Gout and Uric Acid: From Disease to Transporters to Therapy

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Disclosures/Conflict of Interest

• Previous employee of Ardea Biosciences, Inc., a wholly-owned subsidiary of AstraZeneca PLC

• Member of ARTA Bioscience Advisory Board

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History of Selective Uric Acid Reabsorption Inhibitor (SURI) Discovery and Development

• Ardea was a small biotech company conducting early trials in Oncology and Antivirals

• Phase 1 antiviral clinical trial
  - Treated patients were exhibiting unusual, very significant decreases in serum uric acid levels
    • Higher FEUA – affecting uric acid excretion

• One metabolite was highly excreted to urine; this metabolite was purified
  • It was a URAT1 inhibitor – a renal uric acid transporter important for maintaining uric acid levels in humans

• Lesinurad was approved in 2015 for the treatment of gout

• Verinurad is a second generation compound in development

FEUA=fractional excretion of uric acid.
Production and Excretion of Uric Acid
(What Is Uric Acid?)

Endogenous purines (~66%)

Exogenous (dietary) purines (~33%)

Humans have high serum uric acid 4.0–6.0 mg/dL

Overall uric acid excreted via gut: 30%

Overall uric acid excreted via kidney: 70%

Urate reabsorbed (90%)

Urate excreted (10%)

Humans Have Higher sUA Levels Than Most Other Animals

- Great apes and humans lack uricase\textsuperscript{1,2}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure.png}
\caption{Graph shows mean sUA levels of various primates obtained from the San Diego zoo. The values for mean sUA in American men and women were obtained from the NHANES study. NHANES. National Health and Nutrition Examination Survey.}
\end{figure}

\textsuperscript{1}Graph shows mean sUA levels of various primates obtained from the San Diego zoo.
\textsuperscript{2}The values for mean sUA in American men and women were obtained from the NHANES study.
\textsuperscript{3}NHANES. National Health and Nutrition Examination Survey.

\begin{enumerate}
\end{enumerate}
Conversion of Tyrosine-365 (Y) to Phenylalanine-365 (F) in URAT1 Occurred Relatively Late in Evolution

<table>
<thead>
<tr>
<th>Species</th>
<th>AA 365: F/Y</th>
<th>Urate transport Km (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>Y</td>
<td>857*</td>
</tr>
<tr>
<td>Baboon</td>
<td>F</td>
<td>79</td>
</tr>
<tr>
<td>Human</td>
<td>F</td>
<td>122</td>
</tr>
</tbody>
</table>

Statistical significance compared to human URAT1 was determined using the unpaired Student’s t-test * P < 0.0001.
Gain of High URAT1 Uric Acid Affinity Occurred at Same Time in Evolution as Loss of Uricase Activity

How did we know to examine the evolution of amino acid 365? - Mapping inhibitor binding site on URAT1

Conclusions

• Multiple proteins changed function to accommodate higher levels of uric acid
• There was selective pressure for this advance, and gout in humans was an unintended consequence
• Many hypotheses exist, though empirical data favors the notion that uric acid enhances the ability to store energy (fat) when living in a limited resource environment
Disadvantages of Higher sUA: Prevalence of Comorbidities Rises With Increasing sUA

Figure indicates prevalence of comorbidities according to sUA level in participants with and without hyperuricaemia from the NHANES 2007–2008.
CKD, chronic kidney disease; NHANES, National Health and Nutrition Examination Survey.

Asymptomatic Hyperuricemia: MSU Crystal Accumulation/Deposition

- MSU crystal deposition occurs prior to clinical manifestations of gout\(^1\)
- Deposition is greater in patients with asymptomatic hyperuricemia than with normal uric acid levels\(^2\)

DECT scan showing MSU crystal deposition (green) surrounding the right first MTP and along left midfoot in a patient with asymptomatic hyperuricemia\(^3\)

DECT, dual energy computed tomography; MSU, monosodium urate; MTP, metatarsophalangeal.

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Gout – A Urate Crystal Deposition Disease

![Image of gout symptoms with inflamed joints.](image-url)
Inefficient Renal Excretion of Uric Acid (Low FEUA) Is a Characteristic of Gout Patients

Data from Phase 1 clinical trials

FEUA, Fractional excretion of uric acid.

Renal Transport and Excretion of Urate

Renal Transport and Excretion of Urate

- Urate is completely filtered through the glomerulus into the proximal tubule\textsuperscript{1,2}

\textsuperscript{2} Pittman JR et al. \textit{Am Fam Physician.} 1999;59(7):1799–1806.
Renal Transport and Excretion of Urate

- URAT1 is a major transporter in the tubule system responsible for uric acid reabsorption and maintaining overall uric acid homeostasis\textsuperscript{3,4}

Renal Transport and Excretion of Urate

- Uric acid that is not reabsorbed by the kidneys is excreted in the urine
  - Of uric acid excreted, 70% is renal and 30% is intestinal

Renal Transport and Excretion of Urate

- Normal uric acid excretion is approximately 10% of the filtered load\(^1\)

In patients with gout, increased reabsorption (via URAT-1, etc) occurs, resulting in under-excretion of uric acid (~5% of filtered load)\(^1\)
Renal Transport and Excretion of Urate

- Inhibiting the mechanisms associated with reabsorption can increase uric acid excretion\(^1\)

GOUT PATIENT URIC ACID EXCRETION

- **URAT-1 inhibition**
- Probenecid
- Benzbromarone
- Lesinurad
- Verinurad
- Arhalofenate

**Filtration**

**Reabsorption**

**Secretion**

**Excretion**
Verinurad (RDEA3170)
A Highly Potent ULT in Phase II

- Potent uric acid lowering potential
- Potential for substantial uric acid lowering with low-dose combinations compared to high dose (up-titrated xanthine oxidase inhibitor)
URAT1 Phenylalanine 365 Is Crucial for High Affinity Verinurad Interaction

Y365F is a gain of function mutation (10 X increased affinity)
Human URAT1 Residues 35, 365, and 481 Interact to Enhance Affinity for Verinurad

<table>
<thead>
<tr>
<th></th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rat URAT1</td>
<td>41</td>
</tr>
<tr>
<td>r-N35S</td>
<td>10</td>
</tr>
<tr>
<td>r-Y365F</td>
<td>2.9</td>
</tr>
<tr>
<td>r-M481I</td>
<td>11</td>
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<tr>
<td>r-N35S/Y365F</td>
<td>0.47</td>
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<tr>
<td>r-N35S/M481I</td>
<td>4.43</td>
</tr>
<tr>
<td>r-Y365F/M481I</td>
<td>0.59</td>
</tr>
<tr>
<td>r-N35S/Y365F/M481I</td>
<td>0.14</td>
</tr>
<tr>
<td>human URAT1</td>
<td>0.025</td>
</tr>
</tbody>
</table>
URAT1 Inhibitors

Verinurad (RDEA3170)

lesinurad

benzbromarone

probenecid

sulfinpyrazone
URAT1 Binding Assay Demonstrates Competitive Binding by Inhibitors

- Bind $^3$H-verinurad to membranes from HEK-293T cells expressing URAT1
- $^3$H-verinurad binding to URAT1 is...
  - Displaced by URAT1 inhibitors, at potencies similar to the inhibition of URAT1 transport activity
  - Specific and high affinity

Differential Importance of Individual URAT1 Residues Is Due to Alterations in Binding Affinity

% $^3$H-verinurad bound

[cold compound] log M

<table>
<thead>
<tr>
<th>construct, compound</th>
<th>$IC_{50}$ (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>URAT1, verinurad</td>
<td>0.008</td>
</tr>
<tr>
<td>URAT1 benzbromarone</td>
<td>0.063</td>
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<tr>
<td>F449Y verinurad</td>
<td>0.005</td>
</tr>
<tr>
<td>F449Y, benzbromarone</td>
<td>0.69</td>
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</table>
Phe-365 and Other Residues That Interact With Inhibitors Project Within the Transporter Channel

Images of human OAT1 3-dimensional model\(^1\) with the cytoplasmic surface facing the viewer – equivalent URAT1 residues highlighted

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Conclusions

• Multiple uricosuric agents compete for direct interaction at a single site on URAT1

• Binding pocket is likely centrally located within the core of the transporter

• Amino acids within the binding pocket have differing effects on the affinity of different uricosuric agents
Verinurad lowers serum uric acid (sUA) by increasing fractional excretion of uric acid (FEUA) in humans

A single 40 mg dose of verinurad lowered baseline sUA levels by up to 60% for a sustained time period

Verinurad increased the FEUA in a dose-dependent manner, with a half-maximal effective plasma concentration of 0.022 µM (22 nM)

Experiments were performed in healthy human volunteers.
Study 204 (Phase 2a, US): PK/PD Study of Verinurad in Combination With Febuxostat

<table>
<thead>
<tr>
<th>POPULATION</th>
<th>Gout subjects with sUA ≥8.0 mg/dL, who are either ULT-naïve or have previously received ULT, N=64</th>
</tr>
</thead>
</table>
| PRIMARY OBJECTIVES | • To assess the PK and PD effect of verinurad + febuxostat  
• To evaluate the safety and tolerability of verinurad + febuxostat |

<table>
<thead>
<tr>
<th>≥14 days</th>
<th>Day 1–Day 7</th>
<th>Day 8–Day 14</th>
<th>Day 15–Day 21</th>
<th>Day 22–Day 28</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Colchicine 0.6 mg QD</td>
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**Cohorts**

<table>
<thead>
<tr>
<th>Cohorts 1–4</th>
<th><strong>Washout period</strong>*</th>
<th><strong>RANDOMIZE</strong></th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>FBX 40 mg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>verinurad + FBX 40 mg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>verinurad + FBX 80 mg</td>
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<tr>
<td></td>
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<td>FBX 80 mg</td>
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<td></td>
<td>verinurad + FBX 80 mg</td>
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<td></td>
<td>verinurad + FBX 40 mg</td>
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<tr>
<td></td>
<td></td>
<td>FBX 40 mg</td>
</tr>
</tbody>
</table>

*Cohort 1 (verinurad 10 mg): PK data available for 13 subjects; PD data available for 10–11 subjects
Cohort 2 (verinurad 15 mg): PK/PD data available for 12 subjects
Cohort 3 (verinurad 5 mg): PK/PD data available for 12 subjects
Cohort 4 (verinurad 2.5 mg): PK/PD data available for 8–9 subjects
Cohort 5 (verinurad 10, 15 or 20 mg): Adaptive design (n=11)

*Washout period of at least 14 days for subjects who have previously received ULT.

FBX, febuxostat; PD, pharmacodynamic; PK, pharmacokinetic; QD, once daily; ULT, urate-lowering therapy.
Study 204 (Phase 2a, US): sUA Lowering With Verinurad + Febuxostat

- There was a consistent dose trend between 40 mg and 80 mg febuxostat in the range of 2.5–10 mg verinurad

FBX, febuxostat; sCr, serum creatinine.
**Study 204 (Phase 2a, US):**

**Urinary Urate Excretion With Verinurad + Febuxostat**

- Urinary urate excretion with verinurad + febuxostat was comparable to or lower than baseline at all time points.
- No sCr elevations ≥1.5 x baseline were observed.

FBX, febuxostat; sCr, serum creatinine; VERU, verinurad.
Summary

• Inhibition of URAT1 by verinurad is dependent on high affinity interaction with F365, S35 and I481
• Verinurad, a SURI that inhibits URAT1, directly improves poor fractional excretion of uric acid in gout patients
• A medical need exists for effective and well tolerated URAT1 inhibitors for combination therapy
• Verinurad is a potential new therapy for hyperuricemia associated with gout used in combination with a xanthine oxidase inhibitor (e.g., allopurinol, febuxostat)