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 (HbSS). In the deoxygenated state, HbSS is labile and undergoes auto-oxidation and polymerization to generate rigid and irreversible sickled erythrocytes leading to vaso-occlusive crises and severe pathophysiology in SCD patients.

- Hydroxyurea (HU) augments fetal hemoglobin production, decreases irreversibly sickled cells and painful events, and is the only approved therapy for SCD patients. However, approximately one-third of SCD patients do not respond to HU therapy. Recent studies have shown dose-limiting myelosuppression with HU treatment and patients still suffer from numerous other complications.

- ACE-356 (lupipartect) is a modified active type 1b receptor-fc fusion (ActRIIB-Fc) protein that binds and inhibits signaling by certain members of the TGF-β superfamily involved in negative regulation of erythropoiesis (Suragani et al., Nature Med. 2014). In a murine model of β-thalassemia, treatment with ACE-356 (murine ortholog of ACE-356) reduced hemichromes on RBC membranes, diminished ROS, improved RBC lifespan and thus ameliorated anemia and mitigated complications of β-thalassemia syndrome (Suragani et al., Blood 2014).

- In this study, we evaluated the effect of RAP-356 as a monotherapy or combination therapy with HU in the murine model of sickle cell disease (HbSS).

Methods

- SCD transgenic mice (Wu et al., Blood 2006) expressing human globin genes (hβu/hβu, hAβF/hAβF and hfy/hfy) were used in the study. Non-symptomatic heterozygotes (hAβF/hβu) were used as controls.

- SCD (HbSS) mice were dosed with RAP-356 (10 mg/kg, i.p) and vehicle control (TBS) twice weekly for 6-12 weeks. A combination treatment with HU (100 mg/kg, i.p) and RAP-356 (100 mg/kg, i.p) twice weekly for 8 weeks was performed and compared with vehicle or HU monotherapy treated SCD mice.

- CBC, serum chemistry, reticulocytes, reactive oxygen species, annexin V binding and blood smear analysis were carried out at various time points as indicated in figure legends. Oxygen status in blood smear samples was determined by CD-Dimetry. Histopathological analyses on H&E stained tissue sections was carried out following study termination.

- Hemin (45μM) was injected to vehicle or RAP-356 pretreated (3 months) SCD mice to induce extracellular hemin crisis and acute chest syndrome (ACS) associated lethality (Samit Ghosh et al., JCI 2013).

Results and Discussion

Figure 1. RAP-356 (1mg/kg) treatment to SCD mice for 6-weeks reduced splenomegaly, reticulocytosis, PS exposure on RBC membranes, ROS, total serum bilirubin, cell free hemoglobin, BUN. RAP-356 treatment also reduced irreversibly sickled cells and improves RBC morphology. N = 5/group. * P < 0.05, ** P < 0.01 vs SCD + TBS. Enumeration approximately 2000 cells/group for sickle cell analysis.

Figure 2. RAP-356 (10mg/kg, 6-weeks) treatment to younger SCD mice (6-week old) improves oxygen carrying capacity of hemoglobin and prevents end organ damage. N = 5-6/group. * P < 0.05 vs SCD + TBS. (A) Hemolysis and sickling of RBC causes anemia and reduced oxygen transport to various tissues in SCD. Treatment of SCD mice with RAP-356 decreases hemolysis and sickle thereby improving anemia and oxygen carrying properties of hemoglobin that would potentially result in improved oxygenation to tissues.

Figure 3. RAP-356 treated SCD mice offer resistance from extracellular hemin crisis induced lethality. Acute chest syndrome (ACS) is one of most common causes of hospitalization in SCD patients. Infusion of hemin causes acute RBC hemolysis and sudden death due to ACS in SCD mice undergoes extensive analysis. RAP-356 (10mg/kg) treatment for 3 months significantly extended survival time (by 3-fold) compared to vehicle treated SCD mice indicating reduced acute intravascular hemolysis and greater resistance against acute chest syndrome compared to treated vehicle SCD mice. These studies suggest a likely improvement against complications or increased resistance to naturally occurring episodes of ACS in patients with SCD on lupipartect treatment. N = 6-8/group. * P< 0.05 vs SCD + TBS

Figure 4. Combination treatment of HU with RAP-356 to SCD mice improves disease pathology compared to HU monotherapy N = 4-5/group. * P < 0.05, ** P < 0.01. ***P<0.001 vs SCD + TBS. (A) Treatment of SCD mice with HU alone reduced spleen weight and reticulocytosis, and also reduced hemolysis and phosphatidyl serine exposure on RBC membranes compared to vehicle treated mice. Combination treatment of RAP-356 together with HU further enhanced the decrease in splenomegaly, phosphatidyl serine exposure and hemolysis compared to vehicle treatment.

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