A Novel Recombinant Oral Urate Oxidase (UrOx) ALLN-346 Reduces Severe Hyperuricemia and Normalized Hyperuricosuria in Nephropathic UrOX Knockout (UrOxKO) Mice

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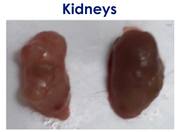
Hyperuricemia, Gout and Present Therapies

- An abnormally high level of blood uric acid, hyperuricemia, is the main cause of the painful inflammatory arthritis associated with gout.
- A growing body of evidence also suggests a role for hyperuricemia not only in gout, but also in the genesis and progression of chronic kidney disease (CKD), hypertension, coronary artery disease (CAD), and metabolic syndrome.[1,2]
- Renal excretion is the major route of uric acid elimination, but the gastrointestinal (GI) tract plays an increasingly recognized role in urate homeostasis, especially in CKD where renal uric acid excretion is impaired and approximately 50-70% of urate is secreted via intestine.[3-5]
- Existing urate lowering therapies (ULT) prevent production of uric acid (xanthine oxidase inhibitors), increase its excretion (uricosurics), or degrade it directly (intravenous uricase). Each has limitations in efficacy and/or tolerance that contribute to refractoriness to therapy in gout.
- Here, we targeted gut elimination of urate *in vivo* with ALLN-346, an orally administered, engineered urate oxidase (UrOx) optimized for proteolytic stability in the GI tract.
- We tested ALLN-346 in UrOxKO mice[6], a model with severe hyperuricemia, hyperuricosuria, and uric acid crystalline obstructive nephropathy.

Urate Oxidase Knockout (UrOxKO) Mouse Phenotype

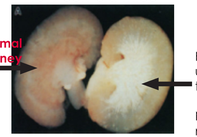
- **Severe hyperuricemia:**
  - ~14 mg/dL (6-20 mg/dL); with maintenance dose of allopurinol (ALLO), plasma urate is reduced to ~4 mg/dL (normal ~2 mg/dL)
- **Severe hyperuricosuria:**
  - ~4-7 mg/24h, varies daily; with maintenance dose of ALLO, plasma urate is reduced to normal range of 2-3 mg/24h
  - Mice renally excrete 20-40 fold more uric acid than humans based on kg body mass
- Mice develop diabetes insipidus, increase water intake, and excrete higher urine volume (~4-15 mL/24h)

Kidneys



Lobulated, pale, amorphous shape, multiple cysts; on maintenance dose of 150 mg/L ALLO

16-week-old mice



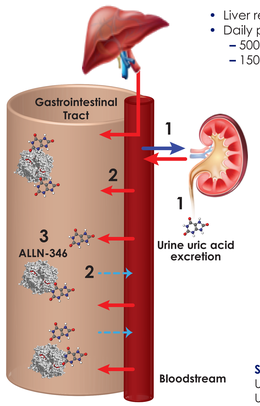
Kidney from UrOxKO mice with uric acid crystal deposits in renal tubules and collecting ducts

No tophi or crystals in joints, normal liver

Source: Wu, et al 1994

ALLN-346: Recombinant Urate Oxidase

- Novel recombinant, specific urate-degrading enzyme optimized for stability in the GI tract and with a mechanism of action suitable to reduce systemic urate burden
  - Orally administered
  - Specifically targets degradation of urate along the GI tract



- Liver releases urate into the bloodstream
- Daily production:
  - 500-700 mg from purine metabolism
  - 150-200 mg from diet

- **Healthy kidneys** filter ~70% of daily uric acid turnover; the remaining ~30% are secreted via intestine
- **In CKD**, extra-renal, intestinal elimination increases up to 50-70%, becoming a major route of urate elimination[3-5]
- Crystal deposition can cause inflammation and associated kidney damage, further contributing to decline in glomerular filtration rate

Source: Illustration adapted from Coe, Fred. "Control of Urine Oxalate Excretion." Kidneystones.uchicago.edu. University of Chicago, March 2017.

1. Kidneys filter urate from the blood; it is either reabsorbed into the circulation or is excreted into the urine
2. Urate is secreted from the circulation into the intestine, and it is partially reabsorbed
3. ALLN-346 mechanism of action is to degrade urate along the GI tract

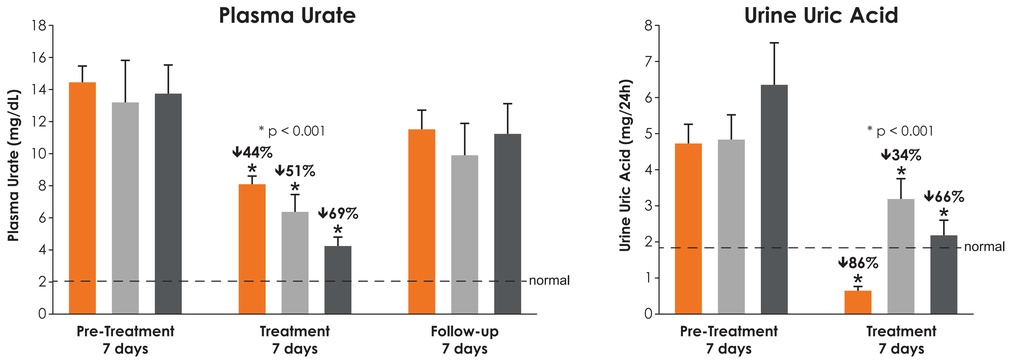
Study Design and Results

Objectives: Demonstrate effect of ALLN-346 on reduction of plasma urate and of urine uric acid excretion

Pre-Treatment: 7 days	Treatment: 7 days	Follow-up: 7 days
Discontinue ALLO		Discontinue Treatment
UrOxKO Mice n = 25	ALLN-346 (n = 8) ALLO 50 (n = 8) ALLO 150 (n = 9)	ALLN-346 (n = 8) ALLO 50 (n = 8) ALLO 150 (n = 9)

Treatment: ALLN-346 mixed with food (~700 units/day); ALLO (50 mg/L or 150 mg/L) in drinking water

Figure 1: Significant Reduction in Plasma Urate and Urine Uric Acid with ALLN-346 or ALLO Therapy



Shown is mean (standard error), $p < 0.05$ for difference between pre-treatment to treatment, paired t-test

Conclusions and Future Steps

- Oral therapy with ALLN-346 was well-tolerated and resulted in significant reduction of plasma urate and normalization of urine uric acid excretion after one week of treatment in an animal model of severe hyperuricemia.
- The effect on plasma urate reduction was similar to the ALLO dose of 50 mg/L, and the effect on urine uric acid excretion was superior to the maintenance dose of ALLO 150 mg/L which is required to sustain this animal model.
- The underlying physiology of hyperuricemia and the extrarenal pathway of uric acid elimination corresponds to the ALLN-346 mechanism of action of degradation urate along the GI tract.
- Future experimental studies will address the effect of ALLN-346 on fractional excretion of uric acid (FEUA) and potential renoprotection.

References: 1) Terkeltaub Nature 2010, **2)** Benn et al Front. Med 2018, **3)** Xu et al Pharm Biology 2016, **4)** Sorensen et al Arthritis Rheumatology 1965, **5)** Sorensen et al. Nephron 1975, **6)** Wu et al PNAS 1994

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