Abstract Submission

29. Non-malignant hematopoietic disorders

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RAPID REVERSAL OF RED BLOOD CELL SICKLING PROMOTED BY PEGYLATED CARBOXYHEMOGLOBIN BOVINE GAS TRANSFER PROPERTIES

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Background: Sickled Red blood cells (RBCs) have increased rigidity restricting their passage through the microvasculature and cause vasoocclusive crisis (VOC). PEGylated carboxyhemoglobin bovine (Sanguinate™; SG) was designed to release carbon monoxide (CO) to reduce vasoconstriction, counteract inflammatory responses as well as deliver O_2 to hypoxic tissues. Early intervention of VOC with SG treatment could limit the crisis event and reduce pain severity while providing a timely crisis resolution.

Aims: SG treatment effects were evaluated under controlled conditions to determine its capacity for gas exchange with RBCs obtained from healthy and Sickle Cell Disease (SCD) volunteers.

Methods: Carboxyhemoglobin and oxyhemoglobin levels were monitored to determine dose and time effects as well as the repetitive capacity of SG to facilitate gas transfer processes. RBC treated samples were analyzed by light microscopy and image capture flow cytometry to visualize and quantify the effects of SG treatment on reversing sickled SCD RBC. PEG bovine serum albumin (PBSA) product was used as a control.

Results: SG addition to normal oxygenated RBC resulted in CO and O_2 exchange between RBC and SG that followed mass balance and reached equilibrium in closed system. Kinetic analysis revealed SG rapidly transferred its CO component to oxygenated RBC with concomitant O_2 loading of SG. Using experimentally loaded RBC with CO and SG with O_2 produced similar reciprocal gas exchange results. Additionally the primary RBC/ SG reaction products were isolated and cycled demonstrating the ability of SG to continually facilitate gas transfer through multiple exposure events. Similar studies using SG, oxygenated SG (produced by an RBC exchange reaction) or PBSA control were conducted with SCD RBC. Sickling was induced by incubation of RBC in a hypoxic chamber for 3 hours prior to SG or control treatments. After 2 hours of treatment, cells were fixed by addition of glutaraldehyde. Photomicroscopy showed a marked reduction in the sickled RBC population with both SG or oxygenated SG treatments but not with the PBSA control (Fig. 1). Results from imaging flow cytometry further supported the microscopy findings and revealed a significant quantitative reduction in the percentage of sickled RBC levels.

Image/Pictures:

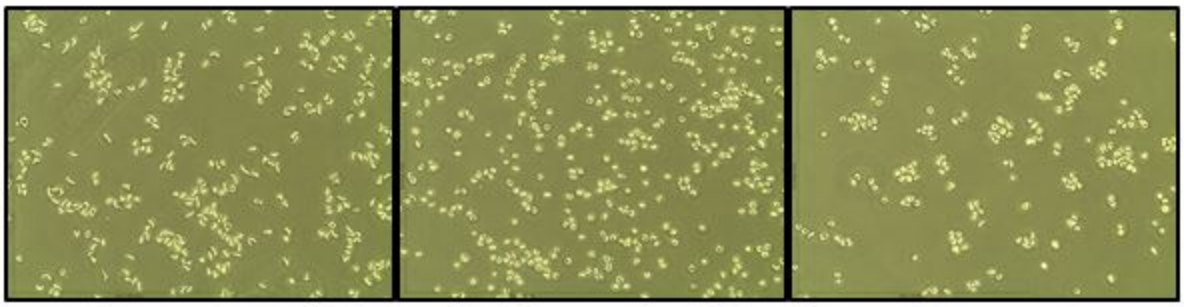

Fig. 1 *SG Treatment of Sickled RBC.* Hypoxic SCD RBC treated with SG, oxygenated SG or PBSA for 2 hrs prior to fixation and image capture (40X mag).

Summary/Conclusion: RBCs are negatively impacted by repetitive HbS polymerization/de-polymerization cycling and treatments that reverse RBC sickling during a VOC event could be expected to provide broad clinical benefits. SG was designed to promote CO and O_2 transfer in a concentration dependent manner providing physiological supplementation of O_2 transport/delivery in conditions of hemolytic or ischemic anemia.

Additionally since ASH 2015, anti-inflammatory activity on LPS activated samples has been quantified by qPCR and flow cytometry of a selected panel of inflammatory markers. SG pre-treatment of normal and SCD whole blood significantly decreased inflammatory cytokine RNA and protein levels. Studies are ongoing examining the effect of SG on hypoxia induced inflammation.

These *ex vivo* data demonstrated for the first time that under controlled conditions, a therapeutic agent serves as an active gas transport agent providing either CO or O_2 to sickled RBCs, prompting rapid unsickling and significant decrease in inflammation markers. Furthermore, image capture flow cytometry provided a quantitative measurement of sickled RBC fraction decrease. This mechanism may provide a useful biomarker test in future clinical studies to monitor SG treatment effects on SCD patients.

Keywords: Inflammation, Sickle cell