Background:

Pain during vaso-occlusive crisis (VOC) is a hallmark of sickle cell disease (SCD) as doxynagminated (sickled) red blood cells (RBCs) 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 (O2) to SCD doxynagminated 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 morphologies using image-based flow cytometry.

Preliminary Clinical Results:

Shift of Global RBC Population After PEG-COHb Infusion

Ex Vivo RBC Shape Determination by Imaging Cytometry

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

Fractional Changes in RBC Shape

In Vivo

SCD RBC

Hypoxia

Non-SCD

PEG-COHb

Circularity

Ex Vivo

Pre-Treatment

Post-Treatment

Symmetry

Q1

Q2

Q3

Q4

A

Placebo

B

PEG-COHb

Symmetry

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 Q2 to Q4.

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.

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 and Q4 after saline or PEG-COHb infusion. (Pearson correlation coefficient and p values shown).

Relationship Between RBC Shape Change and Pain Scores

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 TFRED (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-COHb-influenced patients (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 populations was correlated only in PEG-COHb participants.
- 8 of 14 of PEG-COHb-treated participants exhibited 40% or greater decrease 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 patients.

For More Information Please Contact: www.prolongpharma.com

The Cytometry images are shown for show authors have been satisfied.

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