Preclinical Characterization of Vadadustat (AKB-6548), an Oral Small Molecule Hypoxia Inducible Factor Prolyl-4-Hydroxylase Inhibitor, for the Potential Treatment of Renal Anemia

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Disclosures

- The authors are employees of Akebia Therapeutics, which funded the studies

Disclaimers

- Vadadustat is an investigational drug. Vadadustat is not approved by the United States Food and Drug Administration or any regulatory authority.
Objective

- To summarize the preclinical pharmacological characterization of vadamustat
HIF and the prolyl-4-hydroxylase domain enzymes

Abbreviations:

O₂ = oxygen
PHD = prolyl-4-hydroxylase domain
HIF = hypoxia inducible factor
EPO = erythropoietin
Hb = hemoglobin
RBC = red blood cell
Vadadustat inhibits recombinant human PHD1, PHD2 and PHD3 at equivalent nanomolar concentrations*

*Measured by Time-Resolved Fluorescence Resonance Energy Transfer (TR-FRET) Assay. Data represent Mean ± SD.

Abbreviations:
PHD = prolyl-4-hydroxylase domain
IC$_{50}$ = half maximal inhibitory concentration
pIC$_{50}$ = negative log of the IC$_{50}$ value in molar

<table>
<thead>
<tr>
<th></th>
<th>Mean (95% Confidence Interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC$_{50}$ value (nM)</td>
</tr>
<tr>
<td>PHD1</td>
<td>15.36 (11.96, 19.73)</td>
</tr>
<tr>
<td>PHD2</td>
<td>11.83 (8.20, 17.07)</td>
</tr>
<tr>
<td>PHD3</td>
<td>7.63 (7.21, 8.07)</td>
</tr>
</tbody>
</table>
Vadadustat-O-glucuronide inhibits recombinant human PHD2 at micromolar concentration*

*Measured by TR-FRET Assay. Data represent Mean ± SD.

<table>
<thead>
<tr>
<th></th>
<th>IC_{50} value (μM)</th>
<th>pIC_{50} value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (95% Confidence Interval)</td>
<td>2.31 (1.74, 3.08)</td>
<td>5.64 (5.51, 5.77)</td>
</tr>
</tbody>
</table>

Inhibition is approximately 200-fold less potent than the parent compound at the IC_{50}

Abbreviations:
PHD2 = prolyl-4-hydroxylase domain 2
IC_{50} = half maximal inhibitory concentration
pIC_{50} = negative log of the IC_{50} value in molar
Vadadustat is a competitive inhibitor of 2-oxoglutarate for recombinant human PHD2*

*Measured by TR-FRET Assay. Data represent Mean ± SD.

Abbreviations:
PHD2 = prolyl-4-hydroxylase domain 2
Vadadustat inhibition of recombinant human PHD2 is not sensitive to iron concentration in vitro*

*Measured by TR-FRET Assay. Data represent Mean ± SD.

<table>
<thead>
<tr>
<th>Vadadustat + 100 nM Fe²⁺</th>
<th>Vadadustat + 1 µM Fe²⁺</th>
<th>Vadadustat + 10 µM Fe²⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC₅₀ (nM)</td>
<td>19.25 ± 5.74</td>
<td>3.91 ± 0.38</td>
</tr>
</tbody>
</table>

Abbreviations:
PHD2 = prolyl-4-hydroxylase domain 2
IC₅₀ = half maximal inhibitory concentration
Vadadustat was shown to stabilize both HIF-1α and HIF-2α in Hep3B and HUVEC cell lines in a dose and time dependent manner*.

Abbreviations:
HIF1α = hypoxia inducible factor-1 alpha
HIF2α = hypoxia inducible factor-2 alpha
Hep3B = human hepatocarcinoma cell line
HUVEC = human umbilical vein endothelial cell

*Measured by Mesoscale Discovery (MSD) Electrochemiluminescence Assay. HIF1α and HIF2α were normalized to total cellular protein (pg/µg). Data represent Mean ± SD.
Erythropoietin (EPO) secretion is increased in vitro after exposure of Hep3B cells to vadadustat*

*Measured by an Enzyme Linked ImmunoSorbent Assay (ELISA) after 24 hrs incubation. Data represent Mean ± SD. + P < 0.05 vs respective DMSO Control, Tukey’s Multiple Comparisons Test

**Abbreviations:**
DMSO = dimethylsulfoxide vehicle
EC$_{50}$ = half maximal effective concentration
Production of vascular endothelial growth factor (VEGF) was not observed to increase in vitro after exposure of Hep3B cells to vadadustat*

*Measured by Enzyme Linked ImmunoSorbent Assay (ELISA) after 24 hrs incubation. Data represent Mean ± SD. + P < 0.05 vs 0.1% DMSO, Tukey’s Multiple Comparisons Test.

**Abbreviations:**
DMSO = dimethylsulfoxide vehicle
Single-dose administration of vadalustat in rats was shown to increase the circulating levels of EPO in a time and dose dependent manner.*

*Measured by Enzyme Linked ImmunoSorbent Assay (ELISA). Data represent Mean ± SD.
Multi-dose exposure to vadadustat in mouse, rat and dog demonstrated increases in hemoglobin and hematocrit.

Duration of treatment of normal animals:
- Mouse = up to 6 months
- Rat = up to 2 years
- Dog = up to 9 months
In mouse, rat and dog, vadadustat had a relatively short half-life and did not accumulate after repeat dosing.

<table>
<thead>
<tr>
<th>Species</th>
<th>Dose Level (mg/kg)</th>
<th>Day</th>
<th>Gender Combined $T_{1/2}$ (h)</th>
<th>Gender Combined AUC$_{last}$ (µg*h/mL)</th>
<th>Accumulation Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>100</td>
<td>1</td>
<td>2.40</td>
<td>234</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>56</td>
<td>1.90</td>
<td>197</td>
<td>0.84</td>
</tr>
<tr>
<td>Rat</td>
<td>120</td>
<td>1</td>
<td>2.09</td>
<td>993</td>
<td>NA</td>
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<tr>
<td></td>
<td></td>
<td>28</td>
<td>2.05</td>
<td>902</td>
<td>0.90</td>
</tr>
<tr>
<td>Dog</td>
<td>120</td>
<td>1</td>
<td>2.86</td>
<td>740</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>28</td>
<td>3.59</td>
<td>776</td>
<td>1.05</td>
</tr>
</tbody>
</table>

NA = Not Applicable
Conclusions

• In the preclinical setting, vadadustat
  – inhibited recombinant human PHD1, PHD2 and PHD3 isoenzymes at equivalent nanomolar concentrations
  – stabilized both HIF-1α and HIF-2α in vitro
  – stimulated EPO production in vitro and in vivo
  – increased hemoglobin and hematocrit in multiple species
  – did not stimulate VEGF production in vitro

• The pharmacology of vadadustat support development for anemia of CKD and ESRD
Possible backup slides
Background and Mechanism of Action

Vadadustat is an orally bioavailable hypoxia-inducible factor prolyl hydroxylase inhibitor (HIF-PHI) in development for the potential treatment of anemia due to chronic kidney disease.

The HIF-PH enzymes are also referred to as EGLN proteins or prolyl 4-hydroxylase domains (PHDs).

Pharmacological inhibition of PHD enzymes lead to the stabilization of hypoxia-inducible factor (HIF), a transcription factor that activates target genes to improve the $O_2$ carrying capacity of the blood.