Rusfertide Analog-PN23114 as a Hepcidin Mimetic Provides Efficacy Benefits in Conjunction with Phlebotomy in Mouse Model for Hereditary Hemochromatosis

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INTRODUCTION

Hepcidin targets the major iron transporter ferroportin, and its deficiency in hereditary hemochromatosis (HH) leads to hyperabsorption of dietary iron and primary iron overload[1,2]. Persistent high transferrin-saturation (TSAT%) results in the occurrence of toxic labile iron that can deposit in organs, causing tissue damage and potential organ dysfunction.

- **Rusfertide, a hepcidin mimetic peptide, has demonstrated potential benefit in reducing the need for therapeutic maintenance phlebotomy in hemochromatosis subjects[3].**
- **In a separate Phase 2 clinical study in Polycythemia Vera, rusfertide essentially eliminated the need for chronic phlebotomy, was able to control hematocrit according to NCCN guidelines, and reverse iron deficiency that had resulted from prior frequent phlebotomies[4].**

CO-TREATMENT OF HEPCIDIN MIMETIC AND PHLEBOTOMY IN HH

- TSAT% values can remain uncontrolled in HH patients who receive chronic phlebotomy to maintain their serum ferritin within normal range.
- Newly diagnosed HH patients most often present with severe iron overload, requiring frequent phlebotomies during the induction phase of therapy that can last for many months, during which TSAT% levels can remain elevated.

Our aim was to evaluate the additive benefits of co-treatment of hepcidin mimetic (Rusfertide analog- PN23114) along with phlebotomy.

Results from our study in mouse model for HH indicate potential value in using Rusfertide therapy to control TSAT%, limit labile iron, and prevent and reverse organ iron deposition.

MOUSE MODEL STUDY METHODS AND RESULTS

METHODS:

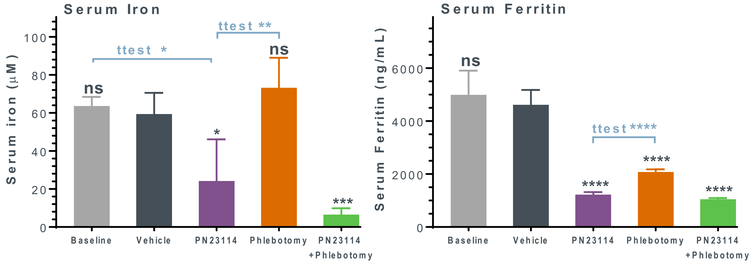
We assessed the effects of hepcidin mimetic treatment on ferrokinetic parameters and organ iron concentrations in a commonly used mouse model for HH, $HJV^{-/-}$ model with homozygous knock-out of HJV, which is a positive regulator of hepcidin.

Male $HJV^{-/-}$ mice ($129S\text{-}Hjv^{tm1Nca}/J$)[5] at 8-12 weeks of age were treated with either PN23114 (7.5 mg/kg, TIW), phlebotomy (~0.3mL blood drawn, QW), or combination of both for a total of ~6 weeks (a separate baseline group was terminated at start of treatment).

PN23114 is a rusfertide analog peptide with identical pharmacokinetic and pharmacodynamic characteristics in rodents. The mice were maintained under diet containing 35ppm–iron from weaning all the way to study termination.

RESULTS:

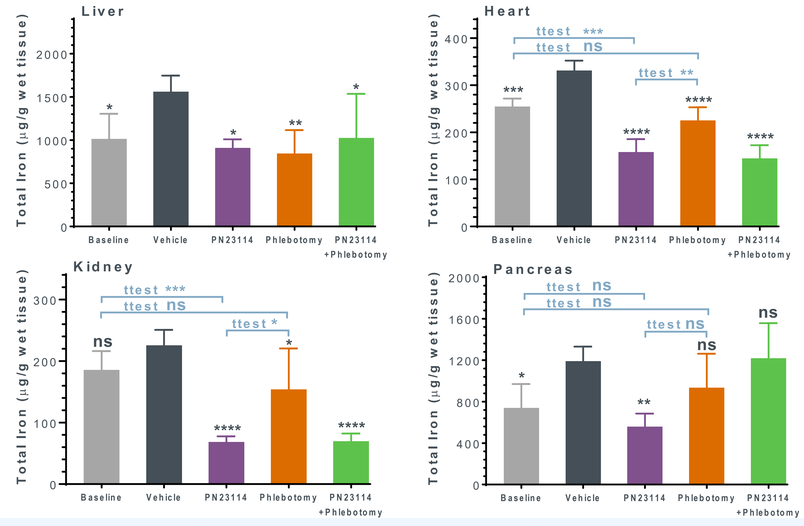
- ❑Treatment with PN23114 alone or in combination with phlebotomy lowered serum iron to normal values (wild type mouse normal value ~30µM)
  - •Serum iron was lowest in the combination group. Weekly phlebotomy therapy alone was not sufficient to reduce serum iron.
- ❑Serum ferritin was lowered with all three treatments (wild type mouse normal value ~1000ng/mL)
  - •Combination was better than PN23114 alone, which in turn was more efficacious than phlebotomy alone.



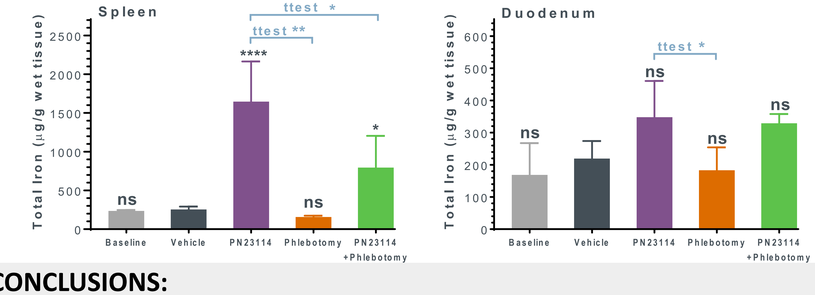
All graphs:
Results from One-way ANOVA with comparisons vs. vehicle are represented by black symbols; Results from pair-wise Welch's t tests are represented by blue symbols

ADDITIONAL METHODS: *Serum iron measurement was by colorimetric method after acidic disassociation of elemental iron from transferrin. Serum ferritin was measured by immunoassay. All measurements were at ~48-hr post-last dose (at trough drug levels). Organ iron concentrations were assessed by ICP-MS method (for total iron). Statistical analysis was performed using One-way ANOVA w/Dunnett's Multiple Comparisons or t test w/ Welch's correction (for pair-wise analysis).*

- ❑Liver iron deposition was prevented in all three treatment groups.
  - •While liver iron in vehicle group had increased compared to baseline group.
- ❑Heart and Kidney iron concentrations were significantly lowered with PN23114 treatment as compared to baseline group.
  - •Phlebotomy by itself was not able to lower heart and kidney iron from baseline.



- ❑PN23114 redistributed iron into spleen and phlebotomy removed iron from the body



CONCLUSIONS:

1. **PN23114 was superior to phlebotomy in controlling serum iron, lowering ferritin, and reducing iron deposition in heart and kidney.**
2. **All three treatments were effective in preventing iron deposition in the liver.**
3. **Combination treatment had additive benefits, with PN23114 driven reduction in tissue iron deposition due to better serum iron control, and phlebotomy driven iron removal from the body.**

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