Mechanism of Action of Vorolanib and Differentiation From Other Anti-Vascular Endothelial Growth Factor Receptor Tyrosine Kinase Inhibitors

Background

- Age-related macular degeneration (AMD) leads to central vision loss. Vascular endothelial growth factor (VEGF) is a key wet AMD (wAMD) mediator.1
- Intravitreal anti-VEGF therapy for wAMD:2-5
  1. Targets only 1-2 ligands:
  2. Requires frequent treatment; and
  3. Patients can lose vision, despite therapy.
- There are several VEGF ligands and several VEGF receptors (VEGFRs), meaning ideal therapies should target all the signaling pathways.4
- Tyrosine kinase inhibitors (TKIs) are pan-VEGFR inhibitors that enable inhibition of all VEGF signaling pathways.7
- Vorolanib is a class II TKI that is a pan-VEGFR inhibitor (Figure 1).1,9

Methods

- Kinase HotSpot™ assay screen (Reaction Biology).
- Half-maximal inhibitory concentration (IC50) values determined (Reaction Biology).
- Melanin binding determined by liquid chromatography–mass spectrometry (Charles River Laboratories).
- In vitro inhibition of angiogenesis assessed in a human umbilical vein endothelial cell (HUVEC) sprouting assay (Reaction Biology).
- In vivo inhibition of angiogenesis assessed using a chorioallantoic membrane (CAM) assay (inoculation).

Purpose

- Differentiation of TKIs is of great interest, so pan-VEGFR inhibitors vorolanib, sunitinib, and axitinib were compared in vitro and in vivo to determine their ability to inhibit VEGFRs and angiogenesis, thereby assessing utility of these TKIs for application as an effective treatment for wAMD.

Results

- The kinase screen results confirmed that all 3 tested TKIs are pan-VEGFR inhibitors (Figure 2).10

Figure 1: Vorolanib Strongly Binds and Inhibits VEGFR2

Figure 2: Kinase Assay Screen for the 3 TKIs

Table 1: Melanin-Binding Data for the 3 TKIs and Chloroquine

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 (nM)</th>
<th>Mean % Bound (Across All Concentrations)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vorolanib</td>
<td>ND</td>
<td>14.3</td>
</tr>
<tr>
<td>Sunitinib</td>
<td>3.0×10^-8</td>
<td>46.7</td>
</tr>
<tr>
<td>Axitinib</td>
<td>3.0×10^-7</td>
<td>56.7</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Inhibition of all VEGF ligands and VEGF receptors by vorolanib, sunitinib, and axitinib

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 (nM)</th>
<th>VEGFR1</th>
<th>VEGFR2</th>
<th>VEGFR3</th>
<th>TIE2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vorolanib</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Sunitinib</td>
<td>3.0×10^-7</td>
<td>56.7</td>
<td>56.7</td>
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</tr>
<tr>
<td>Axitinib</td>
<td>3.0×10^-7</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Figure 3: IC50 Curves for the 3 TKIs Against VEGFRs and TIE2

Conclusions

- The HUVEC sprouting assay showed that all 3 TKIs inhibited in vitro angiogenesis similarly (Figure 4).11

Figure 4: HUVEC Sprouting Assay Results for the 3 TKIs

- The CAM assay confirmed that all 3 TKIs inhibited in vivo angiogenesis similarly. TKIs appeared to inhibit angiogenesis better than the positive control (anti-VEGF antibody, bevacizumab; Figure 5).

Figure 5: CAM Assay Results for the 3 TKIs

- Findings also provide possible points of differentiation: TKIs have differences, and of the 3 studied, only axitinib strongly inhibited TIE2 and only sunitinib bound melanin.
- Conclusions: TKIs are pan-VEGFR inhibitors, which are advantageous over treatments that target 1 to 2 VEGF ligands (Figure 6).

Figure 6: Mechanism of Action of Vorolanib, Sunitinib, and Axitinib

- TKIs have differences, and of the 3 studied, only axitinib strongly inhibited TIE2 and only sunitinib bound melanin.
- Findings also provide possible points of differentiation in the safety profiles among vorolanib, sunitinib, and axitinib.