Iron Restricted Erythropoiesis under Hepcidin Mimetic Treatment (PN23114) Improves Disease Parameters in a Mouse Model for Sickle Cell Disease

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INTRODUCTION

Sickle Cell Disease (SCD) is an inherited hemolytic disorder that results from a single point mutation in the hemoglobin-beta (HBB) gene that encodes for the β-globin subunit of hemoglobin, causing the production of abnormal hemoglobin S (HbS). HbS polymerizes under low oxygen conditions, causing sickling effects on red blood cells (RBCs). Sickling of RBCs initiates vaso-occlusion, downstream inflammatory responses, and hemolysis (Zhang D, Blood 2016). Previous studies have shown that polymerization under low oxygen conditions is extremely sensitive to HbS concentrations within the RBCs (Hofrichter et al, PNAS, 1974; Sunshine HR, Nature 1978). Controlling iron delivery for erythropoiesis provides a mechanism to potentially down-modulate the HbS concentration within RBCs, thereby reducing the potential for HbS polymerization and sickling of RBCs (Parrow N, Blood 2021).

HEPCIDIN MIMETIC THERAPEUTIC IN SCD MOUSE MODEL

- The potent **hepcidin mimetic peptide PN23114** targets the iron exporter membrane protein ferroportin to trigger its degradation, thus preventing iron export from cells.
- A single dose of PN23114 results in >80% reduction in serum iron within 7 hours post-dose in mouse models.
- The pharmacodynamic effect of limiting iron availability for erythropoiesis in the Townes mouse model for human SCD should reduce HbS concentrations within the RBCs, preventing sickling and hemolysis, thereby altering outcomes in the model.
- Nyffenegger N et. al. (Blood, 2022) have demonstrated reductions in mean corpuscular hemoglobin concentration (MCHC) with vamifeport (small molecule ferroportin inhibitor, currently in phase 2 clinical trial for SCD) treatment in Townes mouse model.

TOWNES MOUSE MODEL FOR HUMAN SCD

HbSS Townes model which is genetically altered to contain the human SCD HbSS homozygous mutation (Wu Li-Chen, Blood 2006; JAX stock #013071):

- Exhibit severe hemolysis as indicated by elevated bilirubin and lactate dehydrogenase (LDH) levels, and a shortened RBC half-life compared to wild type (WT) mice.
- Develop vaso-occlusive events and elevated inflammatory responses, consequent to hemolytic RBCs.
- Exhibit anemia with decreased RBCs, hemoglobin, and consequently elevated reticulocytes and extramedullary hematopoiesis

Disclosures: All authors are current or former employees of Protagonist Therapeutics, Inc.

METHODS

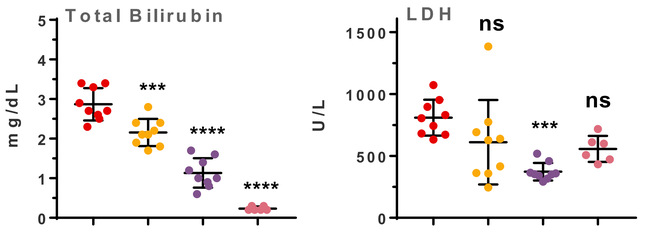
- PN23114 was administered subcutaneously to Townes HbSS mice. Mice were treated over 4 weeks (3 times weekly) with vehicle or PN23114 at 1 or 2.5 mg/kg (vehicle treated WT mice served as controls).
- Serum iron measurement was by colorimetric method after acidic disassociation of elemental iron from transferrin. Hematology analyzer calibrated for mouse samples was used for complete blood counts analysis.
- Statistical analysis was performed using one-way ANOVA w/Dunnett's Multiple Comparisons vs. HbSS Vehicle group; ns p>0.5, * p<0.5, ** p<0.01, *** p<0.001, **** p<0.0001

RESULTS

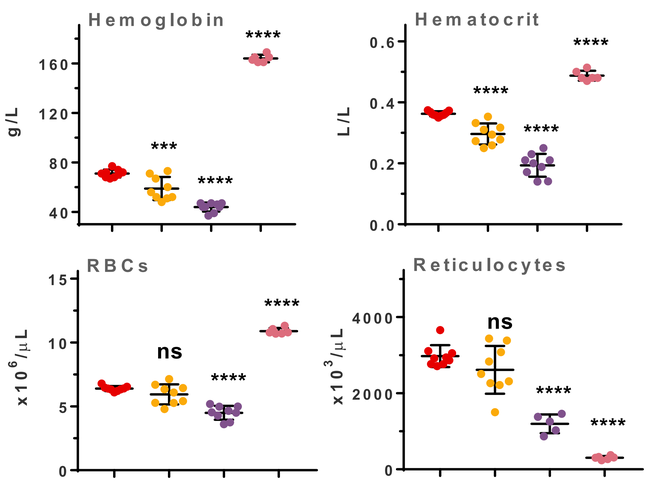
LEGEND: ● HbSS Vehicle ● HbSS PN23114 1 mg/kg ● HbSS PN23114 2.5 mg/kg ● Wild Type Vehicle

Graphs show data for individual animals, along with group mean and standard deviation

❑Total Bilirubin and LDH were lowered with PN23114 treatment compared to the vehicle group, indicating considerable reductions in hemolysis

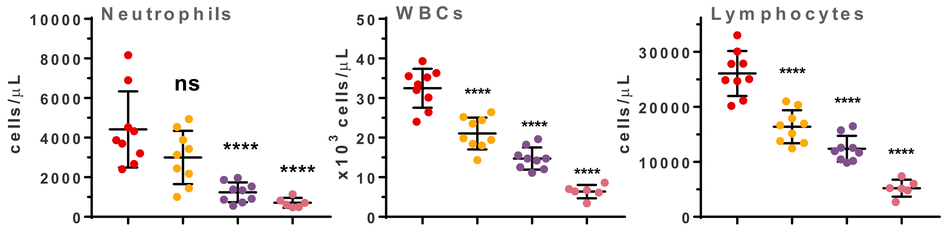


❑Treatment with PN23114 led to reductions in red blood cell parameters

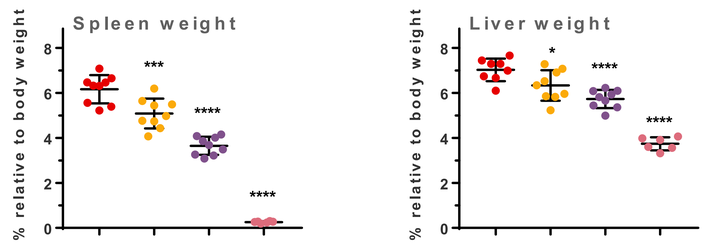


Reductions in RBC parameters were of similar magnitude to published results for vamifeport in the same Townes model (Nyffenegger N., Blood, 2022).

❑Neutrophils are elevated in SCD as a result of inflammatory response to hemolysis and vaso-occlusive events. We observed reductions in neutrophil numbers with PN23114 treatment. Total white blood cell counts (WBCs), and lymphocytes were also reduced with PN23114.



❑ Spleen and liver weights were reduced in the PN23114 treated compared to vehicle groups indicating alleviation of extramedullary hematopoiesis.



CONCLUSIONS:

1. The hepcidin mimetic PN23114 resulted in definitive improvements in several disease parameters in Townes HbSS mouse model for human SCD.
2. Titratability of a subcutaneous hepcidin mimetic peptide will potentially allow for reductions in hemolysis, and with tolerable effects on overall hemoglobin levels.
3. PN23114 is an analog peptide of Rusfertide, with identical PKPD in rodents

➢ **Rusfertide is currently in phase 3 clinical study in polycythemia vera (NCT05210790)**