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Poster P535

Modified ActRIIB-Fc Fusion Protein (ACE-536) Decreases Irreversible Sickle Cells in a Murine Model of Sickle Cell Disease

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Introduction

- Sickle Cell Disease (SCD) is a hereditary disorder caused by a single point mutation in the β-globin gene resulting in sickle hemoglobin variant (β^S/β^S). In sickle cell disease, under hypoxic conditions, sickle hemoglobin in red blood cells (RBC) undergoes aggregation / polymerization which leads to several damaging sequela:
  - Increased red cell hemolysis as reflected in increased bilirubin and cell free hemoglobin along with higher levels of reactive oxygen species (ROS);
  - Increased scramblase activity leading to phosphatidylserine (PS) exposure in the outer membrane leaflet likely increasing RBC adherence to the endothelium, slower vessel transit time and greater red cell deoxygenation;
  - Very short red cell half life which results in newly formed red cells (reticulocytes) comprising as much as 50% of total red cells in circulation;
  - The sickle shaped RBCs are partially filtered out by the spleen resulting in a large increase in spleen mass and a decrease in spleen function but most importantly these misshapen cells cause vaso-occlusion in end organs leading to hypoxia, painful crises and organ damage.
- In clinical studies, hydroxyurea has been shown to decrease hemolysis, ROS, and splenomegaly, as well as reducing the number of irreversibly sickled cells (ISC) by 50-80%. Hydroxyurea is approved to reduce the frequency of painful crisis in SCD, yet patients still suffer from numerous other complications.
- ACE-536 is a ligand trap for members of the TGFβ superfamily that stimulates erythropoiesis (Sako et al., JBC 2010; Suragani et al., Nature Med. 2014). In a murine model of β-thalassemia, treatment with ACE-536 reduced the accumulation of misfolded alpha globin and ameliorated the anemia (Suragani et al., Blood 2014).
- In this study, we investigated the effect of ACE-536 in a mouse model of sickle cell disease where deoxygenated misfolded human sickle beta globin generates sickled RBCs and recapitulates the pathology noted in human sickle cell disease.

Methods

- SCD transgenic mice (Wu et al., Blood 2006) were used in the study. These mice are produced by replacing the mouse globin genes (mα/mα and mβ/mβ) with human globin genes (hα/hα, hβs/hβs and hγ/hγ).
- SCD (hβS/hβS) or non-symptomatic heterozygous (listed as control; hβ/hβs) mice were dosed with RAP-536 (murine version of ACE-536) at 1 mg/kg or vehicle control (TBS) twice per week for up to 3 months starting at 3 months of age.
- CBC, serum chemistry, reticulocytes, reactive oxygen species and blood smear analysis, were carried out at various time points as indicated in figure legends. At study termination, spleen weights were also determined.
- Annexin V staining (measuring binding to cell surface phosphatidylserine) was carried out on peripheral RBCs to determine phosphatidylserine exposure as an indicator of the potential for increased red cell adherence to the endothelium.

Results

Figure 1. RAP-536 treatment decreases reticulocytosis and increases red blood cells in SCD mice (4 weeks). N = 5/group. ### P < 0.001 vs control + TBS , * P < 0.05 vs SCD + TBS.

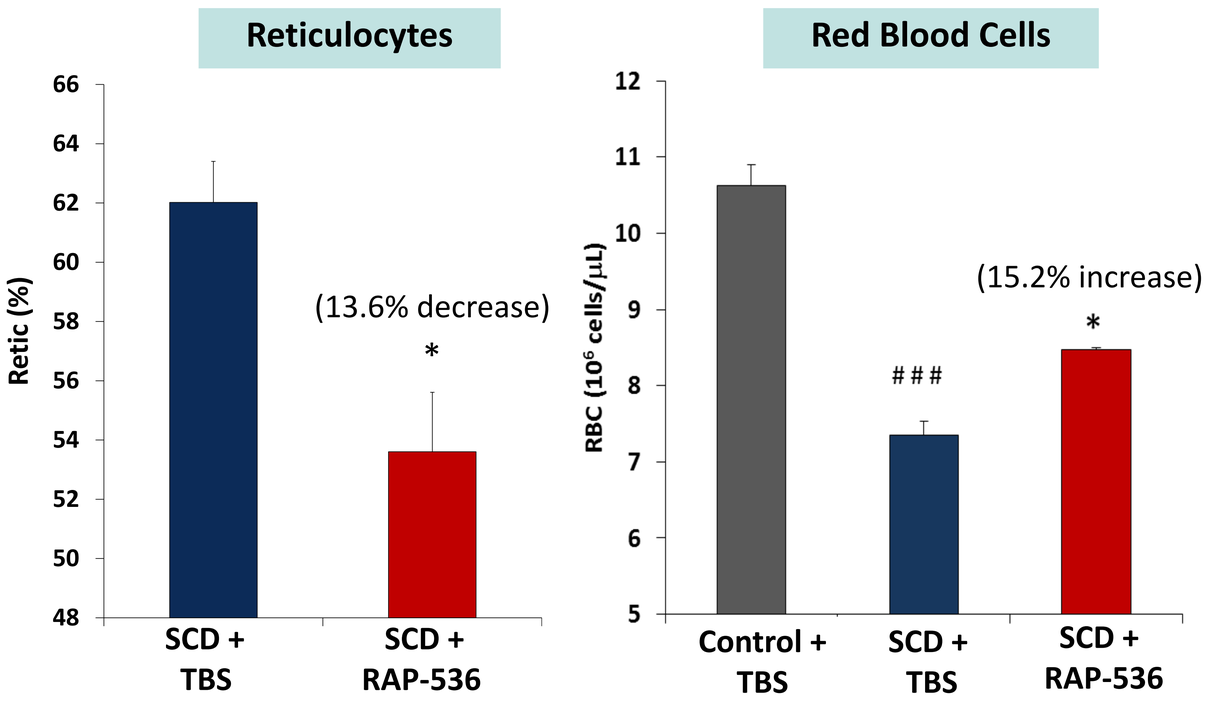
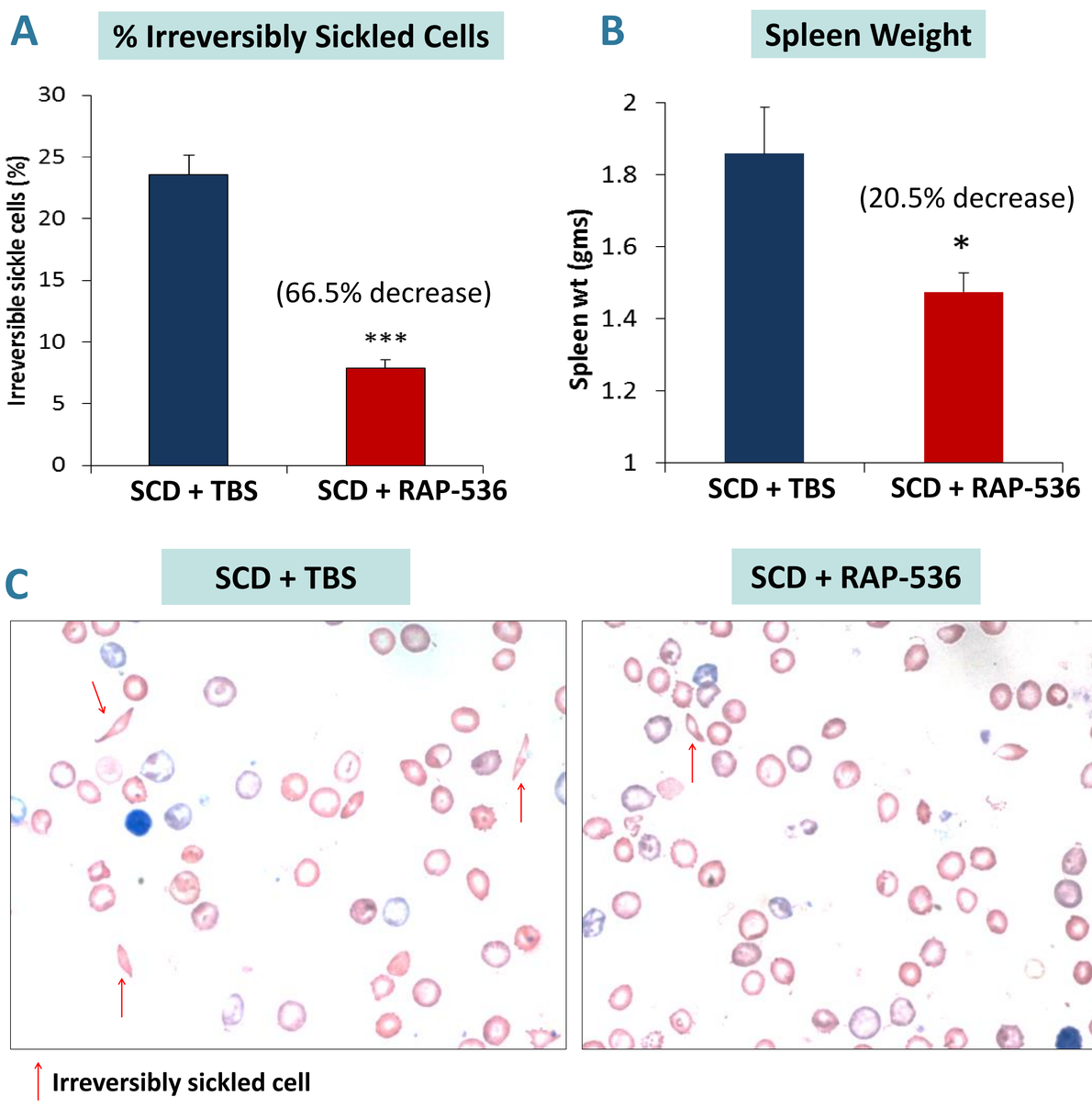


Figure 3. RAP-536 treatment reduces PS exposure on RBC membranes, ROS, total serum bilirubin, cell free hemoglobin and BUN. N = 5/group. * P < 0.05, ** P < 0.01 vs SCD + TBS.

Marker	% Decrease (ACE-536 vs Vehicle)
Annexin V / PS	18.7 ↓
Reactive oxygen species	14.1 ↓
Total bilirubin	17.0** ↓
Cell free hemoglobin	30.7$^{p=0.06}$ ↓
Blood urea nitrogen	19.2* ↓

Figure 4. RAP-536 treatment reduces irreversibly sickled cells (A), reduces spleen weight (B) and improves RBC morphology (C) at 12 weeks. Enumerated approximately 2000 cells /group * P < 0.05, *** P < 0.001 v. SCD + TBS.



Discussion

- In this study we have provided evidence that ACE-536 treatment leads to an improvement in the diverse pathologies noted in the sickle cell disease:
  - Evidence for decreased hemolysis, including decreased bilirubin, cell free hemoglobin and ROS;
  - Potential for decreased RBC "stickiness" as evidenced by decreased PS on outer leaflet of RBCs;
  - Improvement in RBC number with a corresponding decrease in reticulocytosis, consistent with a likely improvement in RBC half-life;
  - Approximately 3-fold decrease in the percentage of irreversibly sickled cells, the primary driver of the pathology of this disease. This decrease is similar to that reported for the only approved therapy for SCD, hydroxyurea (2-4-fold);
  - A decrease in spleen mass likely due to reduced sickling and hemolysis of erythrocytes which is further benefited by mitigation of anemia.
- Future studies will evaluate the effects of combined treatment of ACE-536 and hydroxyurea in addition to detailed mechanistic studies.
- Phase 1 clinical trial of ACE-536 in healthy volunteers completed (Attie et al., Am J Hematol 2014) and ACE-536 is currently being tested in patients with myelodysplastic syndromes and β-thalassemia.

This study was conducted by Acceleron Pharma in collaboration with Celgene Corporation

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