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

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
Clinical Outcomes and Management of PH

Clinical Outcomes

- **PH1**

**ESKD Risk by UOx**

- Less data is available for PH2 and PH3

Clinical Management

**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
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

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)

<table>
<thead>
<tr>
<th>ASSAY</th>
<th>CHK-336 IC$_{50}$</th>
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<tbody>
<tr>
<td><strong>Enzyme</strong></td>
<td></td>
</tr>
<tr>
<td>Human LDHA</td>
<td>0.2 nM</td>
</tr>
<tr>
<td>Mouse LDHA</td>
<td>0.3 nM</td>
</tr>
<tr>
<td><strong>Hepatocyte</strong></td>
<td></td>
</tr>
<tr>
<td>Human Cryopreserved Hepatocytes</td>
<td>121 nM</td>
</tr>
<tr>
<td>Mouse Fresh Hepatocytes</td>
<td>52 nM</td>
</tr>
<tr>
<td>Mouse Cryopreserved Hepatocytes</td>
<td>80 nM</td>
</tr>
<tr>
<td>Rat Cryopreserved Hepatocytes</td>
<td>130 nM</td>
</tr>
<tr>
<td>NHP Cryopreserved Hepatocytes</td>
<td>130 nM</td>
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</table>
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)

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
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

![Increase liver-targeting tissue distribution profile](chart)

** CHK-336 (1 µM) Demonstrates Active Uptake into Human Hepatocytes **

<table>
<thead>
<tr>
<th>Accumulation of CHK-336 (pmol/million cells)</th>
<th>Time (min)</th>
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<tbody>
<tr>
<td>[chart showing active and passive uptake rates]</td>
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</tbody>
</table>

- [chart showing CHK-336 accumulation at different temperatures]
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 pharmacokinetiс 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.
In Vivo Pharmacodynamic Effect of CHK-336 to Inhibit Oxalate Production

Male SD rats ± CHK-336 + $^{13}$C$_2$-glycolate

\[ \text{LDHA} \rightarrow \text{glyoxylate} \]

\[ \rightarrow \text{glyoxylate} \rightarrow \text{LDHA} \]

Urinary oxalate analysis

<table>
<thead>
<tr>
<th>CHK-336 Liver Concentration (µM)</th>
<th>Urinary $^{13}$C$_2$-Oxalate (mg/g creatinine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>0</td>
</tr>
<tr>
<td>CHK-336 1 µM</td>
<td>1</td>
</tr>
<tr>
<td>CHK-336 5 µM</td>
<td>2</td>
</tr>
<tr>
<td>CHK-336 20 µM</td>
<td>3</td>
</tr>
<tr>
<td>CHK-336 50 µM</td>
<td>4</td>
</tr>
</tbody>
</table>

CHK-336 Dose Dependently Blocks on $^{13}$C$_2$-Oxalate Production in Rats

Pharmacodynamic – Pharmacokinetic Relationship of CHK-336 in SD Rats

Liver IC$_{50}$ of ~0.3 µM
CHK-336 Reduces Urinary Oxalate Excretion in an Induced Mouse Model of PH1

Male C57BL/6 mice

Agxt siRNA (0.4 mg/kg IV)
Day 0 & 7

± CHK-336 po, qd 5-days

CHK-336 was Highly Liver Targeted 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.
Generation of a Genetic Mouse Model of PH1

Microinjection
- gRNA + Cas9 mRNA
- Fertilized mouse egg

Targeted Deletion of Agxt Exons 3-8
- Wildtype allele
- gRNA region
- Exon of mouse Agxt
- Knockout region

Legends
- F1
- R1

Founder Breeding
- F1 positives
- Germline transmission

F1 PCR Screening
- Positive for targeted truncated Agxt allele

Sequencing Confirmation
- Confirmed 5678 bp deletion

Homozygous Male PH1 mice
- Urinary Oxalate (mg/g creatinine)
- WT vs Agxt KO

Note: Female ko have mild phenotype
CHK-336 Reduces Urinary Oxalate Excretion in a Genetic Mouse Model of PH1

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.

*** P<0.001 v 0 mg/kg
Comparative Effect of CHK-336 and GO siRNA on Oxalate Production in PH1 Mice

Male Agxt KO Mice (13-14w)

GO siRNA (1, 3, 6 mg/kg, qw) \( ^{13}\text{C}_2\)-glycolate Tracer

CHK-336 (po, qd) \( ^{13}\text{C}_2\)-Ox UOx

GO siRNA FDA approved in late 2020 for PH1

CHK-336, investigational small molecule liver targeted LDHA inhibitor

Clinical significance of blocking LDHA to treat all types of Primary Hyperoxaluria (PH)

PH1 genetic mutation AGXT

PH2 genetic mutation GRHPR

PH3 genetic mutation HOGA1

LDHA

oxalate calcium oxalate kidney stones kidney damage/failure

GO
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

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

- **GO siRNA** (1, 3, 6 mg/kg, qw)
  - $^{13}$C$_2$-glycolate

- **CHK-336** (po, qd)
  - Tracer
  - $^{13}$C$_2$-Ox

Effect of CHK-336 Compared to GO siRNA on $^{13}$C$_2$-Glycolate to $^{13}$C$_2$-Oxalate in PH1 Mice

- **Day 7**
- **Urinary $^{13}$C$_2$ oxalate (mg/g creatinine)**
  - **WT**
  - **PH1**

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
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.
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

Male Grhpr KO Mice (12-15w) ± CHK-336 po, qd, 7 days

UOx

**Effect of CHK-336 in PH2 Mice**

Male Grhpr KO Mice (12-15w)

4 x 24 hr urine collections **Baseline**

4 x 24 hr urine collections **Treatment**

### CHK-336 Reduced Urinary Oxalate in PH2 Mice

**Baseline**

Vehicle CHK-336

**Treatment**

Vehicle CHK-336

**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**
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.
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