Disclosure

The following authors are employees of Protagonist Therapeutics, Newark, California

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All authors are shareholders of Protagonist Therapeutics, Newark, California

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Hepcidin Peptidomimetics - Oral Efficacy in Pre-Clinical Disease Models for Iron Overload and Erythrocytosis

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Introduction

- Hepcidin is the master regulator of iron homeostasis in the body, it regulates iron exporter protein ferroportin.
- Hepcidin mimetic therapies have been proposed to benefit in patients with:
  - aberrant iron homeostasis (e.g. hereditary hemochromatosis - HH)
  - disorders that can be influenced by modulating iron homeostasis (e.g. polycythemia vera - PV)

PTG-300 is a subcutaneous injectable hepcidin peptidomimetic, currently in clinical Phase 2 studies in HH and PV.

Oral presentation: Abstract #482
PTG-300 Eliminates the Need for Therapeutic Phlebotomy in Both Low and High-Risk Polycythemia Vera Patients

Poster: Abstract #1689
Hepcidin Mimetic (PTG-300) Reverses Iron Deficiency While Controlling Hematocrit in Polycythemia Vera Patients

Poster Abstract #2998
Real-World Treatments and Thrombotic Events in Polycythemia Vera Patients: A Retrospective Analysis between 2018-2019 in US Population

Poster: Abstract #2594
Mechanism of Systemic Iron Regulation and Hematocrit Control By Hepcidin Peptidomimetics in Pre-Clinical Models

Oral Hepcidin Peptidomimetics

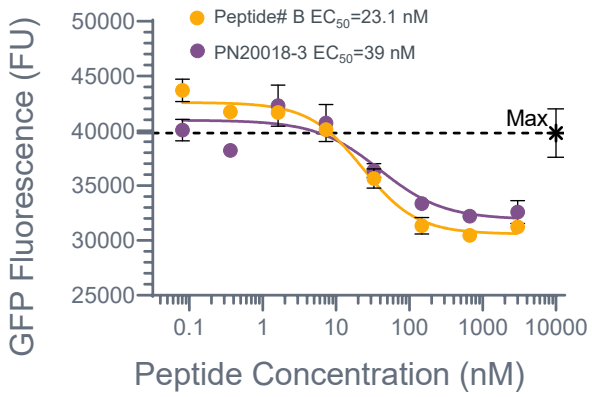
- Oral hepcidin peptidomimetics will potentially mark a paradigm change in management of hereditary hemochromatosis and polycythemia vera.
- In this presentation, we describe orally delivered hepcidin peptidomimetics that are metabolically stable in the gastrointestinal tract, systemically absorbed, and pharmacodynamically active in reducing serum iron parameters in pre-clinical models.
- Further, we demonstrate improvement in disease parameters in two pre-clinical mouse models
  - Hereditary hemochromatosis model ($HFE2^{-/-}$)
  - Erythropoietin (EPO) driven model for secondary erythrocytosis
    (model mimicking aberrant hematological parameters of PV, where JAK2 mutation drives erythrocytosis)

Oral Hepcidin Peptidomimetics Are Potent and Stable Peptides

IN VITRO POTENCY:

Oral hepcidin peptidomimetics are potent in causing ferroportin internalization in HEK-FPN-GFP cells.

Ferroportin internalization assay in HEK-FPN-GFP cells



EC_{50} (nM)	
PN20018	23.8
PN20076	16.5
PN20089	1.4
Peptide# B	19.3

STABILITY:

Oral hepcidin peptidomimetics are stable in matrices simulating gastrointestinal conditions and in mouse plasma.

Degradation assays in biologically relevant matrices

Half-Life (hr)	PN20018	PN20076	PN20089	Peptide# B
SGF with Pepsin	11.8	12.4	n/a	n/a
SIF with Pancreatin	12.5	>24	>24	>24
Mouse Plasma	10.6	>24	>24	>16

SGF: Simulated Gastric Fluids; SIF: Simulated Intestinal Fluids; n/a: not available

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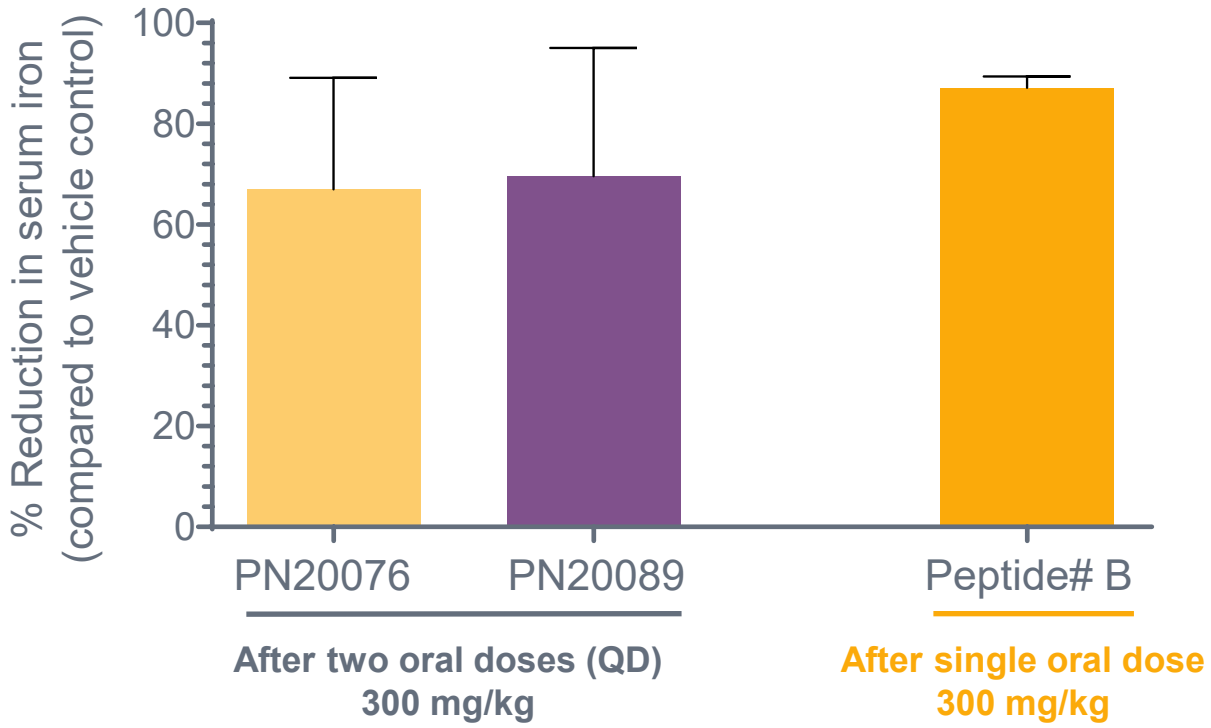
Oral Hepcidin Peptidomimetics Are Pharmacologically Active

Serum Iron Reductions in Healthy Mice

% Reduction in serum iron in healthy mouse at 4.5 hr after oral dosing of peptides



- Peptide# B was optimized from PN20076 for improved potency and permeability
  - showed robust serum iron reduction after single oral dose.

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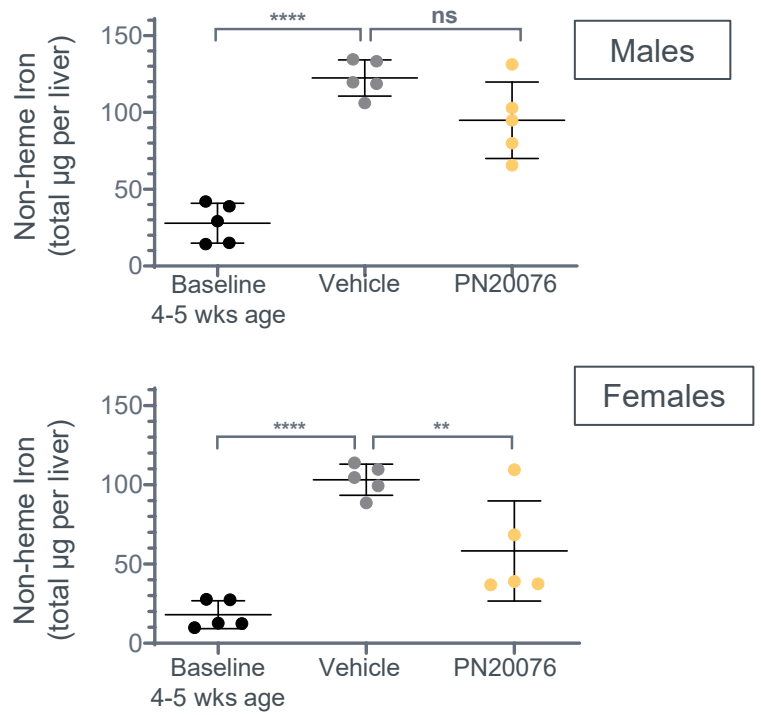
Preclinical POC in A Mouse Model of Hereditary Hemochromatosis

Oral Hepcidin Peptidomimetic, PN20076, Prevented Liver Iron Overload

- In HFE2$^{-/-}$ mice, 2 weeks of iron enriched diet (~ 300 ppm iron) resulted in:
  - Rapid increases in serum iron (2x of normal values, data not shown)
  - Greater than 5-fold increase in non-heme iron concentration in liver
- Concomitant treatment with PN20076 at ~300 mg/kg daily dose, **prevented iron deposition in the liver**
  - Statistically significant difference in female group and trend in males
  - Need longer treatment and probably BID dosing
- HH patients previously phlebotomized to reduce their body iron stores will benefit from a convenient oral therapy for iron overload prevention.

Reduced iron loading in livers of HFE2$^{-/-}$ mice after daily oral dosing of PN20076 for 2 weeks



*Statistical analysis: One-way ANOVA with Dunnet's Comparisons; **** p<0.0001, ** p<0.01, ns non-significant*

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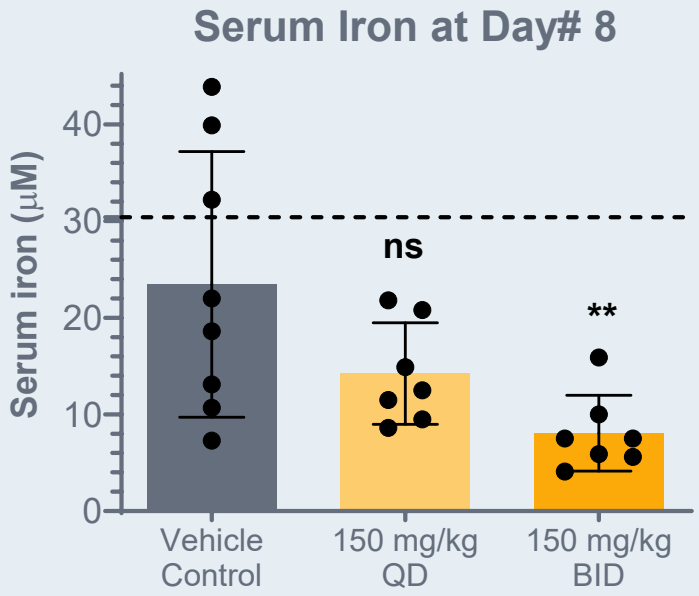
Preclinical POC in Mouse Model for Secondary Erythrocytosis

Oral Hepcidin Peptidomimetic, Peptide# B, is Pharmacodynamically Active in Reducing Serum Iron

EPO driven mouse model for secondary erythrocytosis (Ref: Wang J, Haematologica 2018)

- EPO model mimics the hematological parameters of polycythemia vera where JAK2 mutation drives the erythrocytosis.
- Serum iron had returned to baseline in the QD group on Day# 8.
- BID group showed significant serum iron suppression to < 10 µM on Day# 8.

Oral dosing of Peptide# B at 150 mg/kg in EPO Model



Normal values in mice **not** treated with EPO

*Statistical analysis: One-way ANOVA with Dunnet's Comparisons; ** p<0.01, * p<0.05, ns non-significant*

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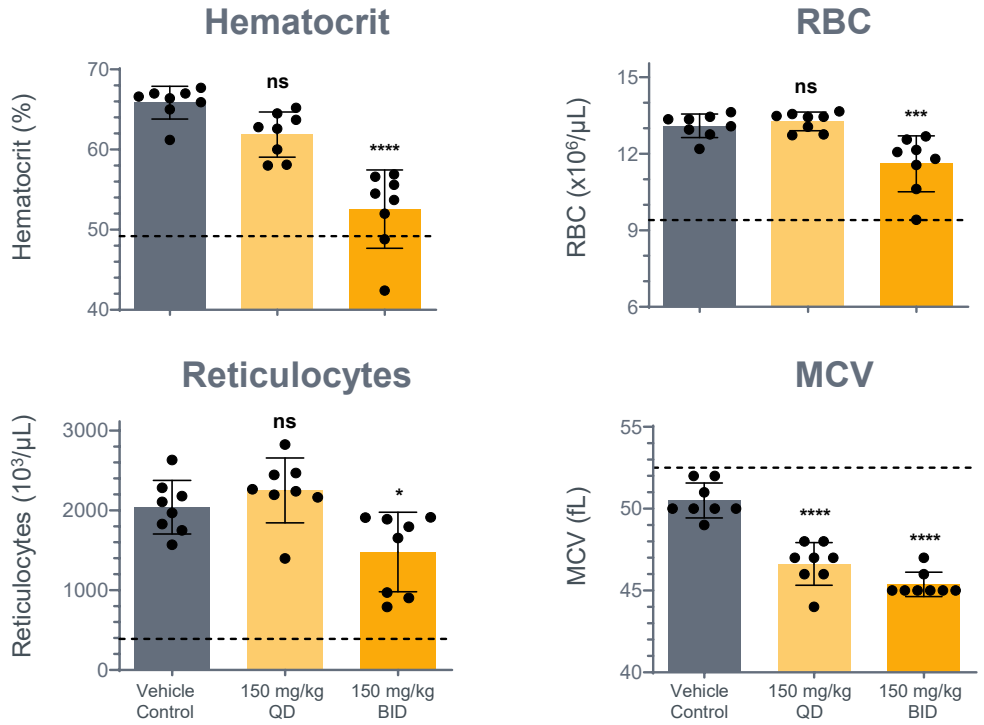
Preclinical POC in Mouse Model for Secondary Erythrocytosis

Oral Hepcidin Peptidomimetic, Peptide# B, Reduced Hematocrit, RBC, Reticulocytes and MCV

- Animals that received BID dosing showed
  - Reductions in hematocrit and hemoglobin (data not shown), with values close to normal levels
  - Reductions in RBC and Reticulocyte counts, although still higher than normal values
  - Reductions in Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin (data not shown) indicating iron restricted erythropoiesis
- Further optimization of oral hepcidin peptides is ongoing to improve potency and permeability.

Oral dosing of Peptide# B at 150 mg/kg in EPO Model



Normal values in mice **not** treated with EPO

*Statistical analysis: One-way ANOVA with Dunnet's Comparisons; **** p<0.0001, *** p<0.001, ** p<0.01, * p<0.05, ns non-significant*

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Conclusions

- We have described the discovery of hepcidin peptidomimetics with oral stability and systemic pharmacodynamic activity (serum iron reduction) in mice.
- Further, in proof-of-concept pre-clinical studies we have demonstrated efficacy of oral hepcidin peptidomimetics in conditions where systemic iron restriction/sequestration is sufficient
  - to prevent liver iron overload in a mouse model of hereditary hemochromatosis ($HFE2^{-/-}$)
  - to control erythropoiesis in a mouse model of EPO-driven secondary erythrocytosis
- Further optimization of oral hepcidin peptidomimetics and treatment paradigm is on-going.
- Early pre-clinical data are indicative of the potential of our oral hepcidin peptidomimetics for hereditary hemochromatosis, polycythemia vera and other conditions that can benefit from modulating iron homeostasis.
- Oral hepcidin mimetics with systemic activity will allow broader access to the treatment of current and new disease indications