Discovery of CHK-336: A First-in-Class, Liver-Targeted, Small Molecule Inhibitor of Lactate Dehydrogenase for the Treatment of Primary Hyperoxaluria

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Primary Hyperoxaluria (PH)
Rare and severe disorder leading to oxalate overproduction and end stage renal disease (ESRD)

Genetic liver enzyme deficiency resulting in excessive oxalate production
- Patients form many calcium oxalate kidney stones
- Median age of kidney failure in PH1 is 23 years
- Only curative treatment is dual kidney-liver transplant; no approved drug therapies

Lower urinary oxalate (UOx) levels associated with reduced risk of ESRD

Clinical proof of concept achieved by injectable siRNA agents (GO-siRNA for PH1 and LDHA-siRNA for PH1/PH2)

No oral, small molecule therapies with potential to treat patients with all types of PH have been reported

Disease Progression of PH
- Abnormal liver metabolism of glyoxylate produces excess oxalate
- Calcium oxalate crystals form in the kidneys
- Decline in kidney function results in systemic oxalosis
- Onset of kidney failure
- Dialysis awaiting dual liver / kidney transplant

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 symptoms¹, therefore liver-targeting with low systemic levels is needed
- Liver-specific LDHA inhibition is expected to be safe and well tolerated

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

Three types of PH caused by different mutations:

- PH1: AGXT (AGT protein)
- PH2: GRHPR (GR protein)
- PH3: HOGA1 (HOGA protein)

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

CHK-336 demonstrates potent inhibition of LDHA in enzyme assays ($IC_{50}$ = 0.1-0.4 nM) and primary hepatocyte assays across multiple species ($IC_{50}$ = 52-293 nM)

CHK-336 also demonstrated tight 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>0.4 nM</td>
</tr>
<tr>
<td>Mouse LDHA</td>
<td>0.1 nM</td>
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<tr>
<td><strong>Hepatocyte</strong></td>
<td></td>
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<tr>
<td>Mouse Fresh Hepatocytes</td>
<td>52 nM</td>
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<tr>
<td>Mouse Cryopreserved Hepatocytes</td>
<td>80 nM</td>
</tr>
<tr>
<td>Rat Cryopreserved Hepatocytes</td>
<td>130 nM</td>
</tr>
<tr>
<td>Monkey Cryopreserved Hepatocytes</td>
<td>130 nM</td>
</tr>
<tr>
<td>Human Cryopreserved Hepatocytes</td>
<td>131 nM</td>
</tr>
<tr>
<td><strong>PH1 Cell</strong></td>
<td></td>
</tr>
<tr>
<td>Mouse Agxt Knockdown Hepatocytes (Oxalate Production)</td>
<td>293 nM</td>
</tr>
</tbody>
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Compound design and sub-nM potency guided by structural biology and X-ray crystallography of LDHA-inhibitor complexes.
Design of a Liver-Targeted LDHA Inhibitor

- In order to reduce the potential for any muscle-related toxicities, as is observed with complete loss-of-function of LDHA, the Chinook team engineered a liver-targeted tissue distribution profile.

- Strategy involves incorporating moieties that are recognized by liver-selective uptake transporters and reducing non-specific passive permeability.

- CHK-336 demonstrates active uptake into human, monkey and rat hepatocytes.
Pharmacokinetic and Pharmacodynamic Properties 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.

Well-profiled pharmacodynamic effect in mice and rats driven by liver concentrations: liver $EC_{50}$ of $\approx 3 \, \mu M$.

Human PK predictions suggest CHK-336 has the potential to be a low, once-daily oral dose in humans.
CHK-336 Produced Significant and Dose-dependent Reductions in Urinary Oxalate in a PH1 Mouse Model

- A mouse model of PH1 was generated by CRISPR-Cas9 deletion of exons 3-8 of Agxt; these mice exhibited elevated urinary oxalate as expected.

- CHK-336 was dosed orally, once-daily for 7 days in male Agxt KO mice and urinary oxalate concentrations were compared to a vehicle control group.

- Low, oral, once-daily doses of CHK-336 significantly reduced urinary oxalate; majority of treated mice reached the normal range observed in wild-type mice.

- Analysis of liver concentrations of CHK-336 resulted in a PK/PD relationship with a liver EC$_{50}$ of 1 – 5 μM CHK-336, consistent with rat liver PD values.

- Similar data generated in a mouse Agxt knockdown model of PH1.
Non-Clinical Safety Assessment Supports Continued Advancement of CHK-336 into IND-enabling Studies

Excellent in vitro Safety Profile
- Low risk of hERG mediated QT prolongation ($IC_{50} > 30 \, \mu M$)
- Non-mutagenic (negative in 5-strain AMES up to 5000 µg/well)
- Excellent off-target selectivity profile (<50% inhibition at 10 µM for 86 target panel, >450-fold selectivity for LDHA)

Low Drug-Drug Interaction (DDI) Potential
- Low risk of CYP-mediated DDI
  - No CYP3A4 inhibition or time-dependent inhibition ($IC_{50} > 30 \, \mu M$)
  - No CYP3A4 induction in hepatocytes ($IC_{50} > 10 \, \mu M$)

Promising Non-GLP in vivo Safety Profile
- Non-GLP in vivo safety studies suggest wide therapeutic margins over anticipated efficacious exposures
- Doses up to 1000 mg/kg/day explored in 14-day rat study
Conclusions

- Targeting LDHA, the terminal step in hepatic oxalate synthesis, represents a potential therapeutic strategy for all forms of PH, as well as other disorders arising from oxalate overproduction.
- By potently blocking LDHA and engineering a liver-targeted tissue distribution profile, CHK-336 represents a potentially safe and effective oral small molecule for the treatment of primary hyperoxaluria.
- CHK-336 shows robust efficacy in a PH1 mouse model at low, once-daily oral doses including the ability to reduce elevated urinary oxalate levels to the normal range.
- The non-clinical safety assessment of CHK-336 conducted to date supports continued advancement into IND-enabling studies.

CHK-336 is a first-in-class oral LDHA inhibitor with the potential to treat all subtypes of primary hyperoxaluria as well as other disorders arising from oxalate overproduction.