**Background:** In Sickle Cell disease (SCD), a single amino acid substitution in the β-globin chain converts HbA to sickle genotype HbS. This genetic change promotes HbS polymerization upon deoxygenation that can promote occlusion of small blood vessels that is often associated with increased blood viscosity and circulatory inflammation. PEGylated-carboxyhemoglobin (PEG-CO-Hb; SANGUINATE) was designed as a novel therapeutic agent to initially release carbon monoxide (CO) and then transfer oxygen (O₂) to hypoxic tissues and cells. Delivery of either CO and/or O₂ to hypoxic, sickled red blood cells (RBCs) should return cells to a more normal cell morphology and help re-establish normal blood flow and rheology. PEG-CO-Hb was shown to mediate transfer of either a CO or O₂-CO mixture and restore normal morphology to hypoxic, sickled RBCs in vitro. Studies are now focused on the potential therapeutic implications of delaying or slowing sickling, which should maintain normal blood flow through hypoxic microvasculature. Unsickling is expected to be expedited by O₂ transfer by PEG-CO-Hb. To examine these potential therapeutic effects, current in vitro studies examined the effects of time and dose of PEG-CO-Hb to not only reverse, but also prevent or delay sickling by transferring CO as well as expedite O₂ transfer to the sickled RBCs.

**Methods:** Reversal of sickling studies were conducted by deoxygenating RBCs from age matched healthy (control) and SCD volunteers in followed by treatment with either PEG-CO-Hb, fully oxygenated PEG-Hb (PEG-O-Hb) or PEG-BSA for 2 hours. For prevention of sickling studies, fully oxygenated RBC suspensions were treated with increasing amounts of PEG-CO-Hb and then subjected to hypoxia for 3 hours. Time-course effects were quantified by area under the curve (AUC) analysis. O₂ transfer studies were conducted by treating hypoxic, sickled RBCs to increasing concentrations of PEG-CO-Hb and raising the pO₂ from 3.8mm to 40mm. In all studies, the fractions of CO-Hb, O₂-Hb and reduced Hb were determined by co-oximetry and sickled RBCs were quantified by imaging flow cytometry of fixed RBC specimens.

**Summary:** RBCs from patients with SCD undergo a conformational shift upon deoxygenation resulting in HbS polymerization and morphological changes of the RBCs. It is only when the fraction of oxygenated or carboxylated HbS reaches a sufficient level that reversion to normal cell morphology occurs which promotes vascular perfusion. These experiments showed a concentration and time-dependent effect of PEG-CO-Hb ability to deliver both O₂ and CO to sickled RBC. This data suggested that PEG-CO-Hb is a promising gas transfer agent that has the potential to improve sickle cell morphology by reversing sickling; the underlying pathology of sickle cell disease co-morbidities. Phase II clinical studies enrolling patients in Central and South America and in the USA for the treatment of vaso-occlusive crisis. A phase 2 trial in SCD leg ulcers has completed enrollment in the Dominican Republic and Panama.