PEGylated Carboxyhemoglobin Bovine (SANGUINATE®) Restores RBCs Roundness and Reduces Pain During a Sickle Cell Vaso-Occlusive Crisis

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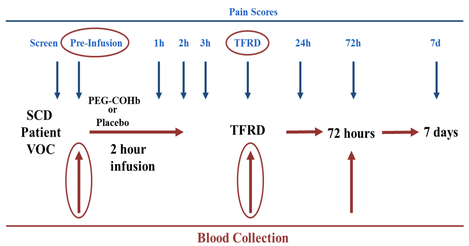
Background:

Pain during vaso-occlusive crisis (VOC) is a hallmark of sickle cell disease (SCD) as deoxygenated (sickled) red blood cells (RBC) aggregate in the microvasculature due to Hemoglobin S (HbS) polymerization. PEGylated carboxyhemoglobin bovine (PEG-COHb; SANGUINATE®) was designed as a gas transfer agent to depolymerize HbS. Prior *in vitro* studies have shown PEG-COHb capability to transfer oxygen (O_2) to SCD deoxygenated RBCs, reversing sickling. A prospective, randomized, single-dose Phase II clinical study is in progress (NCT02411708) evaluating the safety and efficacy of PEG-COHb in the treatment of severe VOC including pain reduction in the ambulatory setting. This research reports on the preliminary evaluation of the levels of RBC sickling following treatment and association with pain score.

Methods:

Participants were randomized 2:1 to a PEG-COHb (320 mg/kg) or saline (placebo) arm in equivalent volume/kg. In addition to standard treatment and IV opioids per institutional practice. Pain was assessed using a 10-point pain scale. Blood was collected to assess RBC shape morphology changes using image-based flow cytometry.

Data Collection: Pain Scores and Blood Samples



Blood was collected at pre-infusion, at TFRD, and at 72-hours post-treatment to test RBC shape, RNA, and Plasma cytokines. RBC shape values and pain-scores at pre-infusion and TFRD are presented here.

KEY:

PEG-COHb: PEGylated Carboxyhemoglobin bovine

Q: Quadrant

TFRD: Time for Readiness of Discharge

For More Information Please Contact:

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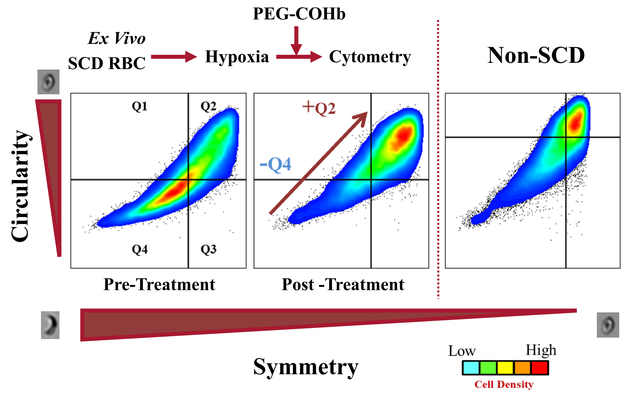
The Conflict of Interest disclosure forms for above authors have been satisfied.

Ex Vivo RBC Shape Determination by Imaging Cytometry

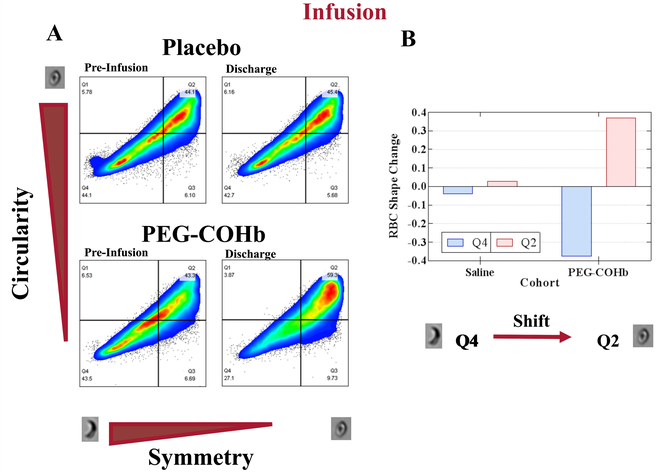


Left: Change in RBC shape profile after *ex vivo* unsickling by PEG-COHb. RBCs from commercially sourced SCD volunteers were deoxygenated for 2 hours in a hypoxic environment and then treated with PEG-COHb, fixed, and subjected to imaging cytometry. RBC circularity is expressed as a function of symmetry, with colors representing event density. Quadrant centers were placed at median values of each parameter of pre-treatment samples, and then applied to post-treatment samples. Change in RBC shape in PEG-COHb post-treatment samples is represented by an arrow as a shift from Q4 to Q2.

Right: RBCs from a non-SCD participant, derived from the same commercial source, are shown for comparison. Quadrant centers were placed at median values of each parameter.

Preliminary Clinical Results:

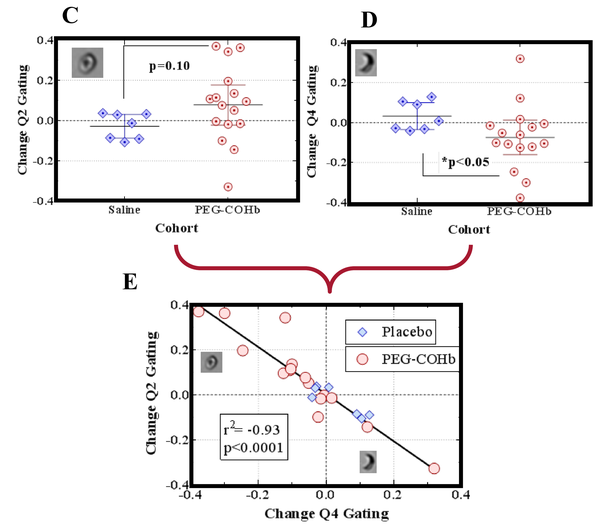
Shift of Global RBC Population After PEG-COHb Infusion



A. Blood samples were collected at pre-infusion (left) and TFRD (right). RBC circularity and symmetry were determined by imaging cytometry and further analysis. Quadrant centers were placed at median pre-infusion values and then applied to TFRD histograms. The percentage of single cell events falling within each quadrant are shown.

B. The fraction of Q2 and Q4 events in TFRD sample were normalized to pre-infusion values and expressed as fractional change. Events falling within Q2 represent more circular and less symmetrical cells, while events falling within Q4 have a negative relationship.

Fractional Changes in RBC Shape

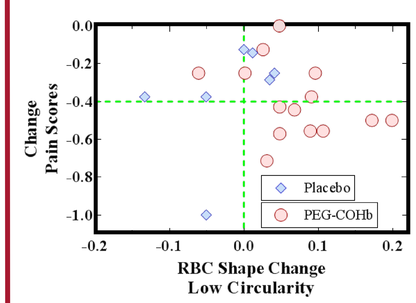


RBC Shape Change ↑ Q2 ↓Q4
Saline-2/7 (28%)
PEG-COHb 10/16 (63%)
(p=0.19, Fishers exact test)

Fractional change in Q2 (**C**) and Q4 (**D**) gating after saline or PEG-COHb infusion. Error bars represent the 95% confidence intervals of the mean. p Values between saline and PEG-COHb were determined by non-parametric test (Mann-Whitney).

E. Negative correlation between the fractional changes in Q2 andQ4 after saline or PEG-COHb infusion. (Pearson correlation coefficient and p value shown).

Relationship Between RBC Shape Change and Pain Scores



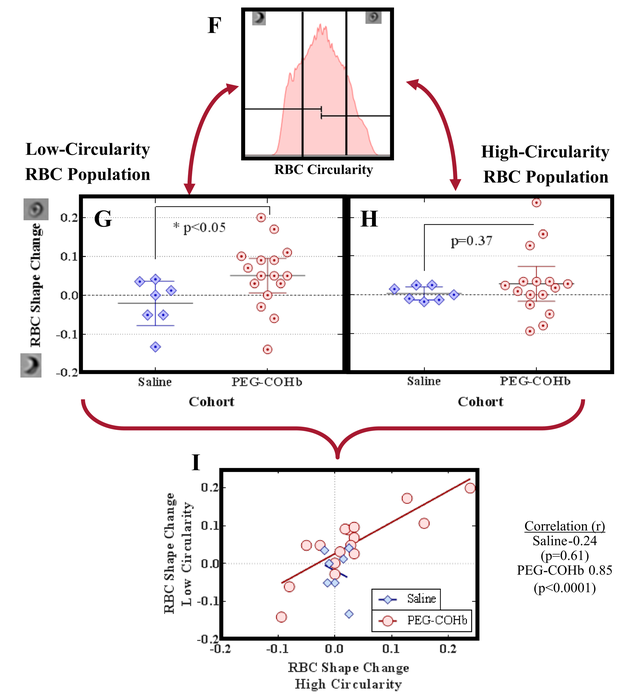
Participants with >40% Pain Score Decrease and
RBC Circularity Increase
Saline 0/7 (0%)
PEG-COHb 8/14 (57.1%)

(*p=0.02, Fishers exact test)

Left: Fractional change in low-circularity population is shown as a function of fractional changes in pain scores at TFRD.

Right: Events in lower right quadrant delineated by green dotted lines (0/7 placebo-vs-8/14, PEG-COHb) denote participants with greater than 40% decrease in pain score and increased RBC circularity values at discharge compared to pre-infusion values.

PEG-COHb Group Show Increased Circularity in High- and Low-Circularity Populations



Correlation (r)
Saline-0.24
(p=0.61)
PEG-COHb 0.85
(p<0.0001)

F. RBC circularity values from each participant were segmented according to population median (high and low). Fractional change of median shape values between pre-infusion (**G**) and TFRD (**H**) in low-circularity population. p values between saline and PEG-COHb were determined by non-parametric test (Mann-Whitney).

I. Normalized values of the low-circularity population are shown as a function of high-circularity values. Positive correlation between the fractional changes of high- and low-circularity RBC populations from PEG-CO-Hb infused participants (Pearson r and p values shown).

Conclusions:

Global RBC population in 10 of 16 of PEG-COHb-treated participants exhibited increased circularity and decreased symmetry consistent with unsickling observed in *ex vivo* experiments.

- Low-circularity RBC population from PEG-COHb-treated participants showed greater shape change after infusion compared to saline control (p<0.05)
- Increase to more normal shape values from high- and low-circularity RBC population was correlated only in PEG-COHb participants
- 8 of 14 of PEG-COHb-treated participants exhibited 40% or greater decrease in pain scores and increased RBC circularity between pre-infusion and time for readiness of discharge compared to 0 of 7 participants in saline cohort (p=0.02)

Results warrant further clinical development of PEG-COHb (SANGUINATE®) for the treatment of acute vaso-occlusive crisis in sickle cell participants.

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