**In Vitro Unsickling and Anti-inflammatory activity by SANGUINATE™ mediated gas transfer**

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**Background:**

Sickle cell disease (SCD) is typified by abnormal red blood cell (RBC) morphology. The abnormally-shaped (sickle) RBCs have difficulty perfusing the microvasculature and are prone to hemolysis. A state of chronic inflammation, poor oxygen delivery and tissue hypoxia are characteristic of SCD and are exacerbated during vaso-occlusive crisis and other acute SCD comorbidities.

SANGUINATE™ (PEGylated carboxyhemoglobin bovine) is designed to release carbon monoxide (CO) to reduce inflammation, inhibit vasoconstriction, as well as deliver oxygen.

**Methods:**

Studies examined gas transfer, anti-sickling, and anti-inflammatory activities using whole blood samples from SCD and healthy volunteers. Carboxyhemoglobin and oxyhemoglobin levels were monitored to determine dose and time effects as well as the repetitive gas transfer capacity of SANGUINATE. Sickling was induced in a low oxygen atmosphere prior to SANGUINATE or control treatments. Unsickling was measured by photo-microscopy and imaging flow cytometry. Anti-inflammatory activity on LPS-activated samples was quantified by qPCR and flow cytometry of selected panel of inflammatory markers.

**Results:**

SANGUINATE was shown to rapidly transfer CO to RBCs with concomitant oxygen loading of SANGUINATE. The gas exchange was rapid and followed mass balance that reached equilibrium in a closed system. Reciprocal results were observed with CO-loaded RBC and oxygenated SANGUINATE. SANGUINATE mediated gas transfer with RBCs through multiple cycles. Both CO and oxygen delivered by SANGUINATE reduced the sickled RBC fraction as compared to controls. Imaging flow cytometry showed SANGUINATE produced rapid, dose-dependent statistically significant reduction in sickled RBC numbers. SANGUINATE pre-treatment of normal and SCD whole blood decreased inflammatory cytokine RNA and protein levels.

**Conclusion:**

The polymerization of the HbS upon deoxygenation causes morphological changes in the RBCs. SCD comorbidities are due to the occlusive and fragile properties of sickled RBCs. Treatments that reverse RBC sickling and reduce inflammation during an acute SCD event such as vaso-occlusive crisis or acute chest syndrome may provide clinical benefits. SANGUINATE was designed to promote CO and O2 transfer to provide physiological supplementation of O2 transport/delivery in conditions of hemolytic or ischemic anemia. Early intervention with SANGUINATE could limit a crisis event and reduce pain severity while providing a timely resolution.

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