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Novel bifunctional inhibitors of xanthine oxidase and URAT1 induce profound hypouricemia in human subjects

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Abstract

Background: We found that a prototype anticancer drug (RLBN1001) induced marked hypouricemia in studies comprising > 350 human subjects. Preliminary exploration suggested dual effects on uric acid (UA) production and excretion. **Objectives**: Given the unusual clinical potency, we sought to: (1) identify mechanism(s) of hypouricemia; (2) clarify structure-activity relationships (SARs) to targets of UA metabolism and genotoxicity; and (3) use these insights to develop analogs that would enhance hypouricemic activity and eliminate genotoxicity, thereby discovering potentially useful treatments for gout. **Methods**: Clinical proof-of-concept (POC) was verified by examining biochemical effects in 50 human subjects treated with RLBN1001. Recursive chemical syntheses were then conducted by exploring SARs using four principal bioassays of activity: renal UA transporters (URAT1 [SLC22A12] and splice variants of SLC2A9 [GLUT9a/b]); xanthine oxidase (XO); and *in vitro* mouse micronucleus (MMN) to detect genotoxicity. **Results**: Over a 15-fold clinical dosing range with RLBN1001, nadir levels of hypouricemia (≤ 1.0 mg/dL) were not dose-related, indicating the minimal effective dose was below the lowest dose examined (100 mg/m^2/d x 5d). At low and high doses, hypouricemia was associated with increased urinary excretion of both UA and total oxypurines. This drug was a potent inhibitor of URAT1 but not GLUT9a/b, a modest inhibitor of XO, and a potent clastogen in the MMN assay. We iteratively synthesized a library of novel analogs and identified new compounds that are potent inhibitors of both XO (i.e., 2-to-3 fold more potent than allopurinol) and URAT1 (5-to-45 fold more potent than lesinurad), but devoid of genotoxicity. One compound showed an effect on urate efflux by GLUT9b, but other compounds showed minimal effects. **Conclusions**: Having established compelling clinical POC with the prototype, we have synthesized a series of unique compounds with strongly enhanced activities that both reduce UA production and enhance UA excretion. Pharmaceutical properties of a lead compound are being optimized with the objective of developing a novel, potential first-line treatment for hyperuricemic patients with gout.

Background

One of us (RPW) conducted first-in-man clinical trials with a novel, putative cytotoxic drug, RLBN1001.[1] While failing to show anticancer promise, we observed the drug was associated with a profound decrease in serum uric acid (UA).[2] Having shown that the drug did not interfere with the assay for UA, we found that this agent exhibited both uricosuria as well as increased excretion of urinary oxypurines, suggesting a second potential effect on UA production. In early studies, we explored the latter finding and showed the drug was a modest inhibitor of xanthine oxidase; however, the combined activities failed to account for the observed magnitude of clinical hypouricemic potency.[2,3] In view of the distinctly unmet medical need, we re-examined this agent to pursue three principal objectives:

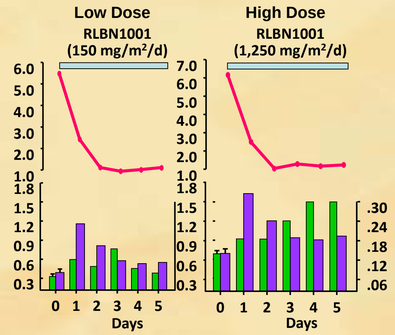
- Elucidate mechanism(s) of cytotoxicity and determine whether this activity could be dissociated from the hypouricemic effect;
- Clarify uricosuric mechanism(s) related to transporter biology;
- Clarify mechanism(s) of decreased UA production.

Clinical Study

Design: RLBN1001 was administered to 50 subjects by continuous IV infusion daily for 5 days in cohorts of 3-6 subjects per dose level. Intra-subject dose escalation was not allowed. Doses are expressed per body-surface area (i.e., mg/m^2). Initial and lowest on-study values of serum uric acid (mean ± SEM) are shown for selected levels over the 15-fold dose range in 50 subjects.

Dose	Serum Uric Acid (mg/dL) Initial Value	Lowest Value
100	6.2 (4.2–6.9)	1.0 (0.8–1.8)
150*	5.4 (3.1–8.1)	1.2 (0.6–3.9)
150	6.2 (3.4–6.2)	1.2 (0.6–3.9)
200	5.8 (3.7–10.5)	0.8 (0.7–1.3)
250	5.8 (4.4–7.8)	1.2 (0.8–1.3)
750	4.7 (3.6–9.0)	1.1 (0.4–1.3)
1000	5.3 (3.8–6.9)	1.2 (0.7–2.5)
1500	4.4 (3.1–7.3)	1.2 (0.7–1.7)
Mean ± SD	5.5 ± 1.9	1.2 ± 0.6

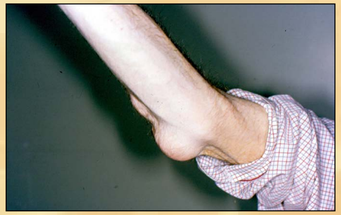
No dose-effect correlation over 15-fold range. Implication: Lowest effective dose was not identified in this study

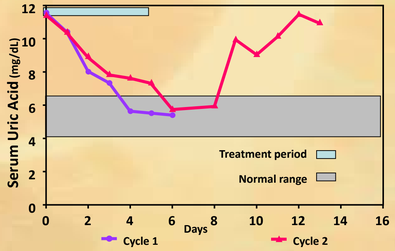


Profound hypouricemia (< 1.0 mg/dl) is associated with increased excretion of both uric acid and oxypurine precursors

Chart shows changes in serum uric acid (mg/dL; top, red); urinary uric acid (mg/Cr [mg]/24 h, bottom, purple; and urinary oxypurines (mmol/Cr [g]/24h, bottom, green.

RLBN1001 Lowers Serum Uric Acid in Severe Tophaceous Gout





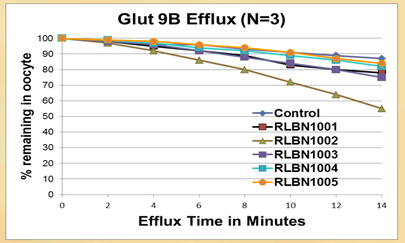
Single patient IND: Treatment with two 5-day infusions (~500 mg/m^2/d) in cycles 3 weeks apart. Nadir not identified due to short infusion/followup. Recovery 48h after treatment cessation noted in Cycle 2.

Methods

We assessed RLBN1001, known human metabolites, and novel compounds developed via recursive syntheses based on iterative knowledge of structure-activity relationships (SARS). We initially assessed whether uricosuria was related to effects on high-capacity renal urate transporters, URAT1 and/or GLUT9a/b. URAT1 inhibition was assayed in CHO-K1 cells stably expressing transfected hu-URAT1 using orotic acid as a substrate probe and benzbromarone as a control (Solvo). SLC2A9 (GLUT9) long and short splice variants were expressed in Xenopus oocytes and urate efflux was assayed as described.[4] We independently developed a method to assess xanthine oxidase inhibition using allopurinol as a control (AMRI). RLBN1001 was negative in the Ames test but strongly clastogenic in the *in vitro* mouse micronucleus assay (MMN); thus, we used the MMN to assess genotoxicity (Cerep, Seattle).

Results

GLUT9b Inhibition by RLBN 1001-1005



One (highly clastogenic) compound (RLBN1002) showed significant (~50%) inhibition of GLUT9b. (No effect was observed on GLUT9a [data not shown]).

Clastogenicity and Comparative Inhibition of URAT1 and Xanthine Oxidase

Controls and Prototype Compound	URAT1 IC50 (μM±SEM) [EXCRETION]]	Xanthine Oxidase IC50 (μM ± SEM) [PRODUCTION]	Mouse Micronucleus [GENOTOXICITY]
Probenecid	9.7	-	ND
Benzbromarone	0.2	-	ND
Lesinurad	53 ± 6*	>300†	ND
Allopurinol	>300†	2.13	ND
RLBN1001	**5.4 ± 1.0**	**274**	**+**

*Proc EULAR Abstract #THU0357, 2008; †presentation estimate

The prototype, RLBN1001, was a moderate inhibitor of URAT1 (~10-X more potent than lesinurad) and a (very) modest XO inhibitor. Metabolites and derivatives were developed by examining recursive SARs from in vitro assays.

Compound Clastogenicity and Inhibition of URAT1 and Xanthine Oxidase

Compound	URAT1 IC50 (μM±SEM)	Xanthine Oxidase IC50 (μM ± SEM)	Mouse Micronucleus
RLBN1001	**5.4 ± 1.0**	**274**	**+**
RLBN2022	1.2	>300	+
RLBN2027	6.3	243	+
RLBN2028	-	206	Negative
RLBN2023	**2.6 ± 0.6**	**1.1**	**Negative**
RLBN2024	**9.4 ± 0.6**	**0.9**	**Negative**
RLBN3022	**3.5**	**1.9**	**Negative**

URAT1 inhibition was well-conserved. A non-clastogenic series of compounds emerged early. Further syntheses showed that – of the 3 assays -- XO inhibition was most sensitive to structural alteration. Novel derivatives of RLBN3022 inhibit XO at nM concentrations.

Conclusions

1. RLBN1001 induces profound hypouricemia in human subjects via bifunctional inhibition of XO and URAT1. A minimal effective dose is ≤ 100 mg/m^2/d (i.e., ≤ 10% of the Phase 2 dose administered to > 300 oncology patients).
2. Novel non-clastogenic derivatives exhibit combined XO and URAT1 inhibition equivalent or superior to allopurinol and lesinurad, respectively.
3. Current studies are optimizing pharmaceutical properties of the RLBN3022 series, thereby enabling once-daily oral dosing.

References

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