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Pilot study of reloxaliase in patients with severe enteric hyperoxaluria and hyperoxalemia

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Enteric hyperoxaluria (EH) is a serious condition that affects ∼250 000 people in the USA and can lead to recurrent kidney stones, oxalate nephropathy and chronic kidney disease (CKD) with a risk of kidney failure requiring chronic dialysis [1–3]. It is characterized by excessive urine oxalate (UOx) excretion ≥40 mg/24 h that is necessitated by increased intestinal oxalate absorption [4] as a consequence of gastrointestinal (GI) conditions associated with fat malabsorption [5]. When estimated glomerular filtration rate (eGFR) declines, plasma oxalate (POx) concentrations rise reflecting systemic oxalate load [6]. In severe courses of disease indicated by markedly increased POx concentrations, oxalate may deposit in various tissues throughout the body, a life-threatening condition called systemic oxalosis [7, 8]. However, to date, therapeutic options are limited with a focus on dietary modifications [4].

Here we describe an open-label, single-arm, multi-site pilot study of reloxaliase in subjects with EH and advanced CKD and an elevated POx (hyperoxalemia), defined by an eGFR <45 mL/min/1.73 m^2 and POx >5 µmol/L [6]. The primary objective of the study was to determine whether reloxaliase can reduce POx and UOx over 12 weeks of therapy. Reloxaliase is a recombinant oxalate decarboxylase enzyme from *Bacillus subtilis* expressed in *Escherichia coli* that catalyses the conversion of oxalate to formate and carbon dioxide. Reloxaliase is orally administered with food, and its mechanism of action is to degrade oxalate along the GI tract, thereby preventing its absorption [9]. Since reloxaliase is not systemically absorbed it has low potential for systemic toxicity.

Subjects received 7500 units of reloxaliase 5×/day with meals and snacks, and were instructed to follow their individual standard diet recommended to them by their physician, making no attempts to change their oxalate, calcium, sodium or fluid intake throughout the study. Efficacy assessments obtained at baseline and at Weeks

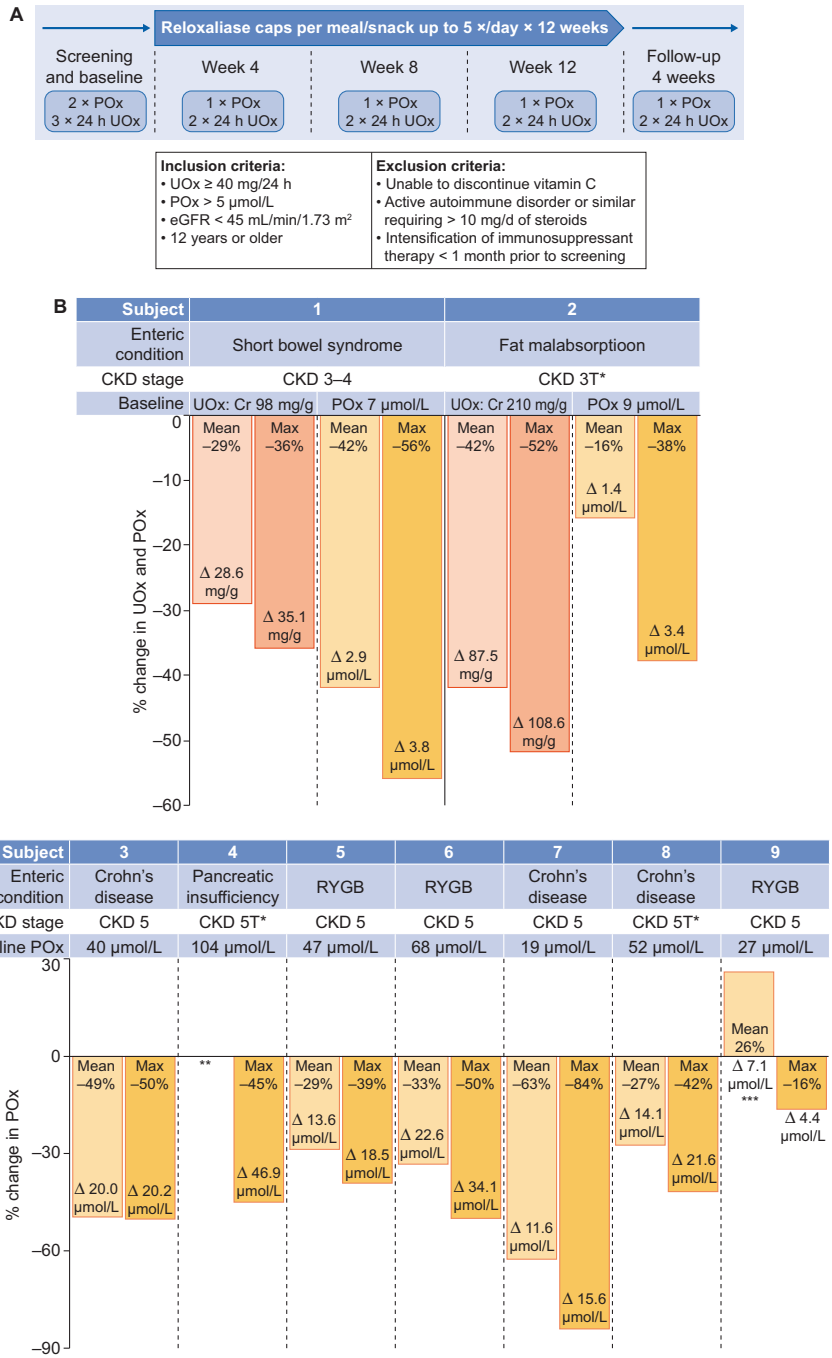

FIGURE 1: Study design and outcomes. (**A**) Study design and eligibility criteria. A Phase 2, open-label, single-arm, multi-site pilot study of reloxaliase treatment for 12 weeks. Eligible subjects collected one baseline POx and two 24-h UOx samples followed by monthly POx and UOx assessment. Blood samples were collected in the morning after an overnight fast and following the longest interval between dialysis treatments in haemodialysis patients. Subjects received 7500 units of reloxaliase 5×/day with meals and snacks. Inclusion and exclusion criteria for the study are shown in the lower panel. (**B**) Reloxaliase reduces both hyperoxaluria and hyperoxalemia in subjects with CKD 3b. Individual patient results of two patients with CKD Stage 3. Shown are two subjects who finished the trial and have all collections; one subject withdrew from the study after 4 weeks of treatment and did not collect a 24-h urine during the treatment period and only collected one POx. These results were included in the mean, max change from baseline. *Post-kidney transplant subject with CKD Stage 3. Δ values in bar graphs are mg/g for UOx and µmol/L for POx. (**C**) Reloxaliase reduces hyperoxalemia in subjects with CKD Stage 5. Individual patient results of seven patients with CKD Stage 5. *Post-kidney transplant subject with CKD Stage 5. **Subject 4 had only one POx sample collection during the study. ***Subject 9 last blood POx was not obtained following the longest interval between dialysis sessions and the dialysis regimen was changed. Δ values in bar graphs are in µmol/L.

4, 8 and 12 included POx, and in subjects with eGFR >15 mL/min/1.73 m^2, two 24-h urine collections (Figure 1A). The primary efficacy analyses were absolute and per cent change in POx and UOx from baseline to the average across Weeks 4–12. In addition, we sought to quantify the extent of systemic formate absorption as a potential by-product of oxalate degradation and determine the extent of reloxaliase absorption (if any) among this group of patients with enteric conditions. Standard safety assessments including treatment-emergent adverse events (TEAEs), concomitant medications, clinical laboratory parameters, vital signs, physical examinations and electrocardiograms were performed throughout. For more details, see Supplementary data for Complete Methods.

The study protocol was approved by the local Institutional Review Boards and Ethics Committees and was conducted at sites in the USA, UK and Germany. The ClinicalTrials.gov identifier is NCT03391804.

Of the 10 EH subjects who were enrolled, 8 completed all aspects of the study. One subject discontinued study drug at Week 9, but completed study procedures and the other withdrew from the study on Week 5. The median (min, max) age was 65.5 (44, 76) years, and seven were male. Bariatric surgery was found to be the underlying disorder in 40% of all participants, three subjects suffered from Crohn's disease, and one each from short bowel syndrome, pancreatic insufficiency and fat malabsorption. In total, 50% of the enrolled subjects reported a history of kidney stones. Three of the subjects had previously received a kidney transplant, and seven had CKD Stage 5 (six were on dialysis). Further clinical characteristics are listed in Supplementary data, Table S1.

Aggregate data of POx and UOx measurements throughout the study are presented in Supplementary data, Table S2, with individual results presented separately for subjects with CKD Stage 3b (Figure 1B, $n = 2$) and subjects with CKD Stage 5 (eGFR ≤15 mL/min/1.73 m^2 or on maintenance dialysis; Figure 1C, $n = 7$). Across all 10 subjects, 12 weeks of treatment with reloxaliase resulted in a decrease in POx from a mean (standard deviation) at baseline of 37.8 (31.6) µmol/L to 23.8 (16.2) µmol/L across Weeks 4–12 (29% reduction).

Two of three subjects with CKD Stage 3b completed the study, revealing that mean 24-h UOx fell from a baseline of 137.3 (62.9) mg/g creatinine ($n = 3$) to 95.9 (37.4) mg/g creatinine ($n = 2$) on reloxaliase treatment, which represents a mean reduction of 29% in Subject 1 and 42% in Subject 2, as shown in Figure 1B (maximal reduction at any time was 36% and 52%). Accordingly, mean POx in these two subjects dropped by 42% and 16%, respectively, under treatment. At follow-up off-treatment, POx was again 6.4 (0.49) µmol/L, similar to the baseline of 6.9 (2.1) µmol/L.

In the subjects with CKD Stage 5, POx decreased from a mean (standard deviation) at baseline of 51.1 (28.5) µmol/L to 29.8 (13.9) µmol/L across Weeks 4–12, representing 29.2% reduction. POx increased to 37.1 (18.6) µmol/L at the follow-up visit off-treatment. There was one dialysis subject who had an increase in POx on treatment (Figure 1C). This subject's blood samples were not obtained following the longest interval between dialysis sessions and the dialysis regimen was changed, both of which may have affected the steady-state POx and contributed to this result.

At Weeks 4 and 8, there was no evidence of elevated formate blood levels or absorption of reloxaliase (≤0.0001% of the daily doses, data not shown). In accordance, reloxaliase was generally well tolerated. No drug-related serious adverse events were reported. TEAEs were most commonly reported in the GI organ system (Supplementary data, Table S3). Two subjects discontinued study drug early: one subject experienced a TEAE (not related to study drug); the subject subsequently decided to discontinue treatment at Week 9. The other subject had a drug interruption due to GI adverse event, which did not recur on rechallenge. The same subject withdrew from the study on Week 5 for reasons unrelated to the study.

To our knowledge, this is the first demonstration of a pharmacological therapy for POx reduction in subjects with EH and advanced CKD. In a disease process characterized by systemic oxalate deposition, reduction of oxalate has the potential to meaningfully benefit patients with this life-threatening disorder. Hence, reloxaliase might also provide an adjunctive therapy for a more successful kidney transplantation in EH patients, as acute oxalate nephropathy is a dreaded complication after surgery due to transient severe hyperoxaluria [10, 11]. Even if the kidney allograft survives this initial period, persistent [11, 12] or new-onset [13] hyperoxaluria can reduce long-term graft survival secondary to recurrent kidney stones and oxalate nephropathy.

UOx and POx are accepted surrogate endpoints in clinical trials in oxalate-related disorders [14], yet we have not demonstrated an effect on a clinical outcome, such as stone events, progression of CKD or death. Further limitations of our study include the small size of the study population, and the fact that it employed an open-label uncontrolled design.

Based on our findings, subsequent studies are warranted to evaluate the effect of reloxaliase on long-term outcomes in EH and advanced CKD such as recurrence of kidney stones, preservation of kidney function and allograft survival.

SUPPLEMENTARY DATA

Supplementary data are available at ndt online.

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AUTHORS' CONTRIBUTIONS

A.T.K., F.K. and J.C.L. contributed to research idea and study design; D.G. and A.T.K. contributed to data acquisition; A.P., D.G., M.T.K., A.T.K., J.C.L. and F.K. contributed to data analysis/interpretation. Statistical analysis: Advanced Clinical. Each author contributed important intellectual content during manuscript drafting or revision, accepts personal accountability for the author's own contributions, and agrees to ensure that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

CONFLICT OF INTEREST STATEMENT

D.G. is employed by Allena Pharmaceuticals. A.T.K. is employed by and has ownership interest in Allena Pharmaceuticals. M.T.K. serves as a consultant to Allena. F.K. serves as a consultant to Allena Pharmaceuticals, Alnylam Pharmaceuticals and Oxthera Pharmaceuticals. He reports personal fees from Advicenne, Sanofi and Fresenius Medical Care. J.C.L. reports receiving grants and other from Allena, and reports serving on the advisory boards of Novobiom and Synlogic, during the conduct of the study. He is a consultant for Allena, Alnylam, American Board of Internal Medicine, Dicerna, Orfan, OxThera, Retrophin, and Siemens; receives funding from Allena, Alnylam, Dicerna, OxThera, Retrophin and Siemens; and serves on the advisory board for Orfan. A.P. reports personal fees from Alnylam Pharmaceuticals. F.K. and J.C.L. were both clinical trial site Principal Investigators of the study.

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Subfertility and early menopause in women with glomerular disease

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Subfertility, an inability to achieve desired conception for an extended period, affects up to one in six couples and leads to considerable psychological distress [1]. Due to an altered hypothalamic–pituitary–ovarian axis, women with advanced chronic kidney disease (CKD) are more likely to experience menstrual irregularities, subfertility and early menopause [2–6].