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

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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 ß-thalassemia and MDS[1,2]
- Rapamycin has previously been shown to increase HbF levels in a mouse model of sickle cell disease[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 ß-thalassemia (th3/+; B6.129P2-*Hbb-b1*^{tm1Unc} *Hbb-b2*^{tm1Unc}/J)

Methods

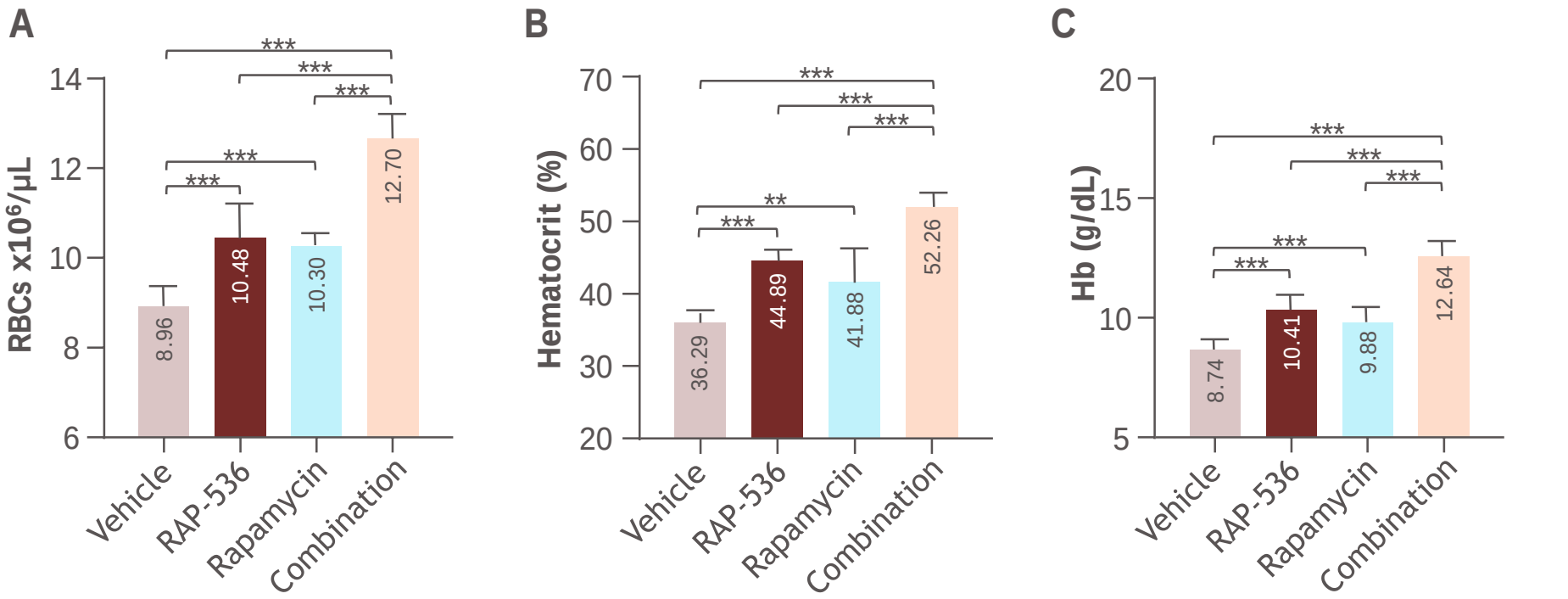
- WT and ß-thalassemic mice were dosed for 14 days
  - RAP-536 was administered at 10 mg/kg dose s.c. twice weekly
  - Rapamycin was administered at 4 mg/kg dose i.p. every day, except for Sundays
- 1 day after the final dose, the mice were harvested, and blood was collected via cardiac puncture
  -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

1. Suragani RNVS, et al. *Blood* 2014;123:3864–3872. 2. Suragani RNVS, et al. *Nat Med* 2014;20:408–414. 3. Khaibullina A, et al. *Blood Cells Mol Dis* 2015;55:363–372.

Results

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



	Average percent increase
RAP-536 vs vehicle	17.0
Rapamycin vs vehicle	15.0
Combination vs vehicle	41.8

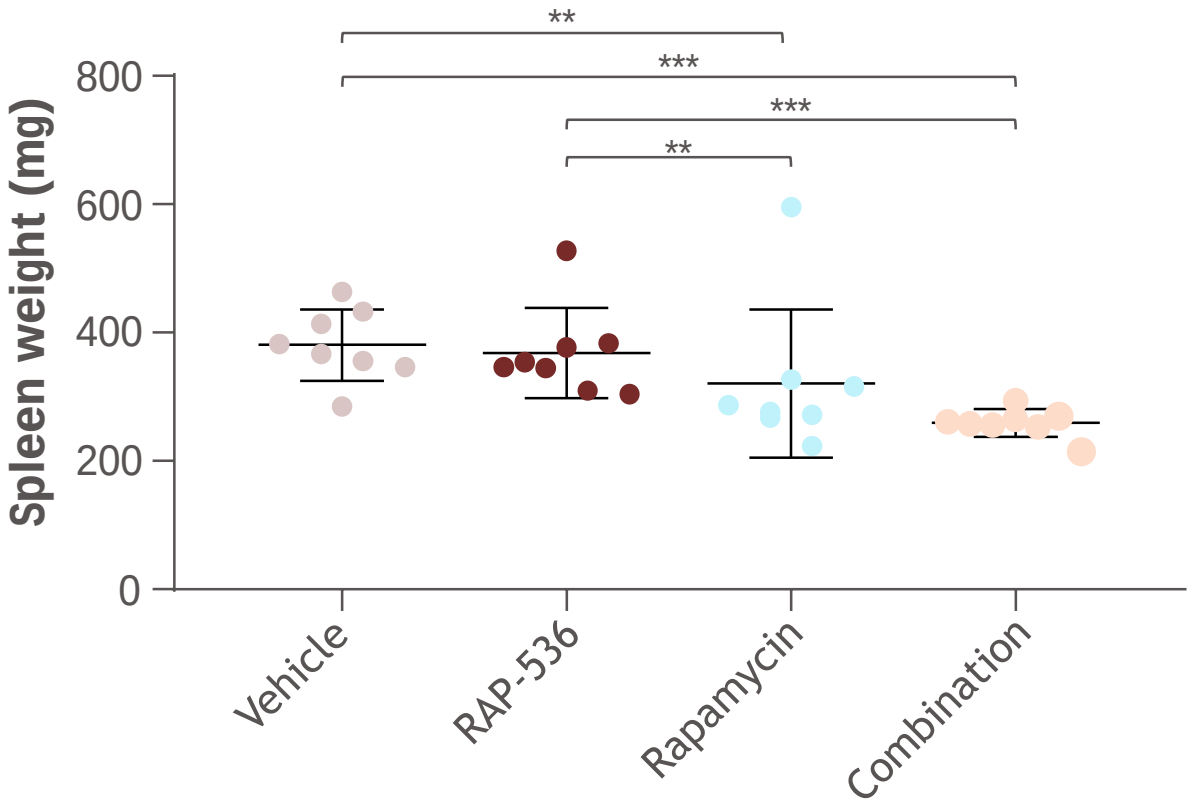
	Average percent increase
RAP-536 vs vehicle	23.7
Rapamycin vs vehicle	15.4
Combination vs vehicle	44.0

	Average percent increase
RAP-536 vs vehicle	19.2
Rapamycin vs vehicle	13.0
Combination vs vehicle	44.7

n = 8 for each group.
$^{**}P < 0.01$; $^{***}P < 0.001$.

Results (cont.)

Figure 2. Effect of RAP-536 and rapamycin on spleen weight in th3/+ mice

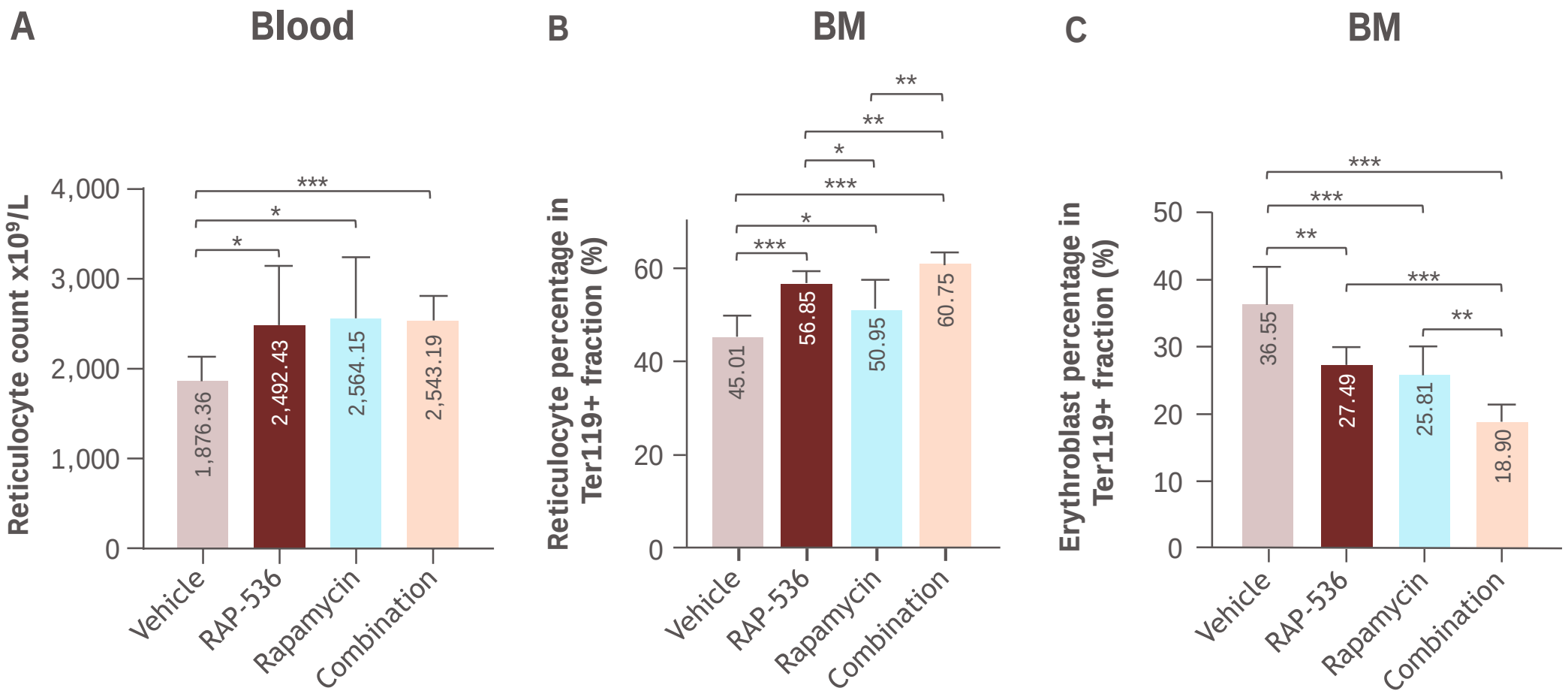


- Splenomegaly was reduced by rapamycin and co-administration of rapamycin and RAP-536

***P* < 0.01; ****P* < 0.001.

Results (cont.)

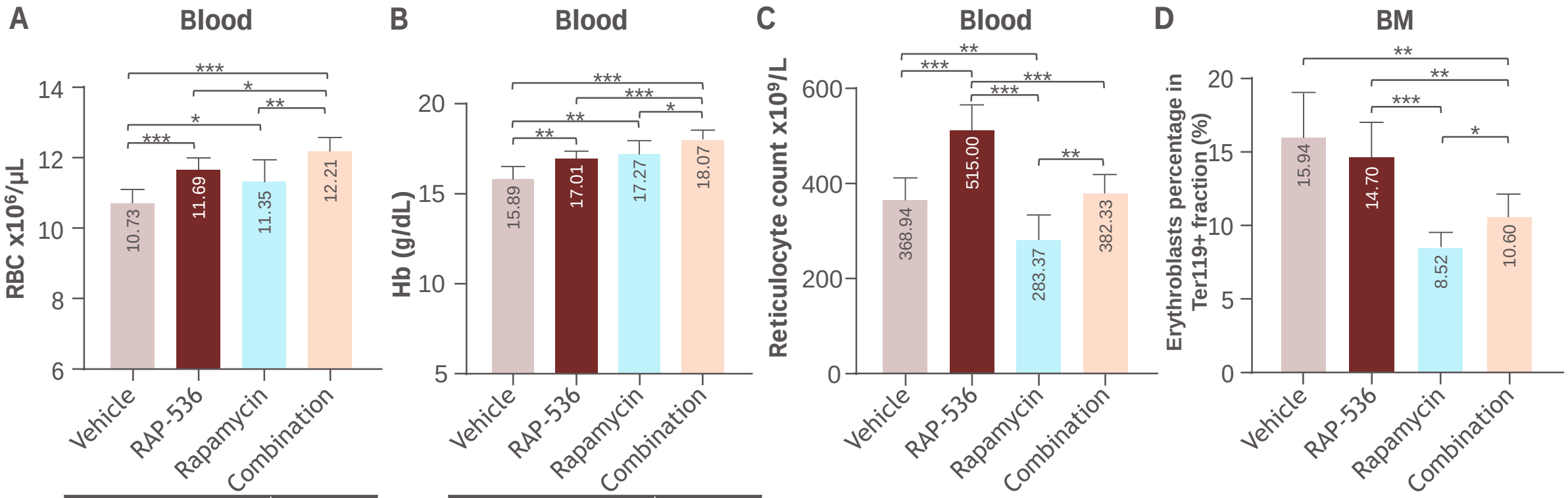
Figure 3. Effect of RAP-536 and rapamycin on (A) blood reticulocyte count, (B) BM reticulocyte percentage, and (C) BM erythroblast percentage in th3/+ mice



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

Results (cont.)

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



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

	Average percent increase
RAP-536 vs vehicle	7.1
Rapamycin vs vehicle	8.7
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 ß-thalassemia (th3/+) mice
- The percent increase in RBC parameters with RAP-536 and rapamycin co-administration in the ß-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 ß-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

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