RAP-536 (Murine ACE-536/Luspatercept) Inhibits Smad2/3 Signaling and Promotes Erythroid Differentiation By Restoring GATA-1 Function in Murine β-thalassemia

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Outline

• Background

• Mechanism of ACE-536
  • Ligand binding profile
  • Molecular Targets
    • RNA-seq analysis
  • GATA1 negative regulation via GDF11

• Conclusions
ACE-536 (Luspatercept) is a Modified ActRIIB Receptor Fusion Protein

- ACE-536 is a fusion protein that consists of a modified activin receptor (ActRIIB) - a member of the TGFβ superfamily - and the Fc of human IgG1
- Inhibits Smad2/3 signaling and acts as a ligand trap
- Robust stimulation of RBC production in mice, rat and cynomolgus monkeys
- Co-developed with Celgene

RAP-536 is murine ortholog of ACE-536
Effect of ACE-536 (Luspatercept) on Erythropoiesis

- ACE-536 treatment increases RBCs – however activity is distinct from EPO
- Effect is focused on the differentiation of erythroid precursors while EPO affects proliferation of BFU-E and CFU-E
- ACE-536 promotes differentiation of Baso, Poly, and Ortho Erythroblasts
- ACE-536 does not affect other cell lineages
- ACE-536 has corrected anemia in various murine preclinical models of ineffective erythropoiesis such as MDS and β-thalassemia

Suragani et al., Nature Medicine 2014
RAP-536 decreases elevated pSmad2/3 and attenuates anemia in a murine model of β-thalassemia (Hbb<sup>−/−</sup>)

RBC

EPO

Reticulocytes

### p < 0.001 vs wt; ** p < 0.01, * p < 0.05 vs th1/th1

Suragani et al., Blood 2014
RAP-536 corrects Ineffective Erythropoiesis and associated co-morbidities in a β-thalassemia mouse model (Hbb−/−)

- **Improved Bone Mineral Density**
  - WT
  - β-thal+TBS
  - β-thal+RAP-536

- **Decreased Liver Iron**
  - WT
  - β-thal+TBS
  - β-thal+RAP-536

- **Reduced Spleen Size**
  - WT
  - β-thal+TBS
  - β-thal+RAP-536

- **Improved RBC Morphology**
  - β-thal+TBS
  - β-thal+RAP-536
RAP-536 decreases elevated pSmad2/3, attenuates anemia and decreased erythroid hyperplasia in a mouse model of MDS

Suragani et al., Nature Medicine 2014
ACE-536 (Luspatercept) inhibits Smad2/3 signaling ligands

<table>
<thead>
<tr>
<th>Smad2/3 signaling Ligands</th>
<th><strong>K\textsubscript{D} (nM) @ 37°C</strong></th>
<th><strong>IC\textsubscript{50} (ng/ml)</strong></th>
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<tbody>
<tr>
<td>GDF-8</td>
<td>3.00</td>
<td>88</td>
</tr>
<tr>
<td>GDF-11</td>
<td>0.71</td>
<td>7.1</td>
</tr>
<tr>
<td>Activin B</td>
<td>0.27</td>
<td>&gt;14,400</td>
</tr>
<tr>
<td>Activin A</td>
<td>No binding</td>
<td>&gt;33,000</td>
</tr>
<tr>
<td>TGF-b1</td>
<td>No binding</td>
<td>&gt;33,000</td>
</tr>
</tbody>
</table>

### Biacore

- **GDF11**
  - Item: ACE536 GDF11 37C_2
  - Ligand: ACE536
  - Sample: GDF11
  - Curve: Fc=4-1
  - Temperature: 37 °C
  - Fit: 1:1 Binding
  - $k_a(1/M_s)$: 9.867E+6
  - $k_d(1/s)$: 0.001537

- **Activin B**
  - Item: ACE536 Activin B 37C
  - Ligand: ACE536
  - Sample: Activin B
  - Curve: Fc=4-3
  - Temperature: 37 °C
  - Fit: 1:1 Binding
  - $k_a(1/M_s)$: 7.601E+7
  - $k_d(1/s)$: 0.02040

- **Activin A**
  - Item: ACE536 Activin A 37C
  - Ligand: ACE536
  - Sample: Activin A
  - Curve: Fc=2-1
  - Temperature: 37 °C
  - No binding
Elevated levels of serum GDF11 detected in patients with MDS and β-thalassemia

<table>
<thead>
<tr>
<th></th>
<th>Normal sera</th>
<th>MDS sera</th>
<th>β-thalassemia sera</th>
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</thead>
<tbody>
<tr>
<td>GDF11 (pg/ml)</td>
<td>Normal: 5</td>
<td>Thalassemia patients: 19</td>
<td>* p&lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>Normal: 6</td>
<td>MDS patients: 8</td>
<td></td>
</tr>
</tbody>
</table>

* p< 0.05
GDF11 and other Smad2/3 ligands contribute to the activity of Luspatercept

Red Blood Cells

Dosage: 10mg/kg, twice/week for 2 weeks, N= 5/group  ** P<0.01, *** P< 0.001 vs VEH

Hemoglobin
Flow cytometric cell sorting of β-thalassemic erythroid precursors (basophilic) for RNA sequencing analysis

- β-thalassemic mice were treated with VEH or RAP-536
- Splenic basophilic erythroblasts were sorted via flow post 16hrs treatment
- RNA was isolated from these cells for RNA-seq
Differentially regulated genes by RAP-536 treatment in β-thalassemic erythroid precursors

- 74 genes were differentially expressed by RAP-536 (absolute fold change >1.5, FDR P-value <0.05)
  - Upregulated pathways:
    - Respiratory Electron Transport
    - Oxidative phosphorylation
    - Porphyrin Metabolism
    - Proteasomal Pathway
  - Downregulated
    - IL-12
Gene Set Enrichment Analysis (GSEA)-Transcription Factors

<table>
<thead>
<tr>
<th>Transcription Factors</th>
<th>Enrichment Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSF_Q6</td>
<td>2.1767392</td>
</tr>
<tr>
<td>GATA_C</td>
<td>1.8235506</td>
</tr>
<tr>
<td>NEF2_01</td>
<td>1.7020864</td>
</tr>
</tbody>
</table>

- Unbiased enrichment analysis - **GATA1, NEF2, and HSF** transcription factors are activated while **NfκB**, etc., are repressed
- 158 GATA1 downstream targets were differentially upregulated by RAP-536 treatment
GATA-1 transcription factor downstream target genes are differentially regulated by RAP-536 treatment

These data indicate that pSmad2/3 negatively regulates erythropoiesis.
Mouse erythroid leukemic (MEL) and murine primary fetal liver erythroid cells as a model

Differentiating erythroid cells (MEL cells treated with DMSO) or Fetal liver cells

- Phosphorylation of Smad2/3
- GATA1 availability
- Reactive Oxygen Species
- What is the role of ACE-536
GATA1 levels are decreased in the nucleus with GDF11 treatment – ACE-536 reverses this effect in MEL cells
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GATA1 levels are decreased in the nucleus with GDF11 treatment – ACE-536 reverses this effect in MEL cells
GATA1 levels are decreased in the nucleus with GDF11 treatment – ACE-536 reverses this effect in murine fetal liver erythroid precursors.
GATA1 levels are restored in the nucleus of β-thalassemic murine BM cells
ROS is induced with GDF11 treatment and reduced upon co-treatment with ACE-536 in MEL cells.
Model - β-thalassemia

- GDF11
- pSmad2/3
- ROS
- C-Cas-3

=Erythroid differentiation is inhibited
Model - β-thalassemia + ACE536

- GDF11
- ACE-536
- ROS
- pSmad2/3
- Nucleus
- GATA1

= Block removed – Proper erythroid maturation

C-Cas-3
Conclusions

- Luspatercept (ACE-536) binds and inhibits signaling by certain Smad 2/3 signaling ligands
- RNA seq analysis revealed that ACE-536 upregulates genes involved in erythroid differentiation through GATA1
- GDF11 increases ROS and limits the availability of GATA1 in the nucleus
- ACE-536 decreases ROS and restores GATA1 availability
- Luspatercept is currently being tested in patients with β-thalassemia and MDS
Acknowledgements

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- Dr. Sherman
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- Celgene Team