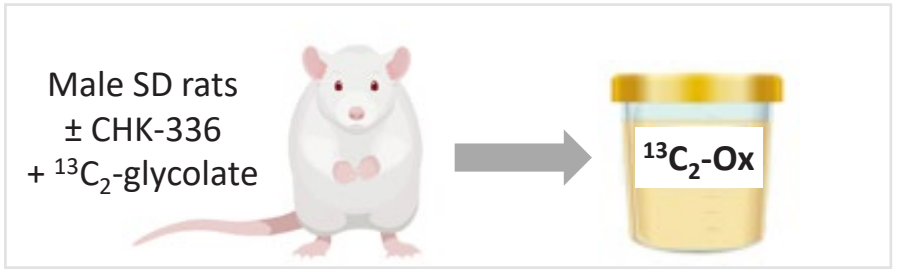
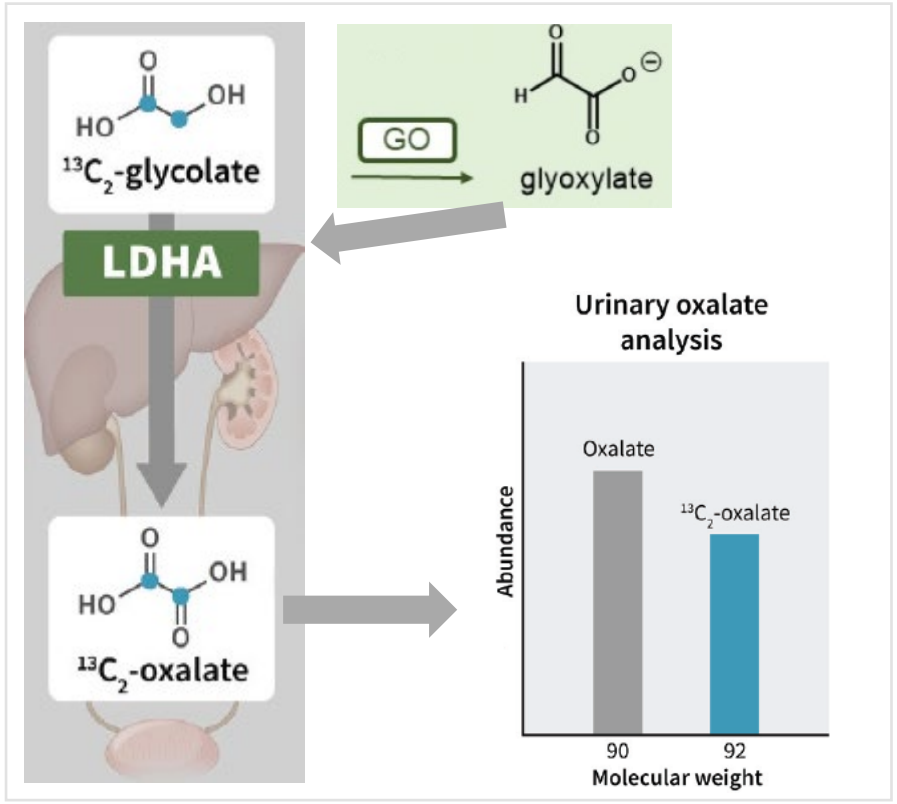
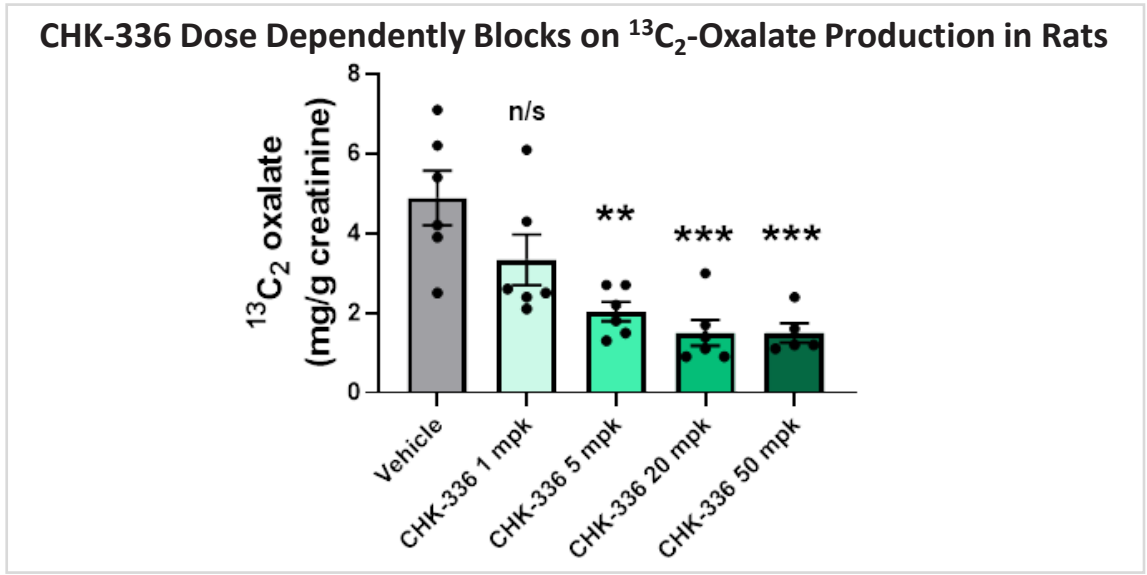
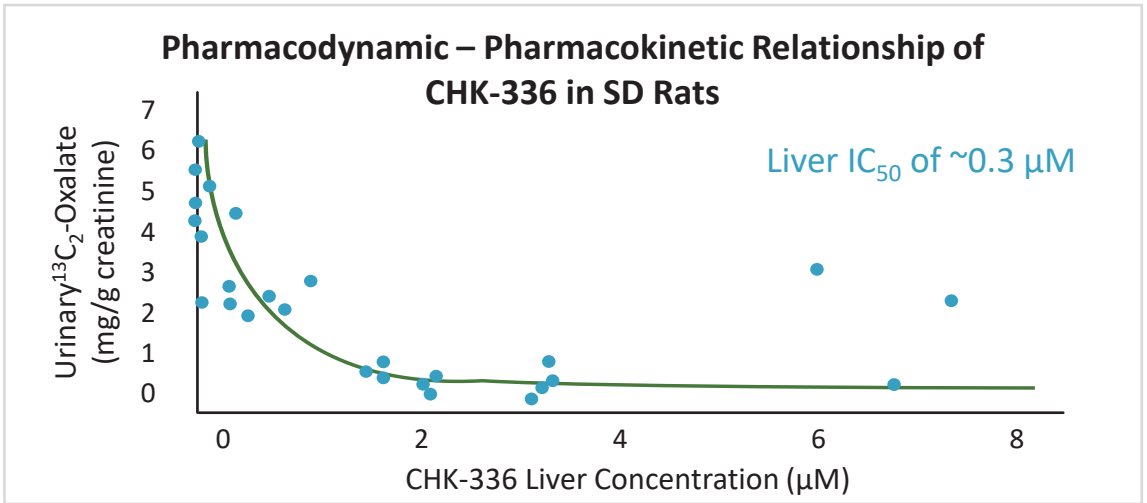
In Vivo Pharmacodynamic Effect of CHK-336 to Inhibit Oxalate Production









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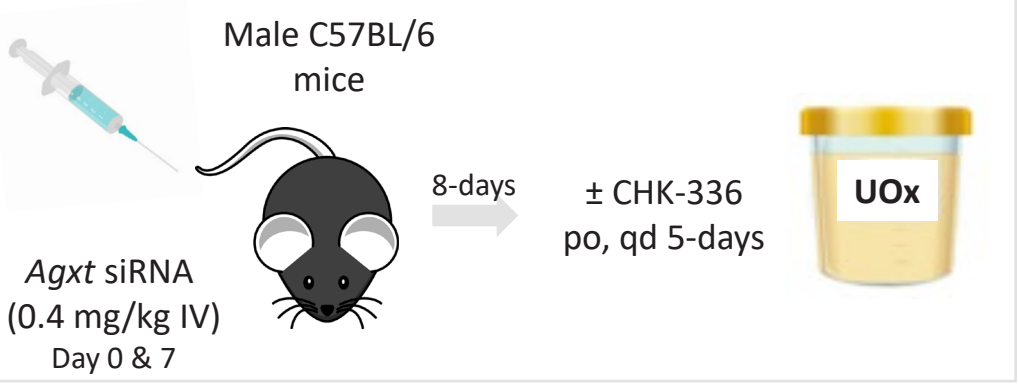
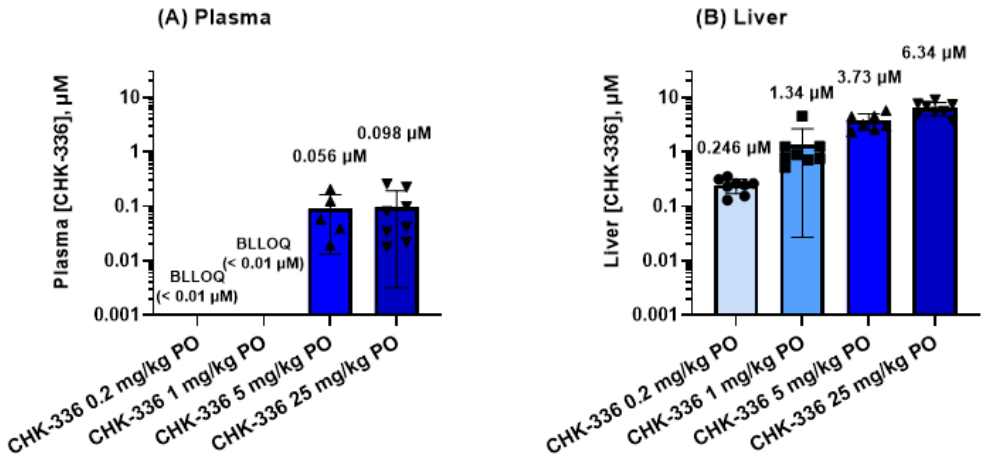
CHK-336 Reduces Urinary Oxalate Excretion in an Induced Mouse Model of PH1

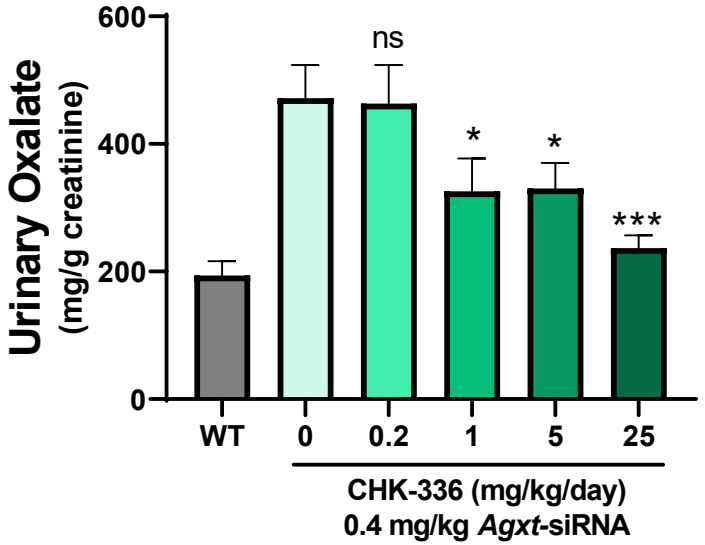


CHK-336 was Highly Liver Targeted in PH1 Mice



Skeletal muscle [CHK-336] BLLOQ

CHK-336 Dose Dependently Reduced Urinary Oxalate in PH1 Mice



In an *Agxt* **knockdown** model of PH1, low, once daily, oral CHK-336 significantly reduced urinary oxalate levels with a highly liver-targeted distribution profile

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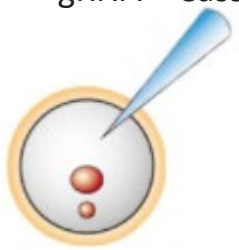
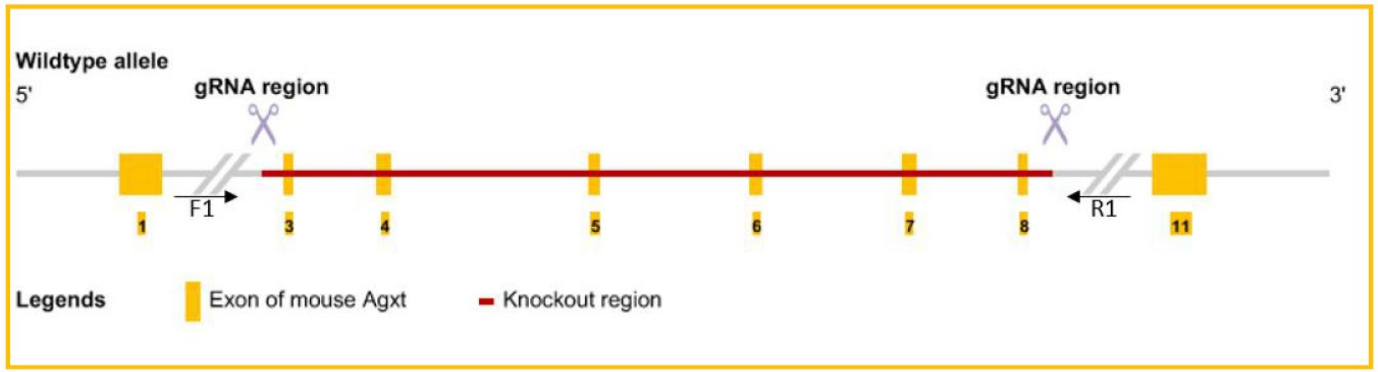
Generation of a Genetic Mouse Model of PH1

Microinjection
gRNA + Cas9 mRNA



Fertilized mouse egg

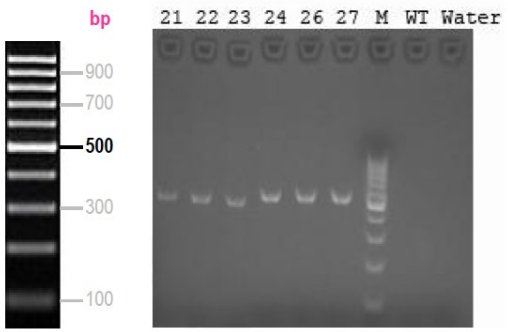
Targeted Deletion of *Agxt* Exons 3-8



Founder Breeding

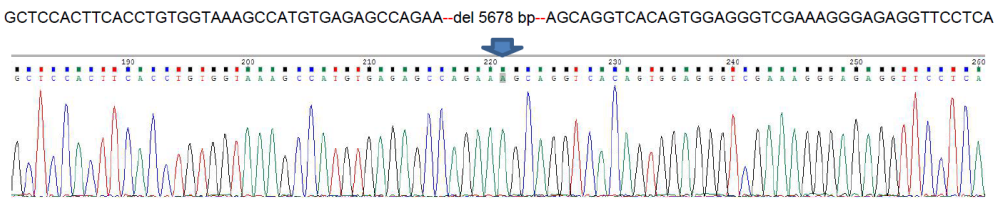
Germline transmission

F1 PCR Screening



Positive for targeted truncated *Agxt* allele

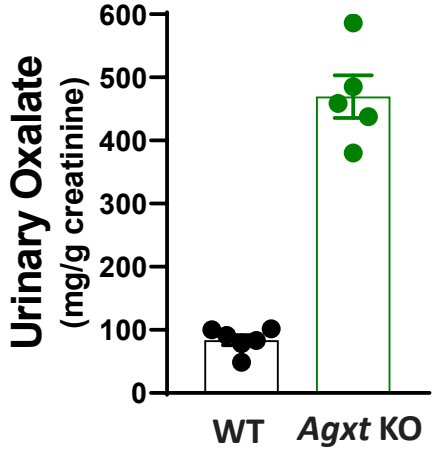
Sequencing Confirmation



Confirmed 5678 bp deletion

Homozygous Male PH1 mice

Note: Female ko have mild phenotype



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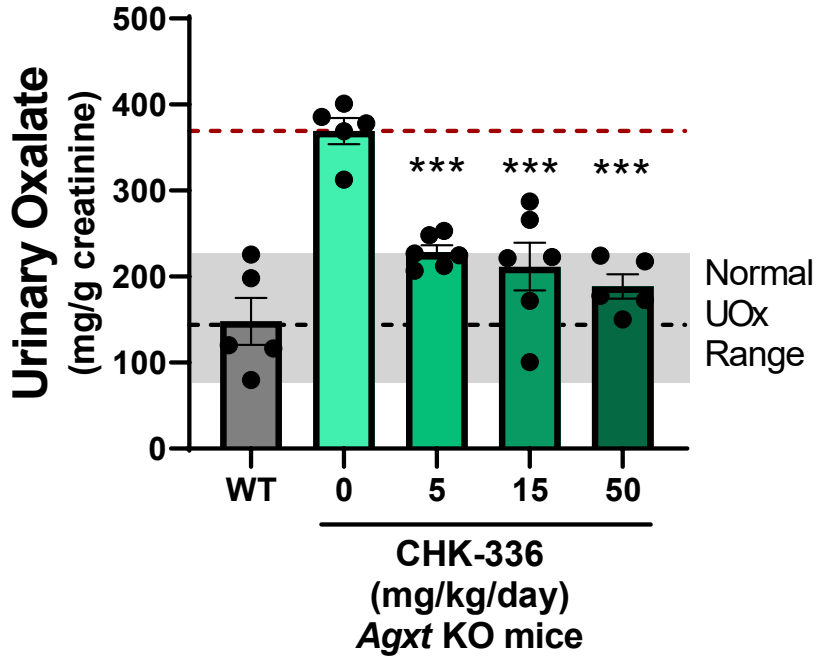
CHK-336 Reduces Urinary Oxalate Excretion in a Genetic Mouse Model of PH1

Male *Agxt* KO
Mice (13-14w)

Randomized to matched Baseline UOx → ± CHK-336 po, qd → 7-days → UOx

CHK-336 Dose Dependently Reduced Urinary Oxalate in PH1 Mice



*** *P<0.001* v 0 mg/kg

In an *Agxt* **knockout** model of PH1, low, once daily, oral CHK-336 significantly reduced urinary oxalate levels with a highly liver-targeted distribution profile

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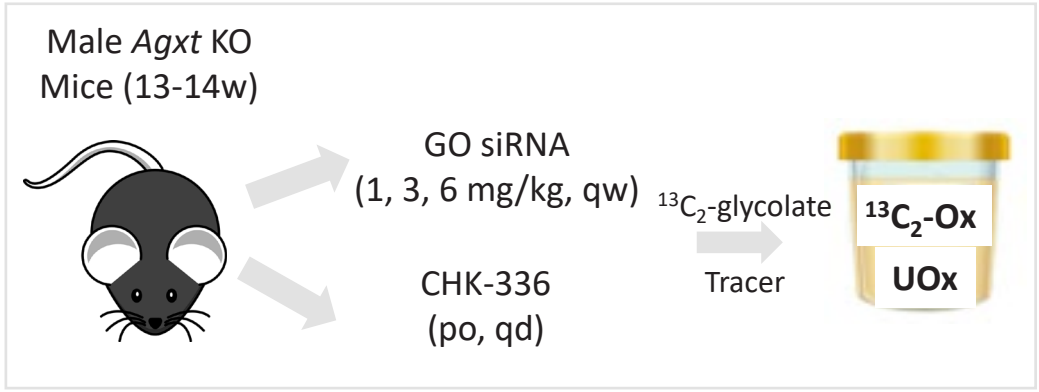
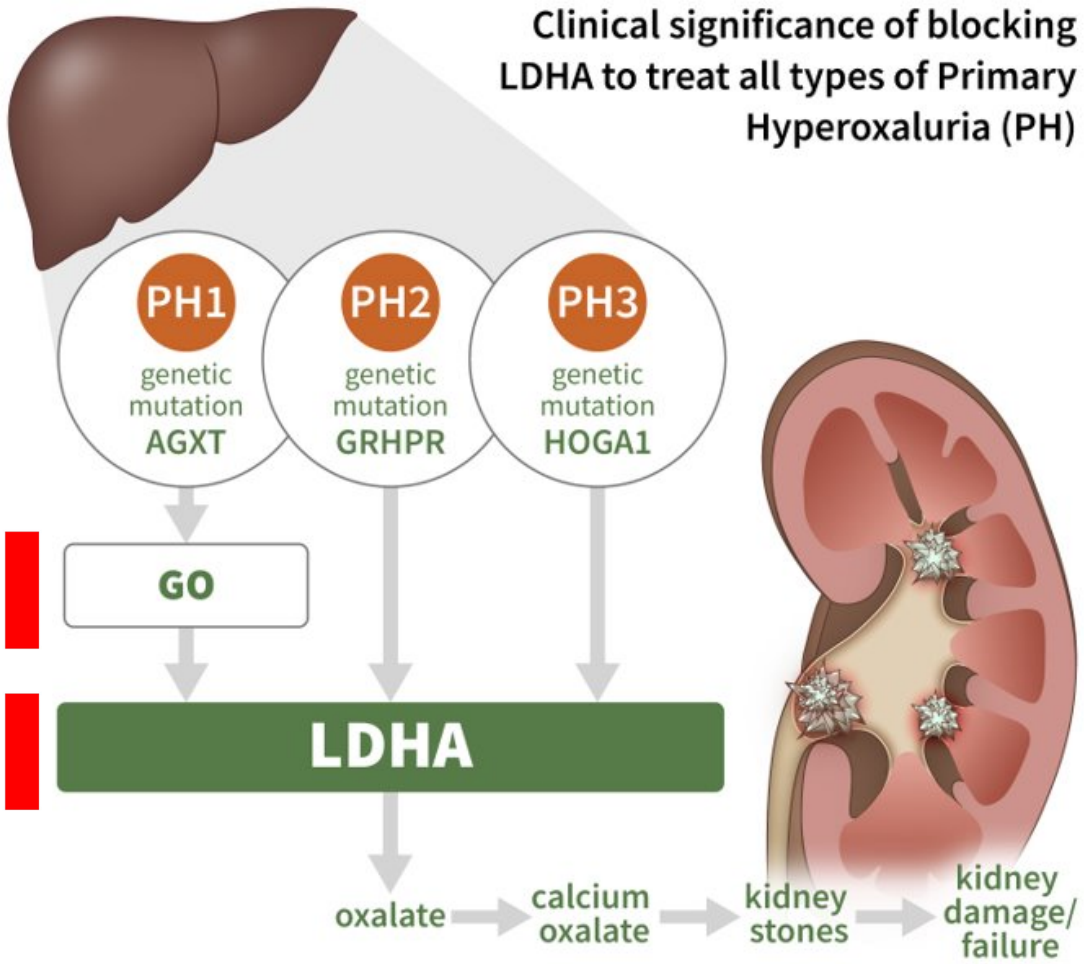
Comparative Effect of CHK-336 and GO siRNA on Oxalate Production in PH1 Mice



GO siRNA FDA approved in late 2020 for PH1

CHK-336, investigational small molecule liver targeted LDHA inhibitor



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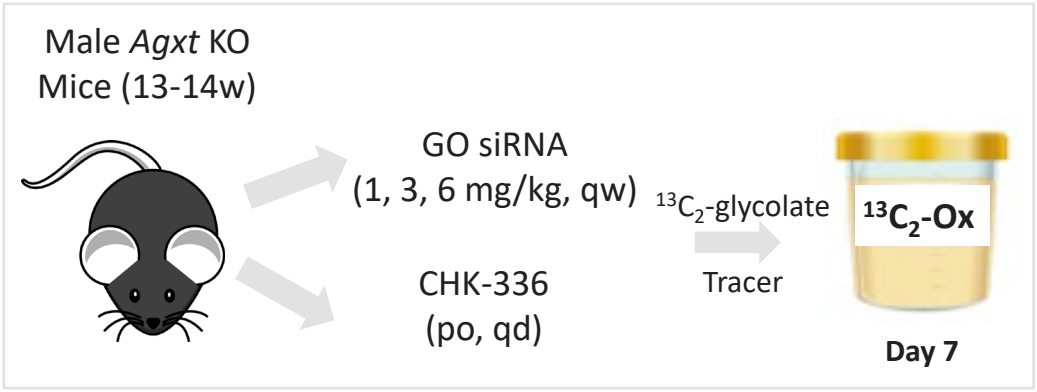
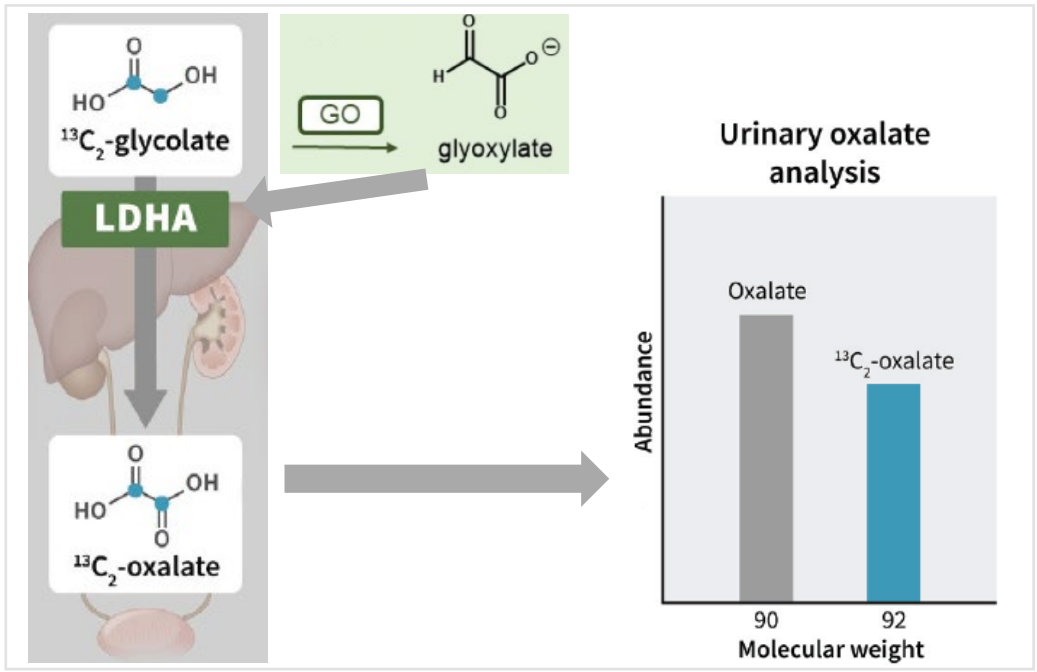
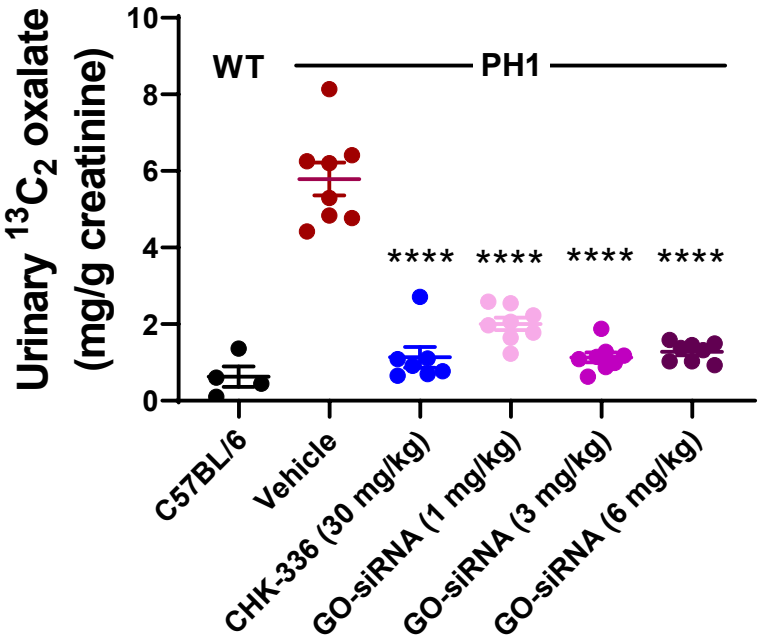
Comparative Effect of CHK-336 to GO siRNA in a Genetic Mouse Model of PH1: Conversion of $^{13}C_2$-Glycolate to $^{13}C_2$-Oxalate





Effect of CHK-336 Compared to GO siRNA on $^{13}C_2$-Glycolate to $^{13}C_2$-Oxalate in PH1 Mice



**** *P<0.0001* v Vehicle

In an *Agxt* knockout model of PH1, low, once daily, oral CHK-336 had comparable inhibition of the conversion of a $^{13}C_2$-glycolate tracer to $^{13}C_2$-oxalate to GO siRNA

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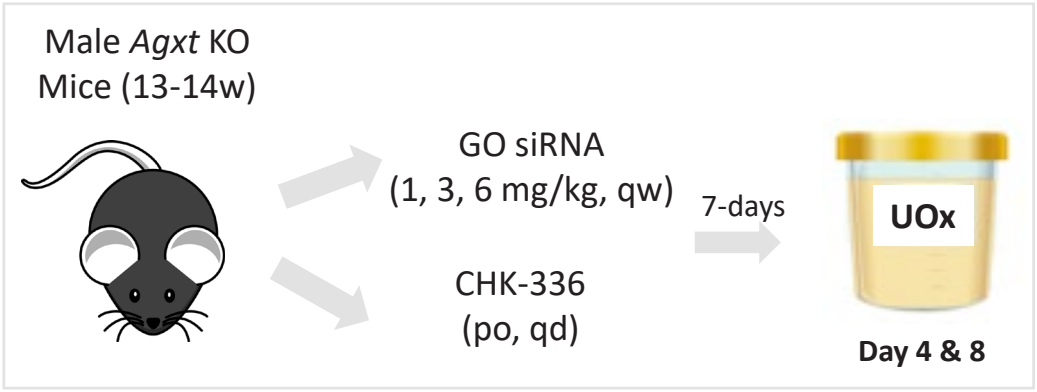
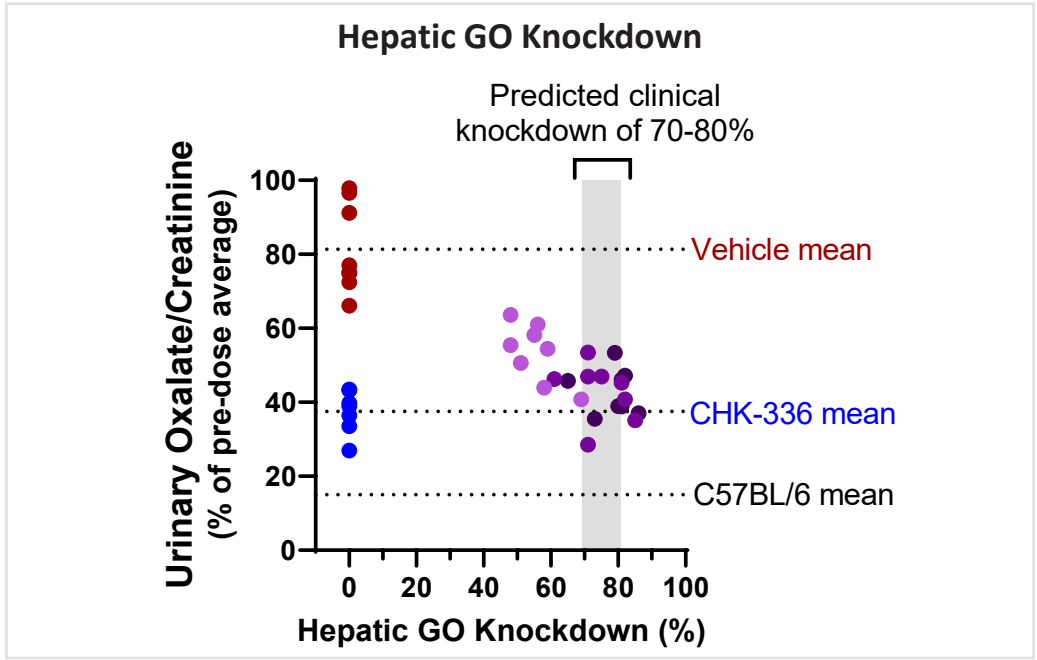
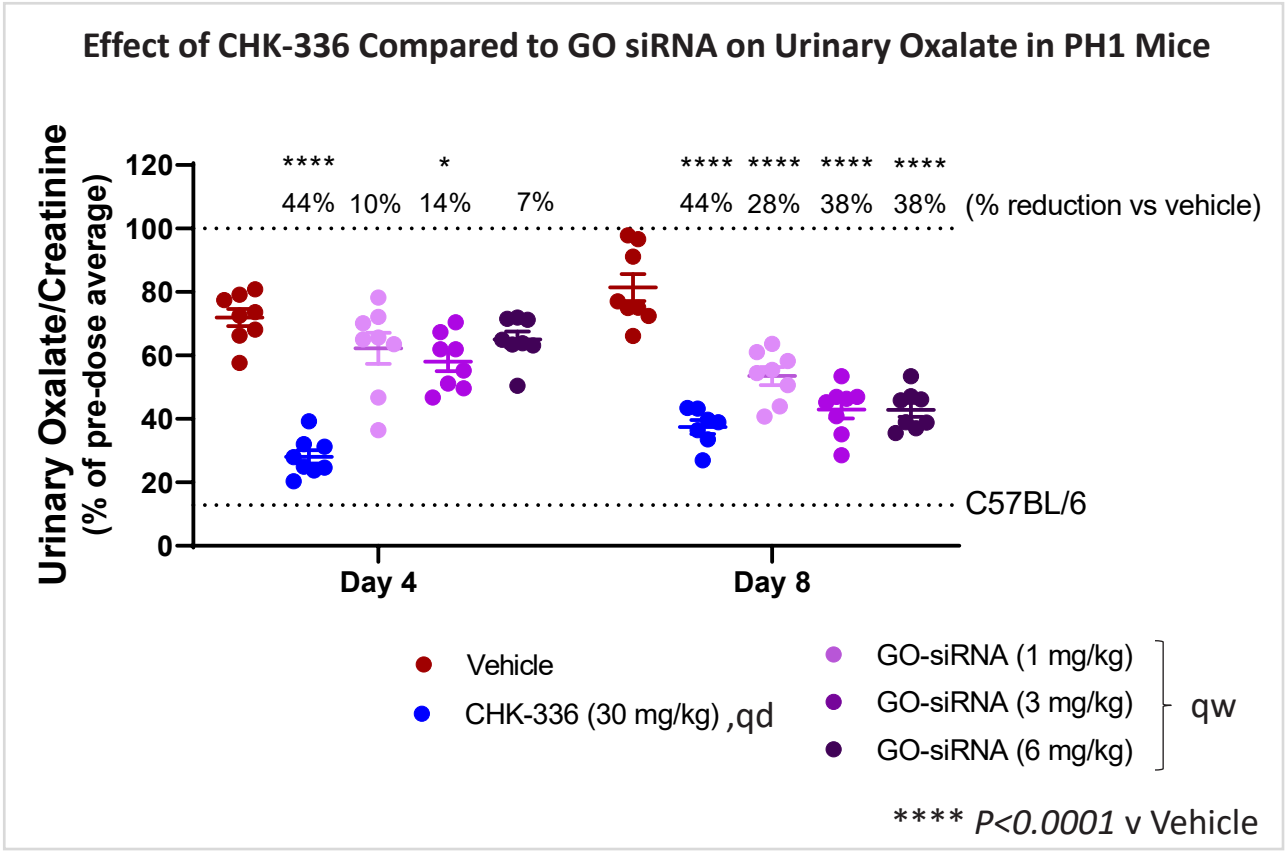
Comparative Effect of CHK-336 to GO siRNA in a Genetic Mouse Model of PH1: Urinary Oxalate Reduction







In an *Agxt* knockout model of PH1, low, once daily, oral CHK-336 had a rapid onset of action and comparable urinary oxalate reductions to GO siRNA

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CHK-336 Reduces Urinary Oxalate Excretion in a Genetic Mouse Model of PH2

Grhpr Knockout PH2 Mice

John Knight, PhD

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Knight et al 2012 Am J Renal Physiol; 302(6): F688-F693

Tissue, 24-h urine, and plasma measurements from 3-mo-old wild-type and GRHPR KO mice

	Wild-Type		GRHPR KO	
	Male	Female	Male	Female
Body wt, g	29.8 (3.0)*	22.7 (2.1)	29.1 (3.9)	25.3 (4.9)
Urine volume, ml	1.37 (0.31)	1.63 (0.33)	1.74 (0.48)	1.27 (0.48)
Urine creatinine, mg	0.627 (0.092)*	0.403 (0.121)	0.484 (0.105)*	0.377 (0.121)
Urine pH	5.9 (0.07)	5.88 (0.19)	5.87 (0.06)	5.74 (0.09)
Urine oxalate, μmol	0.51 (0.21)*	0.30 (0.08)	1.52 (0.26)*†	0.73 (0.18)†
Urine glycolate, μmol	0.44	0.54	0.39	0.56

Effect of CHK-336 in PH2 Mice

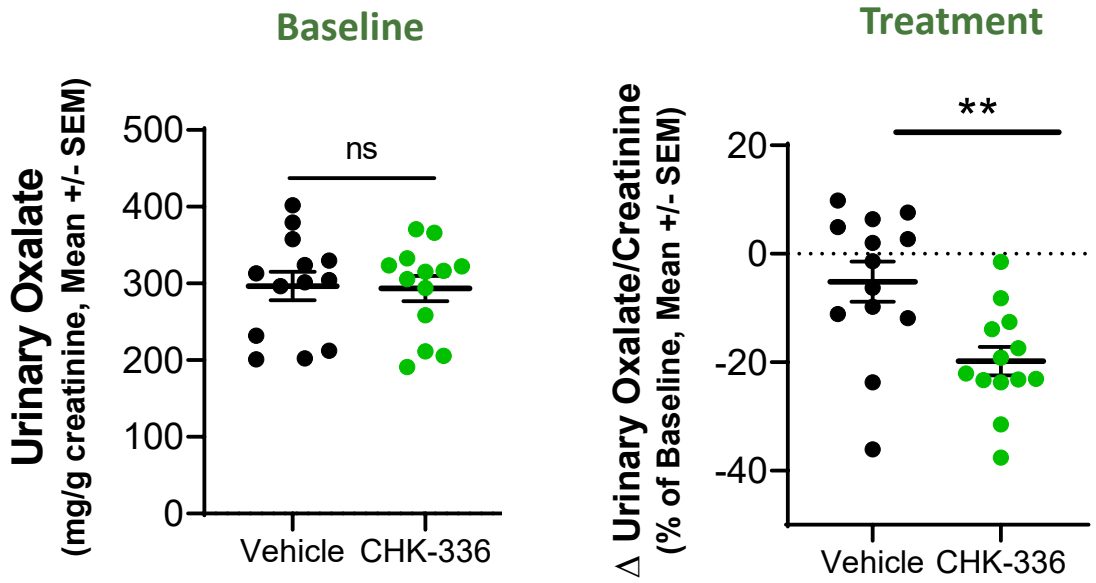
Male *Grhpr* KO Mice (12-15w)

4 x 24 hr urine collections **Baseline**

± CHK-336 po, qd, 7days

4 x 24 hr urine collections **Treatment**

CHK-336 Reduced Urinary Oxalate in PH2 Mice



In a *Grhpr* knockout model of PH2, low, once daily, oral CHK-336 significantly reduced urinary oxalate levels with a highly liver-targeted distribution profile

Knight et al 2012 Am J Renal Physiol; 302(6): F688-F693

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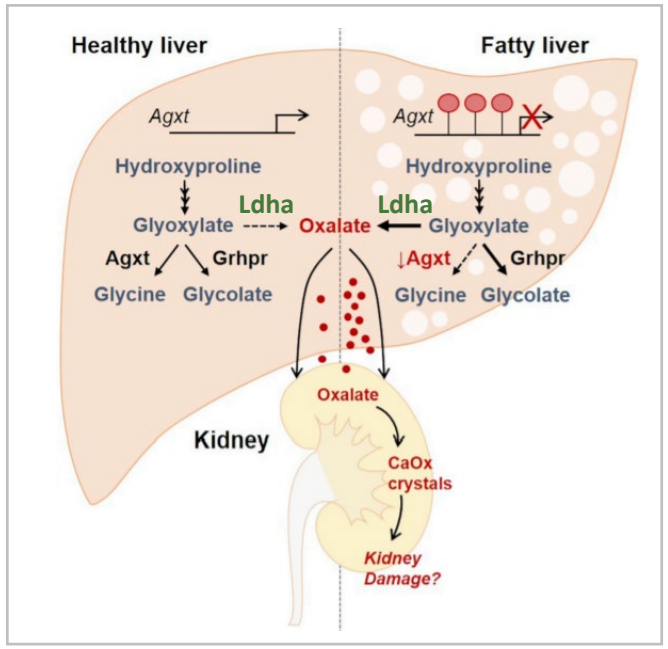
Potential for Mechanisms of Rare Genetic Diseases to Shed Insights on Mechanisms of Common Diseases

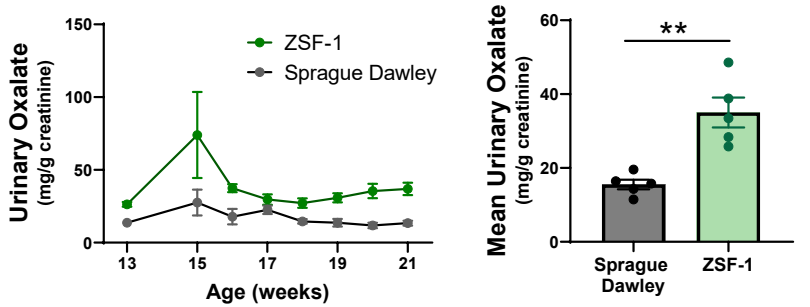
Impaired glyoxylate detoxification in NAFLD has been implicated as a hyperoxaluria risk factor



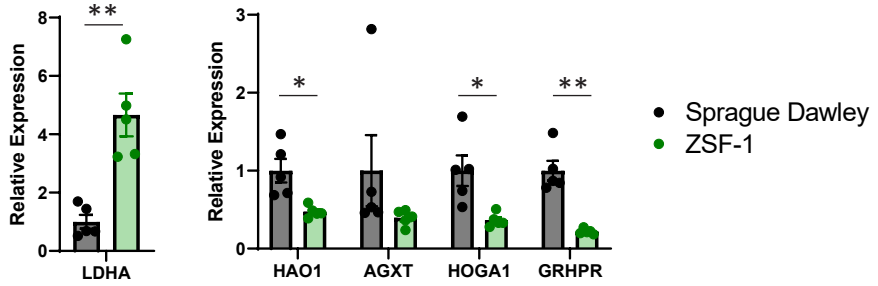
- Hypermethylation and downregulation of *Agxt* (the causal PH1 mutation) observed in mouse and human steatotic hepatocytes
- Steatosis severity in NAFLD adolescents correlates with UOx levels
- NAFLD is associated with increased risk of kidney stones (OR of 1.2 to 5)

Preliminary Chinook data shows elevated UOx in ZSF-1 obese hypertensive diabetic rat model

ZSF-1 rats have increased urinary oxalate



Dysregulated oxalate metabolism pathway



Supports Potential Therapeutic Benefit of CHK-336 in Secondary Hyperoxalurias Associated with Increased Endogenous Oxalate Production

Gianmoena et al Cell Reports 2021

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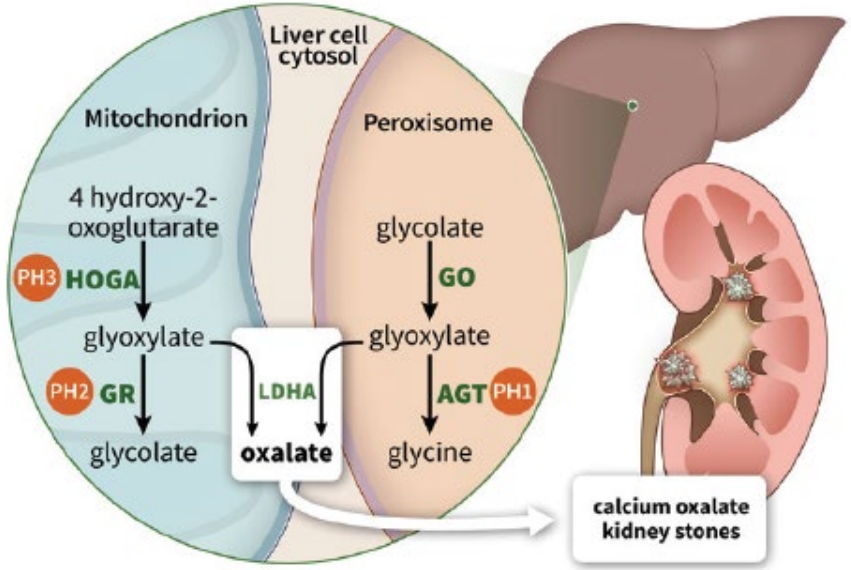
Summary

- PH is a group of devastating genetic diseases of increased hepatic oxalate production that can result in ESKD in young patients
- Lactate dehydrogenase (LDHA) is the final and committed step in production of oxalate from glyoxylate in the liver and is a potential therapeutic target to treat all forms of PH
- CHK-336 is a potent, liver-targeted LDHA inhibitor shown to significantly reduce urinary oxalate excretion in mouse models of PH1 and PH2



IND-enabling GLP toxicity studies have been completed with CHK-336 and a first-in-human single and multiple ascending dose study in healthy volunteers to determine safety, tolerability and PK/PD is anticipated to initiate in H1 2022

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