Prolong Pharmaceuticals™

A Laboratory Interference study of PEGylated Carboxyhemoglobin Bovine (SANGUINATE™) on Assays of Serum Chemistry, Hematology, Coagulation and Blood Gases

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Objective

- PEGylated carboxyhemoglobin bovine (SANGUINATE™ is a dual action carbon monoxide releasing / O_2 transfer agent in development for treatment of ischemic/inflammatory indications such as congenital or acquired hemoglobinopathies and vasculopathies, including Sickle Cell Disease, the α-/β-Thalassemia, and cerebrovascular or peripheral vascular diseases.
- Previous studies have demonstrated that hemoglobin-based solutions and PEGylated proteins exhibit interference in clinical laboratory assays due to the heme groups of hemoglobin which absorb strongly in the visible spectrum. The objective of the current study was to examine clinically-relevant concentrations of SANGUINATE in assays of clinical chemistry, hematology, coagulation, and blood gas measurements.

SANGUINATE™ Overview

An intravenous *therapeutic* which combines the beneficial functions of:

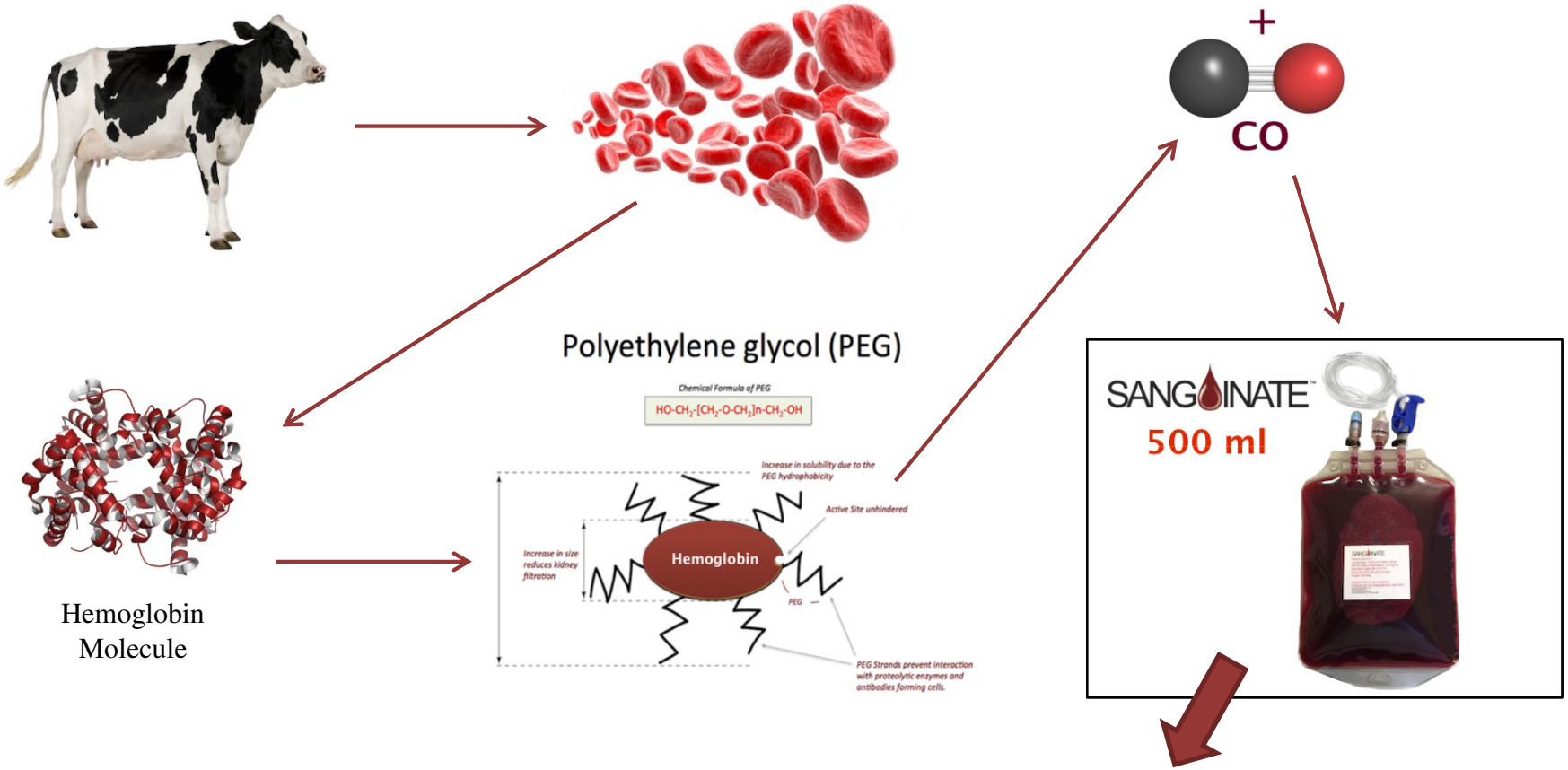
- "Targeted" Oxygen Transfer to hypoxic tissues
- Carbon Monoxide for release into vasculature (anti-inflammatory and vasodilatory)
- Enhanced blood/plasma flow dynamics

that act together to reverse the effects of vascular ischemia and improve perfusion and oxygenation.

SANGUINATE™

SANGUINATE™ What is it?

(PEGylated carboxyhemoglobin bovine)



To treat Anemia, Ischemia and Hypoxia caused by Diseases such as:
Stroke, Subarachnoid Hemorrhage, Myocardial infarction, Vaso-Occlusive Crisis, Sickle Cell Disease & Comorbidities, Thalassemia

Methods

- The EP7-A2 CLSI guideline was used as a basis for the interference studies
- The guidance requires two parts of an interference study (a) identify interference (b) Characterize interference
- According to guidelines, the observed values were used to calculate the interference, and if the value was greater than the confidence interval then the parameter was considered to have interference
- Human Interference study was performed at National Jewish Health Advanced Diagnostics Laboratory (Denver, CO) and interference studies with three species (rat, pig and monkey) were performed at Toxikon Corp., Bedford MA
- The blood samples were collected from healthy volunteers after IRB approval

Methods

- The multispecies study (12-3330-N4) was used in order to show species equivalence
- The multispecies study was also used to characterize interference
- A confidence interval was set using manufacturers guidelines as well as instrument control data
- In general, there were very few differences among the species.
- Any differences that were found were adjusted for by using species specific correction factors.

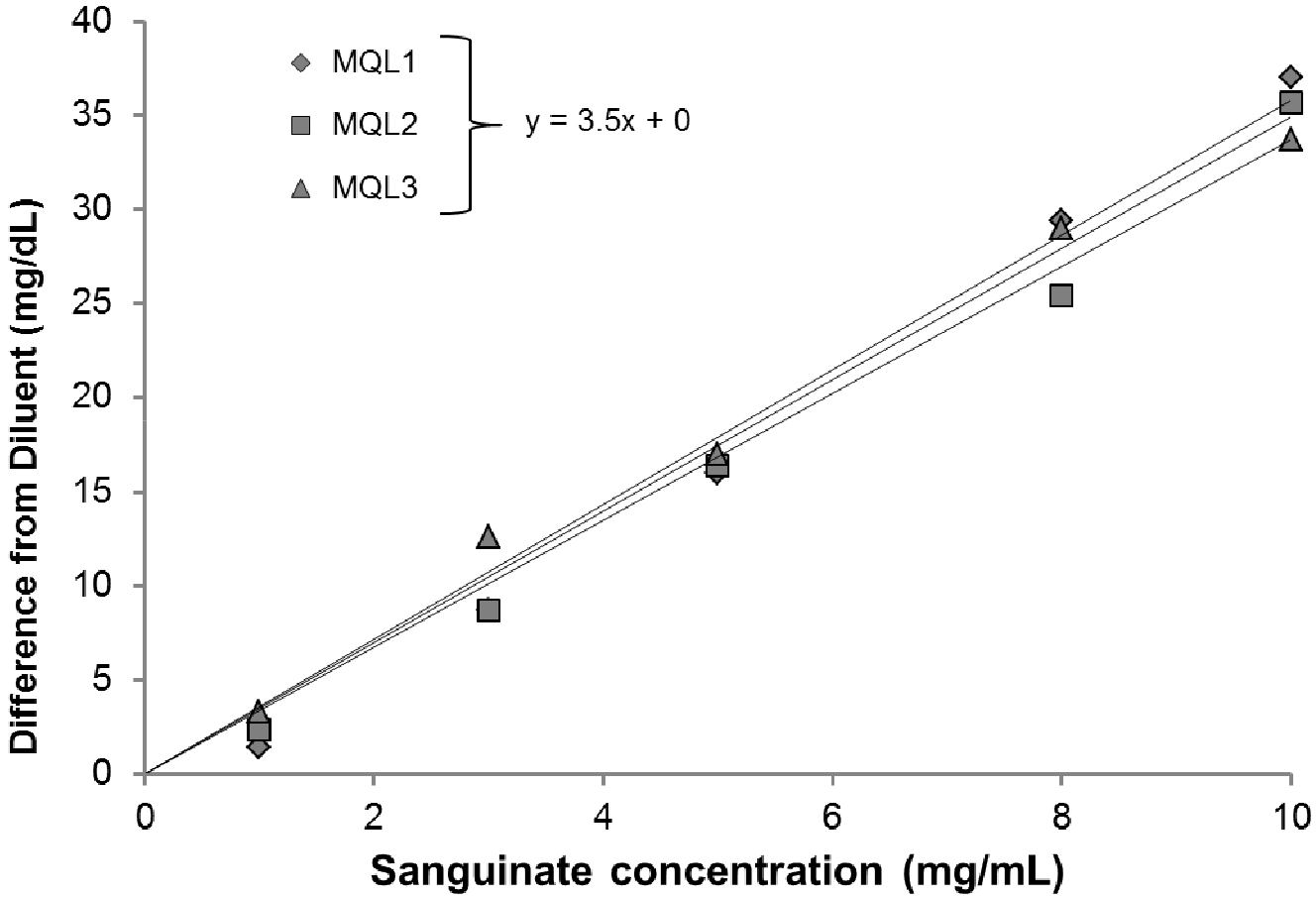
Instrumentation

- Clinical chemistry parameters were tested on an Olympus AU480 instrument (Beckman Coulter, Brea, CA).
- SANGUINATE or diluent was diluted into QC control materials of varying concentrations of analytes (BioRad Multiqual Levels 1, 2 and 3) to determine whether interference differs with varying concentration of analyte.
- Troponin I, Creatinine, BUN were analyzed on the iSTAT® 1 Analyzer (Abbott).
- Hematology parameters were evaluated on a Beckman Coulter LH 750 instrument (Beckman Coulter, Brea, CA) using whole blood drawn in to EDTA and prepared to final SANGUINATE concentrations of 1, 5, or 8mg/mL or equivalent diluent volumes.
- Coagulation parameters were evaluated on an ACL 1000 instrument (Beckman Coulter, Brea, CA) using both QC materials (Instrumentation Labs, HemosIL Controls 1, 2, and 3) and pooled citrated plasma.
- Blood gas parameters were tested using a Radiometer ABL 800 Flex (Radiometer, Copenhagen Denmark) on heparinized whole blood samples. Specimen for blood gas screening were prepared to final SANGUINATE concentrations of 1, 5, or 8mg/mL.

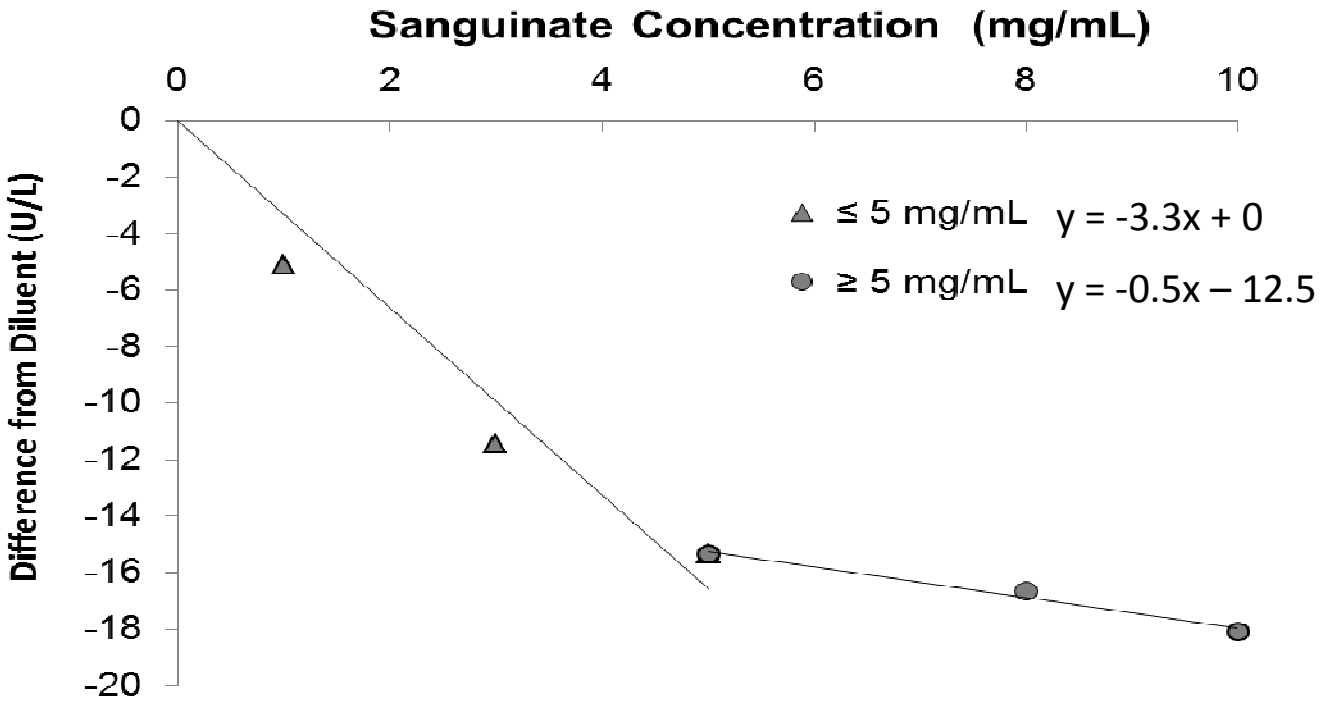
Results

- SANGUINATE elicited a concentration-dependent interference in nine of twenty-eight chemistry assays, with five assays affected with elevated measurements (albumin, A/G ratio, cholesterol, total iron, percent iron saturation), and four assays exhibiting decreased measurements (GGT, globulin, total iron binding, unsaturated iron binding).
- The observed results matched closely the manufacturer's technical documents for hemoglobin interference and correlated well with the reported H-index for hemolysis.
- There was a decrease in automated and manual platelet count due to in vitro clumping of platelets, and a slight increase in aPTT observed.
- Other hematology, coagulation, and blood gas indices were not affected.

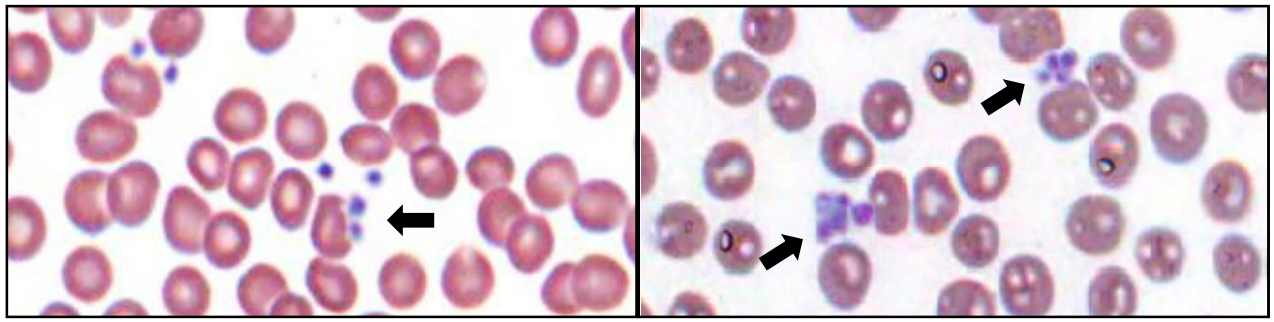
Interference analysis for cholesterol



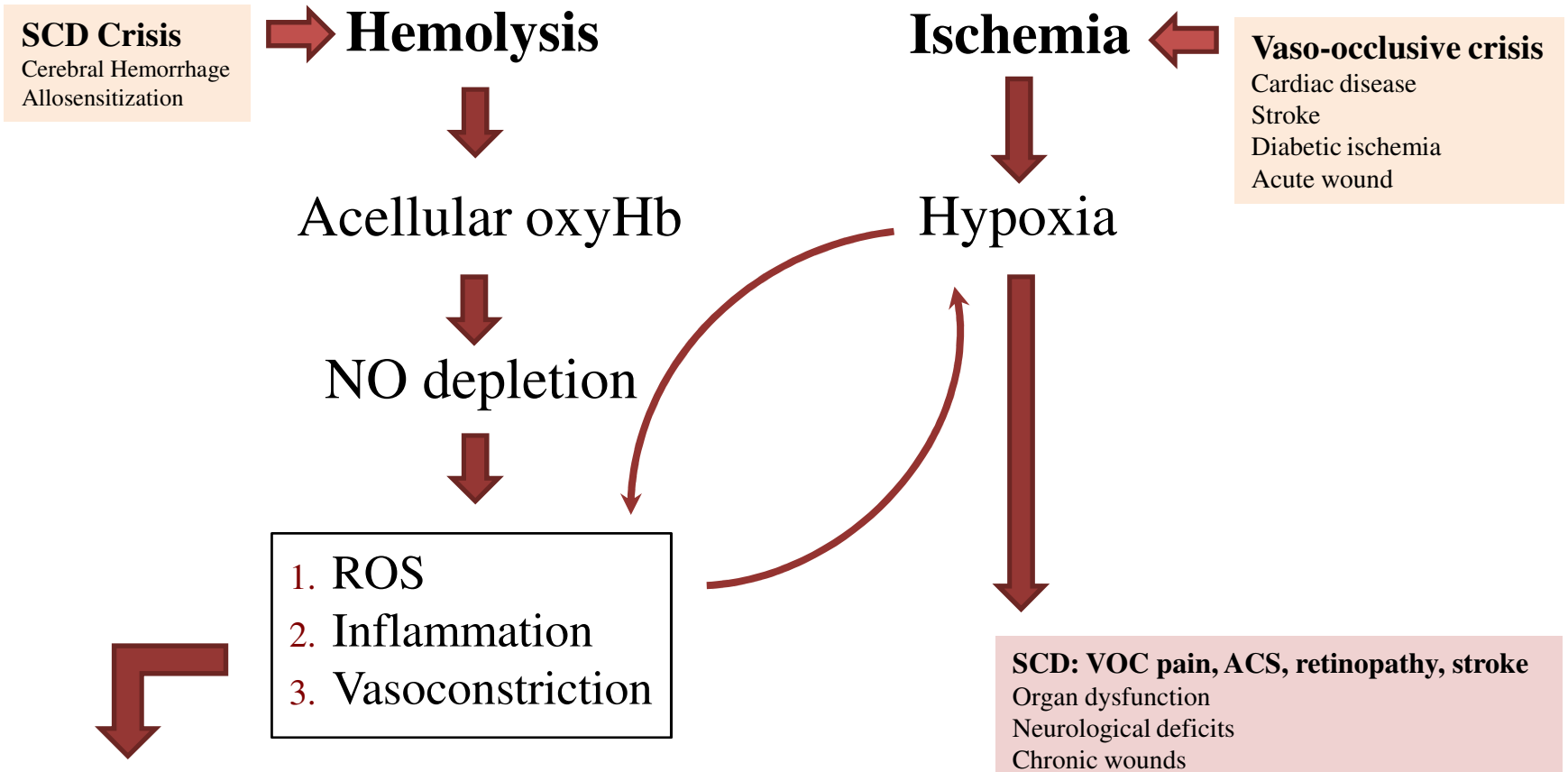
Interference of gammaglutamyl transferase



Clumping of platelets in samples spiked with SANGUINATE™

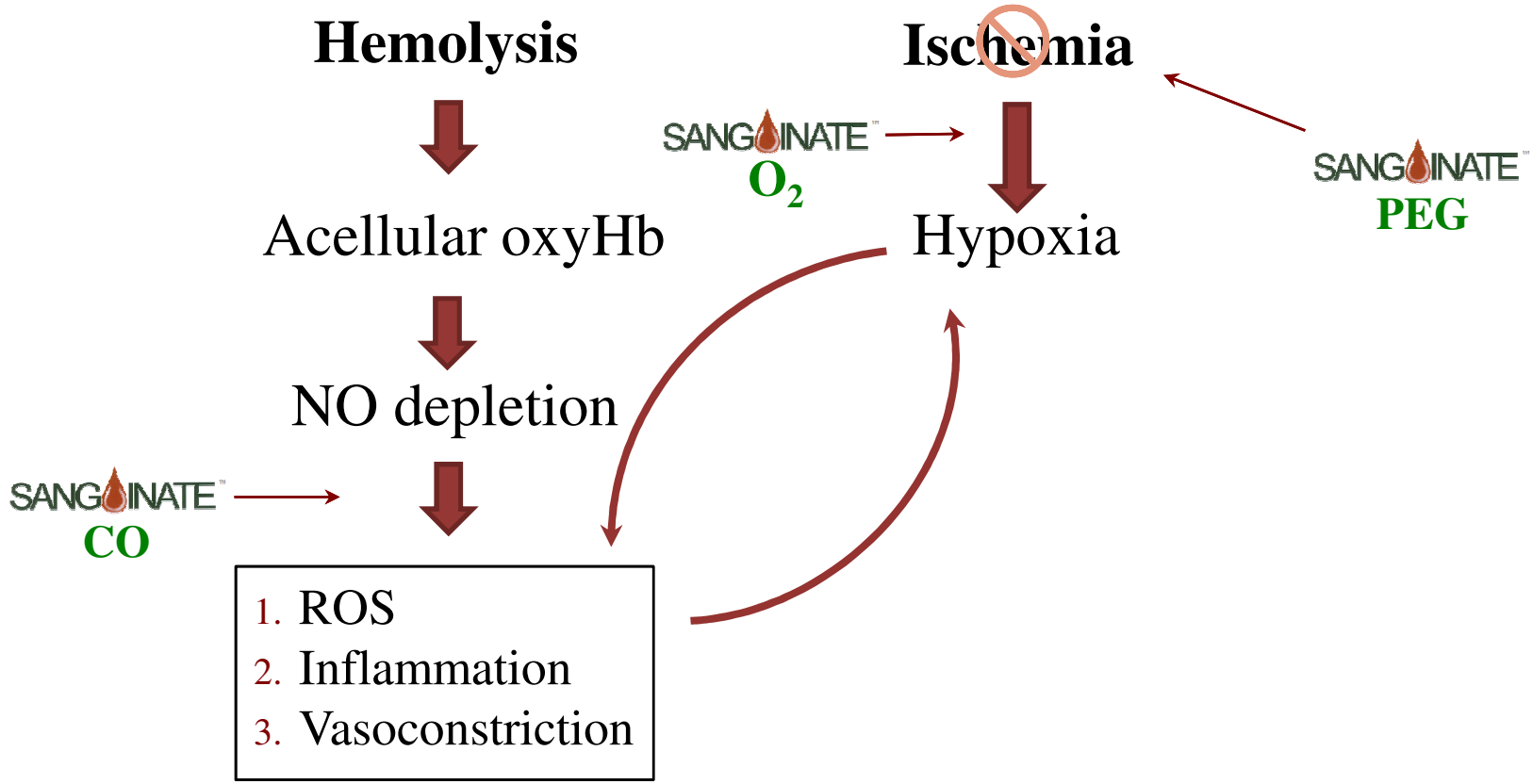


Hemolytic and Ischemic Pathways



SANGUINATE™

Interrupts both the Hemolytic and Ischemic Pathways at multiple points with multiple mechanisms



Clinical Program of SANGUINATE™

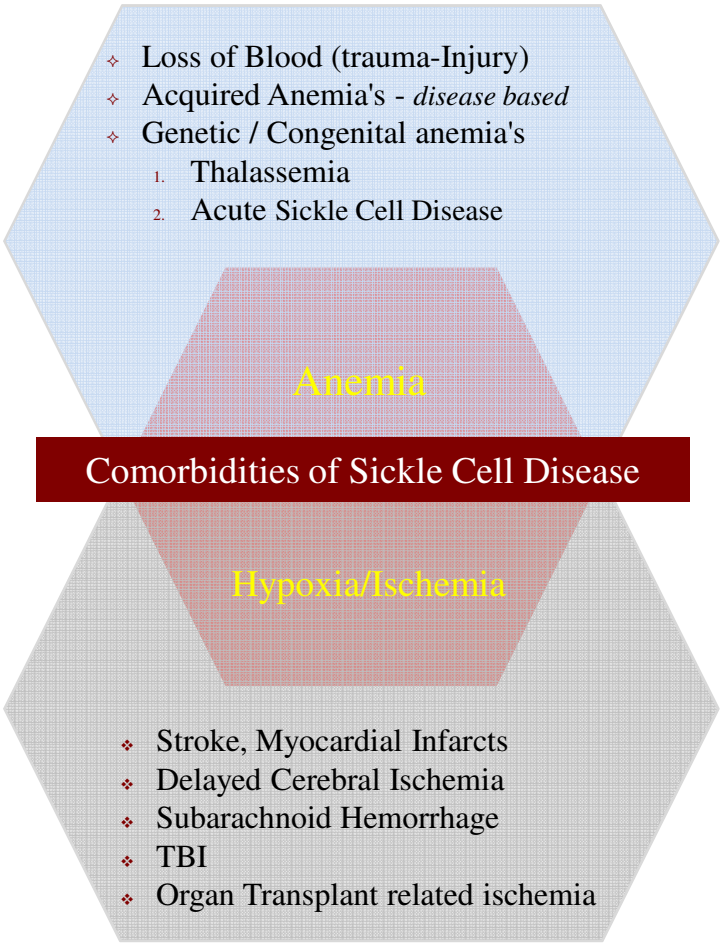
Completed

- 51 subjects/patients treated to date.
- 2 INDs and 3 eINDs (compassionate) approved by FDA.
- Phase I trial in healthy volunteers (Australia) completed and submitted to FDA.
- Phase 1b studies in stable SCD patients (safety) are completed.

On-Going Studies

- Phase 2 study in SCD VOC
- Phase 2 study in SCD leg ulcers
- Phase 2 study in Delayed Cerebral Ischemia
- Phase 2 study in β-Thalassemia

SANGUINATE™ Therapeutic Targets



- **Acute chest syndrome**
- **Pulmonary Hypertension**
- **Allo-immunization**
- **Hyperhemolysis**
- **Vaso-occlusive crisis (pain)**
- **SCD ulcers**
- **Priapism**
- **Dactylitis**
- **Stroke**
- **Cardiovascular Disorders**

Conclusion

- We conclude that none of the analytes used for critical care evaluation exhibits interference due to SANGUINATE, and that the profile of interference requires testing on specific instruments to be used in clinical evaluation of patients.
- Good correlation of the H-index with spiked SANGUINATE concentrations, should provide confidence that clinical laboratory data can be interpreted safely in the presence of SANGUINATE.