Murine TGFβ-antagonist (RAP-1332) Inhibits Fibrosis in a Murine Model of Myelofibrosis

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Polycythemia Vera (PV), Essential Thrombocythemia (ET) and Primary Myelofibrosis (PMF)

- Mutations leading to constitutively active signaling and uncontrolled proliferation of BM myeloid lineage cells and ultimately cause fibrosis

- Janus Kinase2 (JAK2) V617F mutation: 95% in PV, ~50-60% in ET and PMF
  - Calreticulin (CALR): 20-25% in ET and PMF
  - Myeloproliferative leukemia virus (MPL) oncogene: less than 10% for ET and PMF

- Central role of TGFβ signaling pathway in fibrosis\(^1\)

- Murine model of MPN: JAK2(V617F) transgenic expression\(^2\)
  - Elevated RBC (PV), elevated platelets (ET), elevated WBC with progression into Myelofibrosis with age
  - Splenomegaly; mean survival age of ~17 months

\(^1\)Rosemary JA et al., Nature Reviews (2012)
\(^2\)Shu Xing et al., Blood (2008)
Outline

- TGFβ expression and initiation of fibrosis in the JAK2(V617F) murine model

- Treatment of TGFβ antagonist (ACE-1332) in JAK2(V617F) transgenic mice

- Combination treatments of RAP-1332 and ruxolitinib (JAK2 inhibitor) in JAK2(V617F) transgenic mice
JAK2(V617F) transgenic mice display elevated RBC, Platelets, WBC and splenomegaly.
BM Fibrosis starts in JAK2(V617F) transgenic mice at 5-months and worsens with age

Fibrosis (Reticulin, 20x)

Age (months)

2  5  8
Serum levels of TGFβ1 and 3 but not TGFβ2 ligands are elevated in JAK2(V617F) transgenic mice.
ACE-1332 binds tightly to TGFβ1 and TGFβ3 ligands but not to TGFβ2

- ACE-1332 is a protein biologic engineered to bind and inhibit signaling by TGFβ1 and TGFβ3 but not TGFβ2
- Does not bind other ligands in the TGFβ-superfamily
- RAP-1332 is murine ortholog of ACE-1332

### Ligands

<table>
<thead>
<tr>
<th>Ligands</th>
<th>$K_D$ (pM) @ 37°C</th>
<th>$IC_{50}$ (ng/ml) A549 RGA</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGFβ1</td>
<td>5.5</td>
<td>3.8</td>
</tr>
<tr>
<td>TGFβ2</td>
<td>400</td>
<td>No Inhibition</td>
</tr>
<tr>
<td>TGFβ3</td>
<td>2.2</td>
<td>3.1</td>
</tr>
</tbody>
</table>

### Biacore and Cell Based Assay

- **Biacore**
  - Fit: 1:1 Binding
  - $k_a$ (1/Ms): 2.046E+8
  - $k_d$ (1/s): 0.001130
- **Cell Based Assay**
  - Fit: 1:1 Binding
  - $k_a$ (1/Ms): 2.070E+7
  - $k_d$ (1/s): 0.008273
  - $k_a$ (1/Ms): 3.997E+8
  - $k_d$ (1/s): 8.947E-4
Study design: Treatment of JAK2(V617F) transgenic mice with TGFβ antagonist (RAP-1332)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Sex</th>
<th>Age (Mos)</th>
<th>N</th>
<th>Treatment</th>
<th>Dosage</th>
<th>Frequency</th>
<th>Duration (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wt</td>
<td>M</td>
<td>4</td>
<td>15</td>
<td>TBS</td>
<td>Iso volume</td>
<td>Twice weekly</td>
<td>3, 6</td>
</tr>
<tr>
<td>JAK2(V617F)</td>
<td>M</td>
<td>4</td>
<td>15</td>
<td>TBS</td>
<td>Iso volume</td>
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<td>3, 6</td>
</tr>
<tr>
<td>JAK2(V617F)</td>
<td>M</td>
<td>4</td>
<td>15</td>
<td>RAP-1332</td>
<td>10 mg/kg</td>
<td>Twice weekly</td>
<td>3, 6</td>
</tr>
</tbody>
</table>

Treatment initiated before the onset of fibrosis
RAP-1332 treatment for 3 months reduced fibrosis in BM and Spleen (JAK2 V617F mice)

Age of mice at study termination: 7 months
RAP-1332 treatment for 6 months reduced fibrosis in BM and Spleen in JAK2 (V617F) transgenic mice

Age of mice at study termination: 10 months
RAP-1332 treatment for 6 months reduced splenomegaly in JAK2(V617F) mice

**p < 0.01 vs VEH**
RAP-1332 treatment reduced erythroid hyperplasia in Spleen and serum IL-6 levels in JAK2(V617F) mice after 6 months treatment.
## Combination treatment of RAP-1332 and Ruxolitinib in JAK2(V617F) transgenic mice (12 months old)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Sex</th>
<th>Age (Mos)</th>
<th>N</th>
<th>Treatment</th>
<th>Dosage</th>
<th>Frequency</th>
<th>Duration (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>JAK2(V617F)</td>
<td>M</td>
<td>12</td>
<td>9</td>
<td>veh</td>
<td>Iso volume</td>
<td>Twice weekly</td>
<td>3</td>
</tr>
<tr>
<td>JAK2(V617F)</td>
<td>M</td>
<td>12</td>
<td>10</td>
<td>RAP-1332</td>
<td>10 mg/kg</td>
<td>Twice weekly</td>
<td>3</td>
</tr>
<tr>
<td>JAK2(V617F)</td>
<td>M</td>
<td>12</td>
<td>10</td>
<td>Ruxolitinib</td>
<td>60 mg/kg</td>
<td>Twice daily</td>
<td>3</td>
</tr>
<tr>
<td>JAK2(V617F)</td>
<td>M</td>
<td>12</td>
<td>10</td>
<td>Ruxolitinib + RAP-332</td>
<td>60/10 mg/kg</td>
<td>Twice daily/Twice weekly</td>
<td>3</td>
</tr>
</tbody>
</table>

Treatment initiated at late stage of disease
RAP-1332 or Combination treatment with Ruxolitinib reduced BM fibrosis in JAK2(V617F) transgenic mice.
Ruxolitinib or combination treatment with RAP-1332 reduced RBC and splenomegaly in JAK2(V617F) transgenic mice

* P<0.05; ** P<0.01; *** P<0.001 vs VEH
In the JAK2(V617F) transgenic mouse model of myelofibrosis, bone marrow fibrosis starts at 5 months and worsens with age.

Expression of TGFβ1 and TGFβ3 ligands were elevated in JAK2(V617F) transgenic mice consistent with the initiation of fibrosis.

ACE-1332, a protein biologic ligand trap inhibits specific signaling by TGFβ1 and TGFβ3 ligands but not by TGFβ2 or the other members of the family.

Treatment with RAP-1332 before the onset of fibrosis:
- reduced splenomegaly and erythroid hyperplasia
- inhibited fibrosis in BM and spleen
- decreased inflammatory cytokine levels in serum

Combination treatment of RAP-1332 and ruxolitinib decreases fibrosis compared to ruxolitinib monotherapy while retaining the beneficial effects of decreasing splenomegaly.
Acknowledgements

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- Scott Pearsall, PhD
- Ken Attie, MD
- Matt Sherman, MD
- Steve Ertel, MBA
- Ravi Kumar, PhD
- Acceleron Team!
RAP-1332 treatment did not effect RBC, WBC and Platelets or splenomegaly in JAK2(V617F) mice after 3 months of dosing.

- **RBC**
  - Wt: 8.5 ± 0.5
  - JAK2 + VEH: 12.0 ± 1.0
  - JAK2 + RAP-1332: 16.0 ± 1.5

- **Platelets**
  - Wt: 500 ± 50
  - JAK2 + VEH: 800 ± 50
  - JAK2 + RAP-1332: 1200 ± 100

- **WBC**
  - Wt: 10 ± 2
  - JAK2 + VEH: 30 ± 3
  - JAK2 + RAP-1332: 40 ± 4

- **Spleen wt**
  - Wt: 10 ± 2 mg
  - JAK2 + VEH: 60 ± 6 mg
  - JAK2 + RAP-1332: 60 ± 6 mg

N=5/group