Rapamycin (sirolimus) and RAP-536 increase red blood cell parameters through distinct mechanisms in wild-type and thalassemic mice

Melih Acar,¹ Madhulika Jupelli,² Kyle MacBeth,² Martin Schwickart²

¹Formerly Bristol Myers Squibb, Princeton, NJ; ²Bristol Myers Squibb, Princeton, NJ
Presenting author disclosures

M.S.: BMS - current employment and current equity holder in publicly-traded company.
Introduction, objective, and methods

Introduction

• RAP-536, the murine analog of luspatercept, has been shown to induce erythroblast maturation and increase RBC, Hb, and hematocrit levels in WT mice and in murine disease models of β-thalassemia and MDS\textsuperscript{1,2}

• Rapamycin has previously been shown to increase HbF levels in a mouse model of sickle cell disease\textsuperscript{3}

Objective

• To test whether rapamycin and RAP-536 co-administration improves anemia more than single-agent dosing in WT mice and in a mouse model of β-thalassemia (th3/++; B6.129P2-\textit{Hbb-b1\textsuperscript{tm1Unc} Hbb-b2\textsuperscript{tm1Unc/J}})

Methods

• WT and β-thalassemic mice were dosed for 14 days
  – RAP-536 was administered at 10 mg/kg dose s.c. twice weekly
  – Rapamycin was administered at 4 mg/kg dose i.p. every day, except for Sundays
• 1 day after the final dose, the mice were harvested, and blood was collected via cardiac puncture
  – BM and spleen tissues were collected and analyzed by flow cytometry

BM, bone marrow; Hb, hemoglobin; HbF, fetal hemoglobin; i.p., intraperitoneal; MDS, myelodysplastic syndromes; RBC, red blood cell; s.c., subcutaneous; WT, wild type.
Results

Figure 1. Effect of RAP-536 on (A) RBC, (B) hematocrit, and (C) Hb levels in blood of th3/+ mice

n = 8 for each group.

**P < 0.01; ***P < 0.001.
Figure 2. Effect of RAP-536 and rapamycin on spleen weight in th3/+ mice

- Splenomegaly was reduced by rapamycin and co-administration of rapamycin and RAP-536

**P < 0.01; ***P < 0.001.
Figure 3. Effect of RAP-536 and rapamycin on (A) blood reticulocyte count, (B) BM reticulocyte percentage, and (C) BM erythroblast percentage in th3/+ mice

\*\* P < 0.05; ** P < 0.01; *** P < 0.001.
Figure 4. Effect of RAP-536 and rapamycin on (A) RBC counts, (B) Hb levels, and (C) reticulocyte counts in blood, and (D) percentage of BM erythroblasts in WT mice.

<table>
<thead>
<tr>
<th></th>
<th>Blood</th>
<th>Reticulocyte count x10^9/L</th>
<th>BM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle</td>
<td>RAP-536</td>
<td>Rapamycin</td>
</tr>
<tr>
<td></td>
<td>10.73</td>
<td>11.69</td>
<td>11.35</td>
</tr>
<tr>
<td></td>
<td>15.89</td>
<td>17.01</td>
<td>17.27</td>
</tr>
<tr>
<td></td>
<td>368.94</td>
<td>515.00</td>
<td>382.33</td>
</tr>
<tr>
<td></td>
<td>15.94</td>
<td>14.70</td>
<td>8.52</td>
</tr>
</tbody>
</table>

Average percent increase:
- RAP-536 vs vehicle: 8.9%
- Rapamycin vs vehicle: 5.7%
- Combination vs vehicle: 13.8%

n = 7 for each group.

*P < 0.05; **P < 0.01; ***P < 0.001.
Summary

• RAP-536 and rapamycin co-administration increased RBC and Hb levels more than single-agent dosing in both WT and β-thalassemia (th3/+) mice
• The percent increase in RBC parameters with RAP-536 and rapamycin co-administration in the β-thalassemia mouse model (compared to vehicle dosing) is higher than in WT mice
• In WT mice, rapamycin reduces and RAP-536 increases blood reticulocytes suggesting different mechanisms of action in increasing RBC parameters
• Our results show that rapamycin, or another mTOR inhibitor, may be beneficial for the treatment of patients with β-thalassemia in combination with luspatercept

mTOR, mammalian target of rapamycin.
Acknowledgments

• This study was supported by Bristol Myers Squibb
• All authors contributed to and approved the presentation; writing and editorial assistance were provided by Miriam de Boeck, of Excerpta Medica, funded by Bristol Myers Squibb