Disclosure

All authors are employees and shareholders of Protagonist Therapeutics, Newark, California

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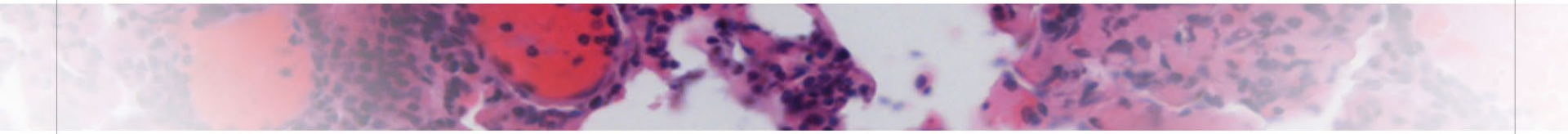

Rusfertide Analog-PN23114 as a Hepcidin Mimetic Provides Efficacy Benefits in Conjunction with Phlebotomy in Mouse Model for Hereditary Hemochromatosis

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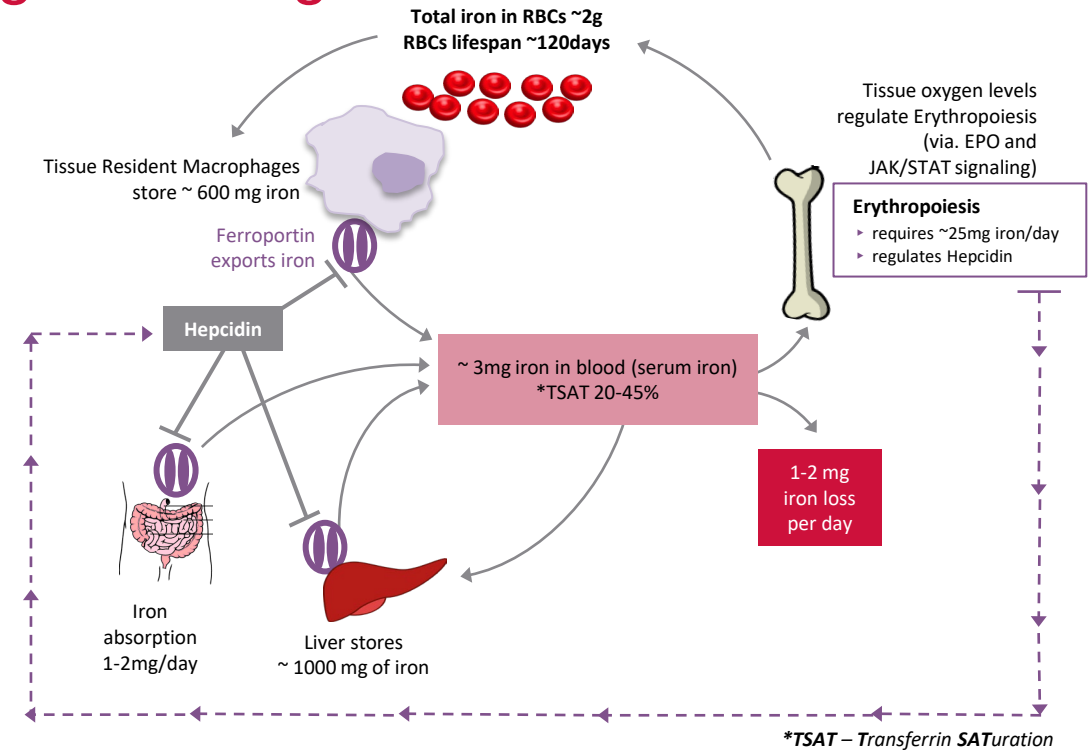
Persistent High Transferrin-Saturation (TSAT%) in HH: Toxic Labile Iron and Organ Damage

- Hepcidin targets iron exporter ferroportin
  - causing its internalization and subsequent degradation
- Deficiency of Hepcidin in Hereditary Hemochromatosis (HH)
  - leads to hyperabsorption of dietary iron and primary iron overload
- Persistent high transferrin-saturation (TSAT%) in HH
  - results in the occurrence of toxic labile iron that can deposit in organs, causing tissue damage and potential organ dysfunction.



*(Nemeth E.; Science, 2004: **306** (5704) 2090 Ramos E.; Blood, 2012: **120** 3829)*

Combining Hepcidin Mimetic Therapy with Phlebotomy in Hereditary Hemochromatosis (HH)

- Hemochromatosis patients undergo phlebotomies to prevent iron overload and to maintain their serum ferritin within normal range
- TSAT% values can remain uncontrolled in HH patients who receive chronic phlebotomy
- Newly diagnosed HH patients may 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 goal was to evaluate the additive benefits of co-treatment of hepcidin mimetic peptide “PN23114” along with phlebotomy in a mouse model for HH
- **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**

Rusfertide, a hepcidin mimetic peptide, has demonstrated potential benefit in reducing the need for therapeutic maintenance phlebotomy in hemochromatosis subjects
(***Kowdley KV, AASLD Hepatology (2021) 74: S1***)

Hereditary Hemochromatosis Mouse Model and Study Design

Mouse model for Hemochromatosis *(Ref: Andrews NC, J Clin Invest. (2005) 115 (8): 2187)*

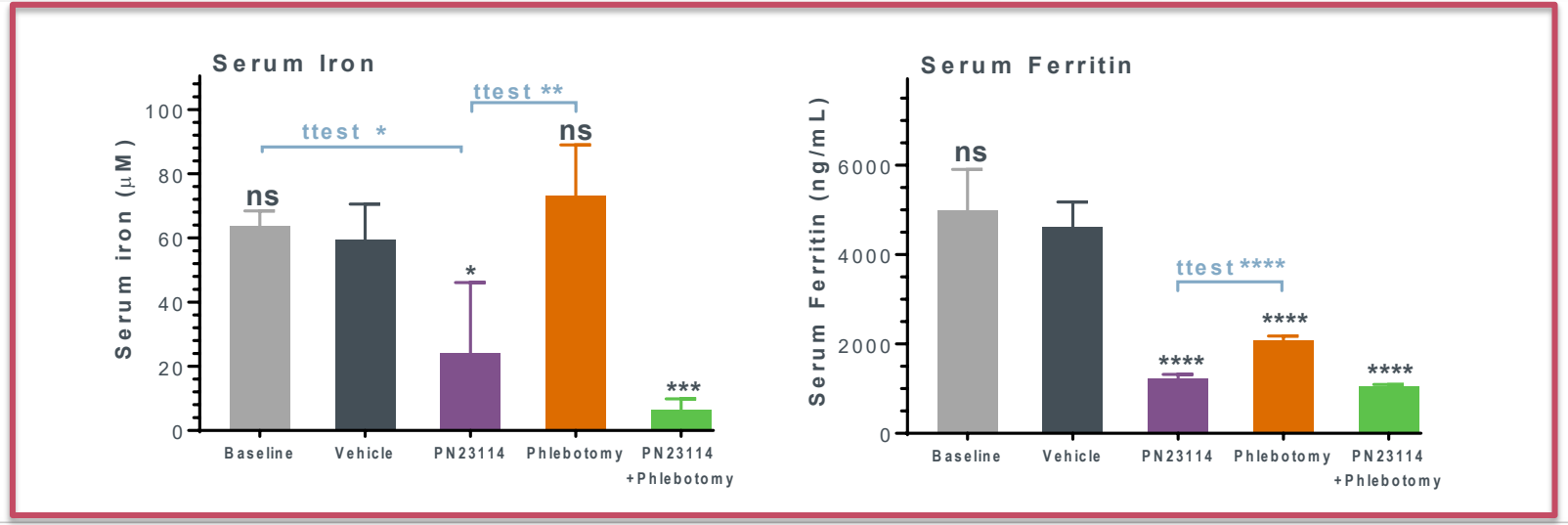
- HJV-/- mice (129S-Hjvtm1Nca/J) with homozygous deletion of hemojuvelin (HJV), which is a co-receptor for hepcidin upregulation
- Male mice recruited into the study were ~8-12weeks of age, by which time they were iron overloaded
  - Maintained under a diet containing >35ppm iron from weaning (~4weeks) to start of treatment

Male HJV-/- mice	Treatment for 6 weeks	Analysis:
➢ 8-12 weeks of iron loading	- PN23114 (7.5 mg/kg; SQ injection; thrice a week) - Phlebotomy (~0.3mL blood draw, QW) - PN23114 + Phlebotomy Separate baseline group terminated at study start	➢ Serum iron, and TSAT% ➢ tissue iron concentrations

**PN23114: Rusfertide analog peptide with similar pharmacodynamic effects.*

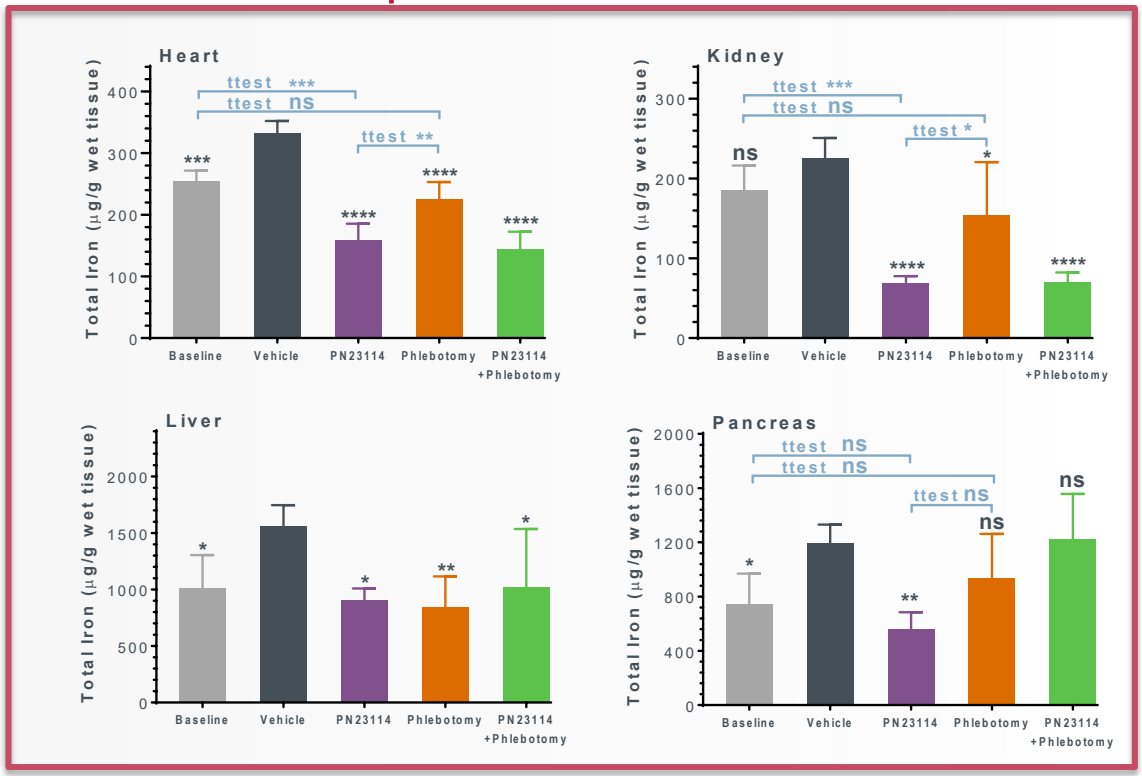
Serum Iron Levels were Normalized with PN23114 Treatment: Phlebotomy alone was insufficient to Lower Serum Iron

- Treatment with PN23114- alone or in combination with phlebotomy- lowered serum iron to normal levels (wild type mouse normal value ~30µM)
  - Weekly phlebotomy therapy alone was not sufficient to reduce serum iron.
  - Serum iron was lowest in the combination group
- 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.



PN23114 Treatment Reversed Iron Overload in Heart and Kidney; All Treatments Strategies Prevented Iron Deposition in Liver

- 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
- Liver iron deposition was prevented in all three treatment groups
  - Liver iron in vehicle group had increased compared to baseline group

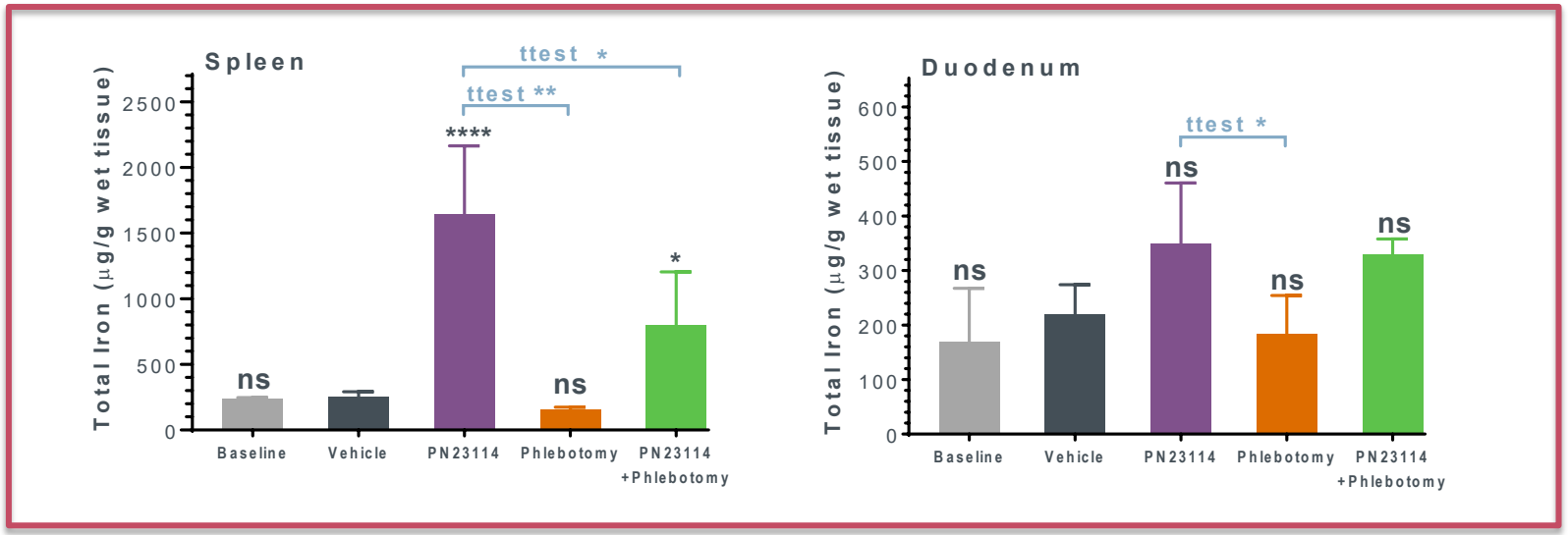


Statistical analysis:

*One-way ANOVA w/Dunnett's Multiple Comparisons; ns p>0.5, * p<0.5, ** p<0.01, *** p<0.001, **** p<0.0001*

PN23114 redistributed iron into spleen while Phlebotomy removed iron from the body

- In the group receiving PN23114 and Phlebotomy, we observe lesser iron sequestration in spleen as compared to PN23114 group



Conclusions

- PN23114 was superior to phlebotomy in controlling serum iron, lowering ferritin, and reducing iron deposition in heart and kidney
- Iron deposition in the liver was prevented in all three treatment groups
  - Liver iron had increased in vehicle group as compared to baseline group
- 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