CD47 Morpholino for Therapeutic Intervention in Beta Thalassemia

Beta thalassemia is characterized by a loss in normal beta globin production. The RBCs of these individuals are small, show less oxygen delivery capacity and have significantly decrease half-life in the circulation. The clinical symptoms of the homozygote form of the disease are consistent with the fundamental defects in RBC size, number, oxygen delivery capacity, and rapid clearance from the circulation and include tissue ischemia, inflammation, decreased exercise capacity and end organ damage in the spleen, liver, bones, lungs, heart and vasculature among other body systems and organs.

These features of the disease are aggravated by a loss of beneficial anti-inflammatory signals in the vasculature including decreased nitric oxide (NO) and increased production of pathologic reactive oxygen species, such a superoxide anion and hydrogen peroxide (1). Further, the most common cardiovascular complication in individuals with beta thalassemia is pulmonary hypertension (2, 3) that, lacking lung-heart transplantation, is fatal. Interestingly, individuals treated with inhaled nitrite, that is converted in NO, showed decreased platelet adhesion (3). Children with beta thalassemia had lower circulating NO associated with increased vascular occlusive episodes (4) and tissue ischemia. Indeed, adult beta thalassemia individuals were noted to have substantial deterioration in the production of vascular NO and decreased vasodilation response to exogenous NO donating drugs (5). Individuals with beta thalassemia also had increased manifestations of pulmonary hypertension with decreased NO precursor metabolism (6, 7). Thus, loss of protective cardiovascular NO is a feature and contributor to the disease spectrum of beta thalassemia.

CD47 is expressed on all cells including vascular cells, on activation by its ligand thrombospondin-1, potently and redundantly inhibits the production and function of vascular NO (8). Separate from this TSP1, via CD47, increases pathologic ROS production (9). However, disruption of the TSP1-CD47 signal decreases inflammation and ROS, increases NO, and migrates pulmonary hypertension (10) and heart failure (11). *Of great relevance to diseased beta thalassemia RBCs, TSP1-CD47 limits RBC deformability and increased RBC destruction* (12).

The CD47 morpholino, through blocking mRNA translation, lowers CD47 protein production in all cells, thus blocking TSP1-CD47 signaling (13, 14). This results in decreased inflammation and thrombosis and increased NO and blood flow to restore tissue and end organ function.

<u>Physiologic advantages of CD47 morpholino treatment in beta thalassemia</u>:

- Improved RBC half-life and flexibility to lessen anemia, decrease transfusion needs and decrease vaso-occlusive crisis
- Decreased inflammation, platelet activation and thrombosis
- Decreased tissue necrosis and leg ulcers
- Increased blood flow to ischemic tissues
- Increased pulmonary blood flow to decrease pulmonary vascular resistance and correct heart failure
- Improved function of failing right and left ventricle to stabilize and mitigate heart failure
- Treats disease target RBC and the end organ damage

<u>Treatment advantages of a CD47 morpholino</u>:

- Ease of administration
- Wide tissue penetration and delivery to the sites of disease and organ injury
- Long half-life (2 weeks)
- Small molecule with no ability to activate the immune system
- No loss of potency over time
- Unique agent with new IP in development; only one human base pair sequence that works
- First in class therapy that targets multiple cells/organs and components of the disease
- Therapeutic uses in gene editing or BM transplant recipients to deal with ongoing organ disease

- Applications to other hemoglobinopathies, such as sickle cell disease, and vascular and cardiovascular diseases (atherosclerosis, hypertension, myocardial infarction, primary pulmonary heart failure, kidney injury, transplant organ injury)

Figure 1 below highlights the negative maladaptive TSP1-CD47 signal and how it accelerates and contributes to the disease processes and complications of beta thalassemia. Of note, TSP1-CD47 has a primary role in driving RBC dysfunction in general through making the red cell membrane stiff and non-deformable and increasing the rate or RBC destruction, thus shortening the half-life of RBCs and increasing transfusion needs. As well, stiffer RBCs will impede flow through capillaries, decrease oxygen delivery by the RBCS and increase tissue/organ ischemia.

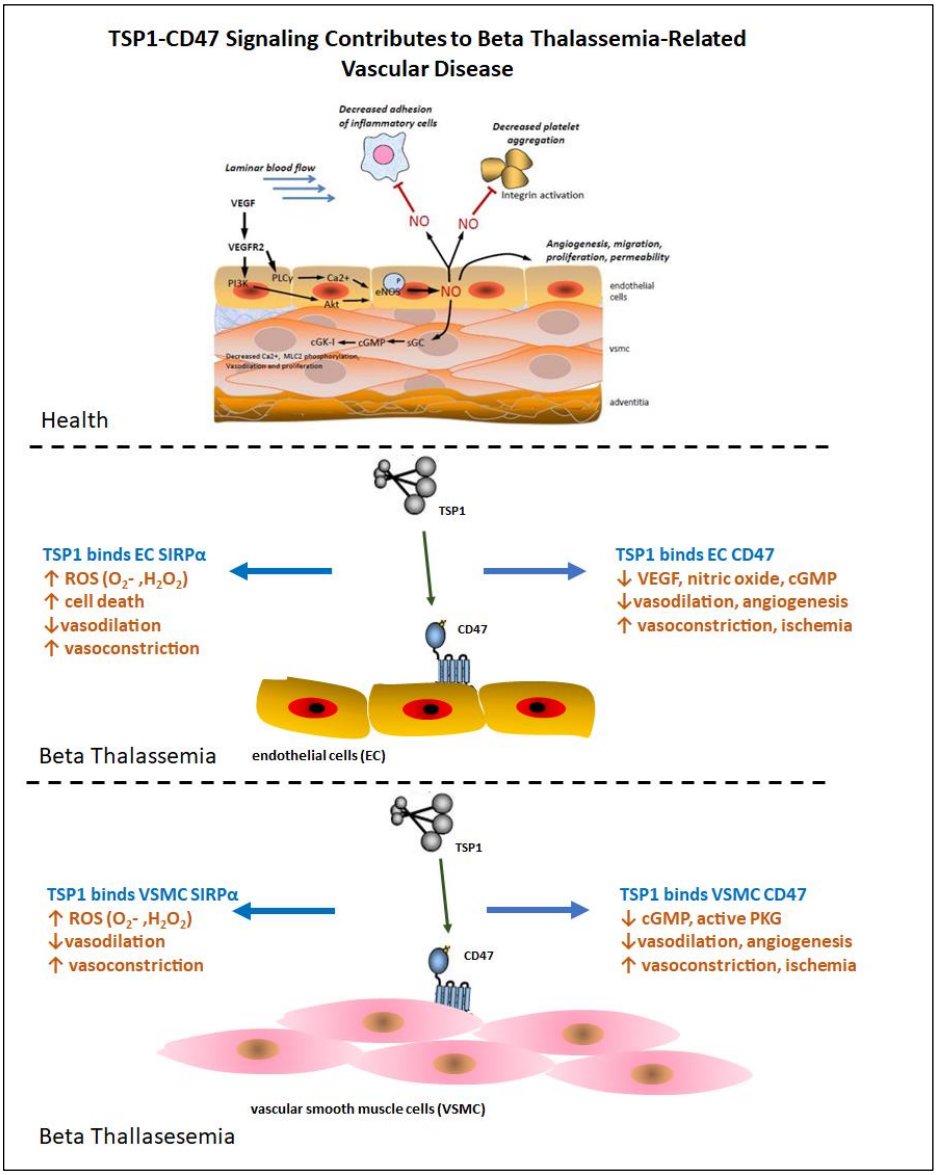

Figure 1. The maladaptive TSP1-CD47 is (1) increased with beta thalassemia and (2) potentiates the known associated defects in the RBCs and elevates (3) the inflammatory environment and degrades vascular function to promote tissue ischemic, pulmonary hypertension and heart failure. Targeting the maladaptive TSP1-CD47 signal with a CD47 morpholino will improve RBC function and half-life, decrease inflammation, increase tissue perfusion and mitigate pulmonary hypertension. TSP1, thrombpsondin-1; VEGF, vascular endothelial growth factor; cGMP, guanosine monophosphate; EV, endothelial cell

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