

S843

**HEPCIDIN MIMETIC PTG-300 FOR TREATMENT OF INEFFECTIVE
ERYTHROPOIESIS AND CHRONIC ANEMIA IN HEMOGLOBINOPATHY
DISEASES**

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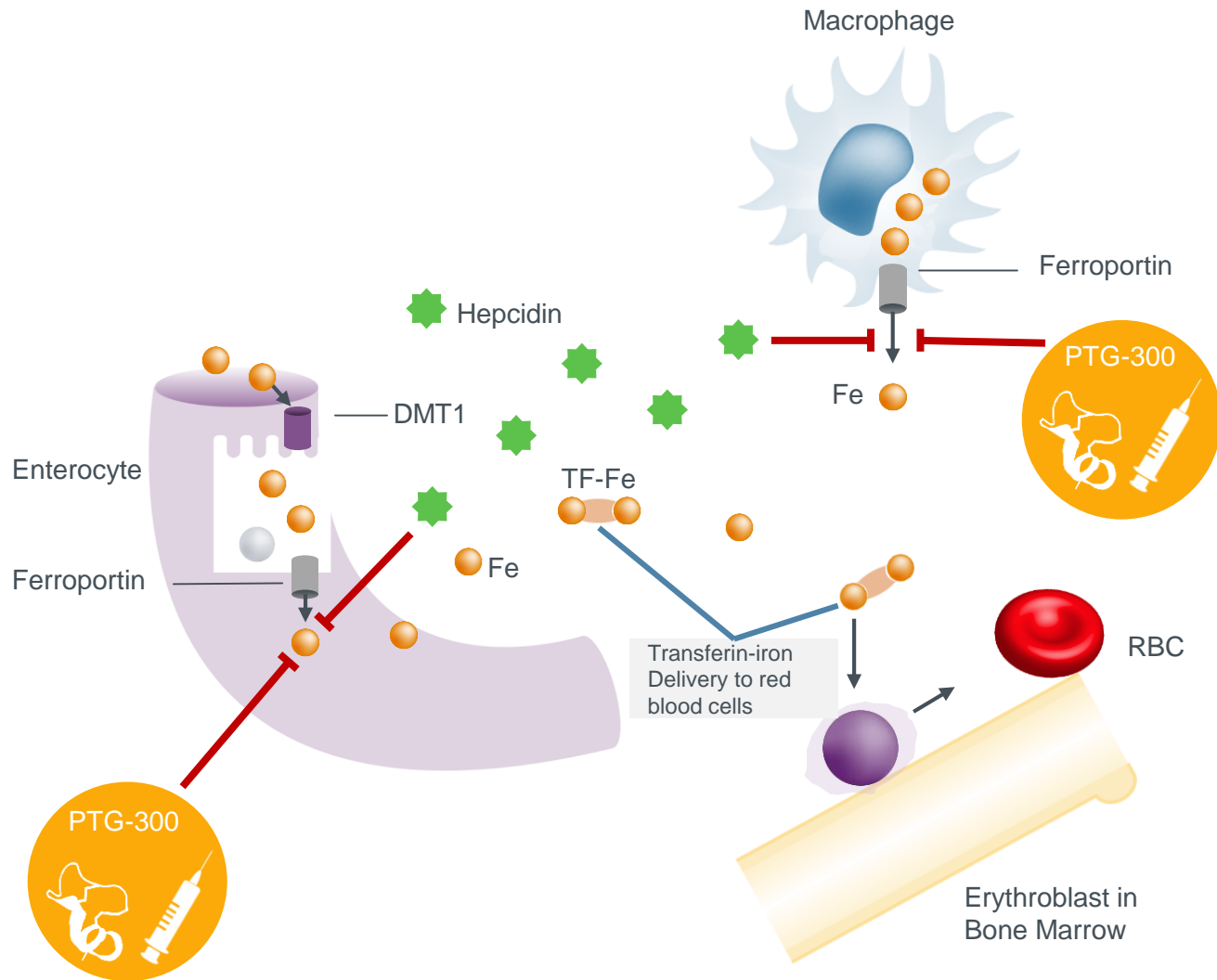
Protagonist Therapeutics, Inc.

Financial Disclosure

- Dr. Liu is an employee of Protagonist Therapeutics, who sponsored these studies

Hepcidin: Regulator of Iron Homeostasis

PTG-300: New Class of Agent – Hepcidin Mimetic



D'Angelo G. Role of hepcidin in the pathophysiology and diagnosis of anemia. *Blood Res.* 2013;48:10-15.

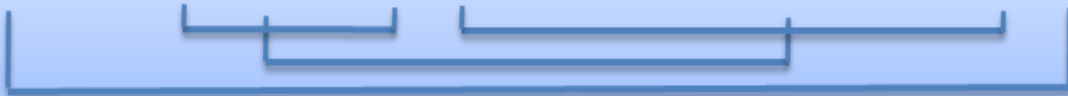
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Treatment for Iron Loading Anemia

- Under conditions of ineffective erythropoiesis (IE), hepcidin levels are suppressed leading to increases in iron absorption from the GI tract and iron export from macrophages
 - Exacerbation of underlying IE through iron toxicity in developing erythrocytes in the bone marrow
- Agents with hepcidin activity may help correct iron distribution abnormalities with beneficial effects on erythropoiesis
- Treatment of anemia will relieve transfusion burden and secondary iron overload which otherwise requires palliative chelator therapy

Hepcidin – Complex Structure

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
Asp-Thr-His-Phe-Pro-Ile-Cys-Ile-Phe-Cys-Cys-Gly-Cys-Cys-His-Arg-Ser-Lys-Cys-Gly-Met-Cys-Cys-Lys-Thr



25 amino acids in length with 4 disulfide bonds, resulting in the complex production of a correctly folded full-length hepcidin

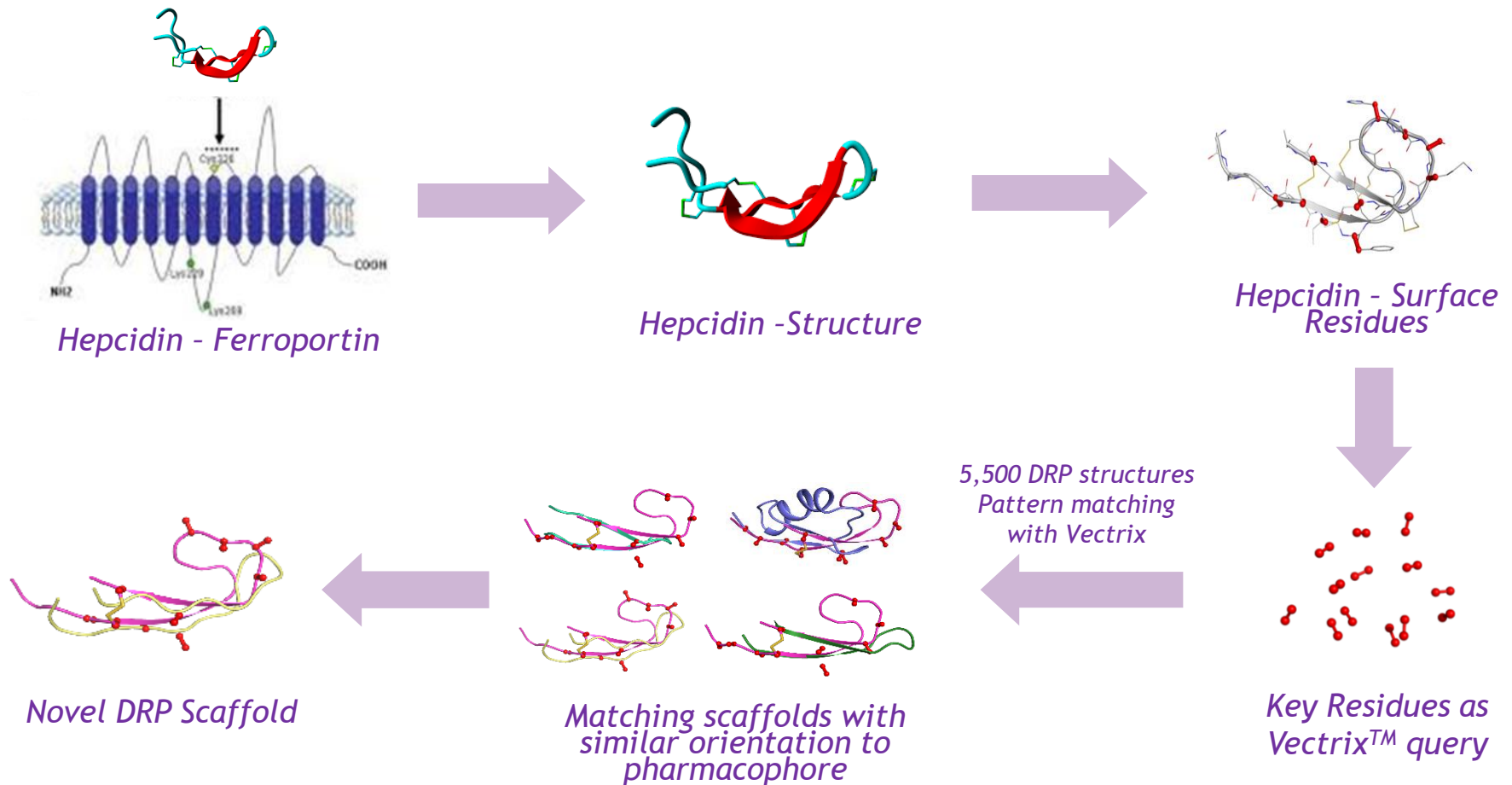
- 105 different folds of 4 disulfide bonds are possible
- Reported : 6-12% folding yield from linear
- Solubility, aggregation and stability issues

Ref: Zhang et al. Peptide Science 2010 94 257 – 264

PTG-300 Discovery of the Hepcidin Mimetic

An example of Vectrix™: Scaffold Hopping

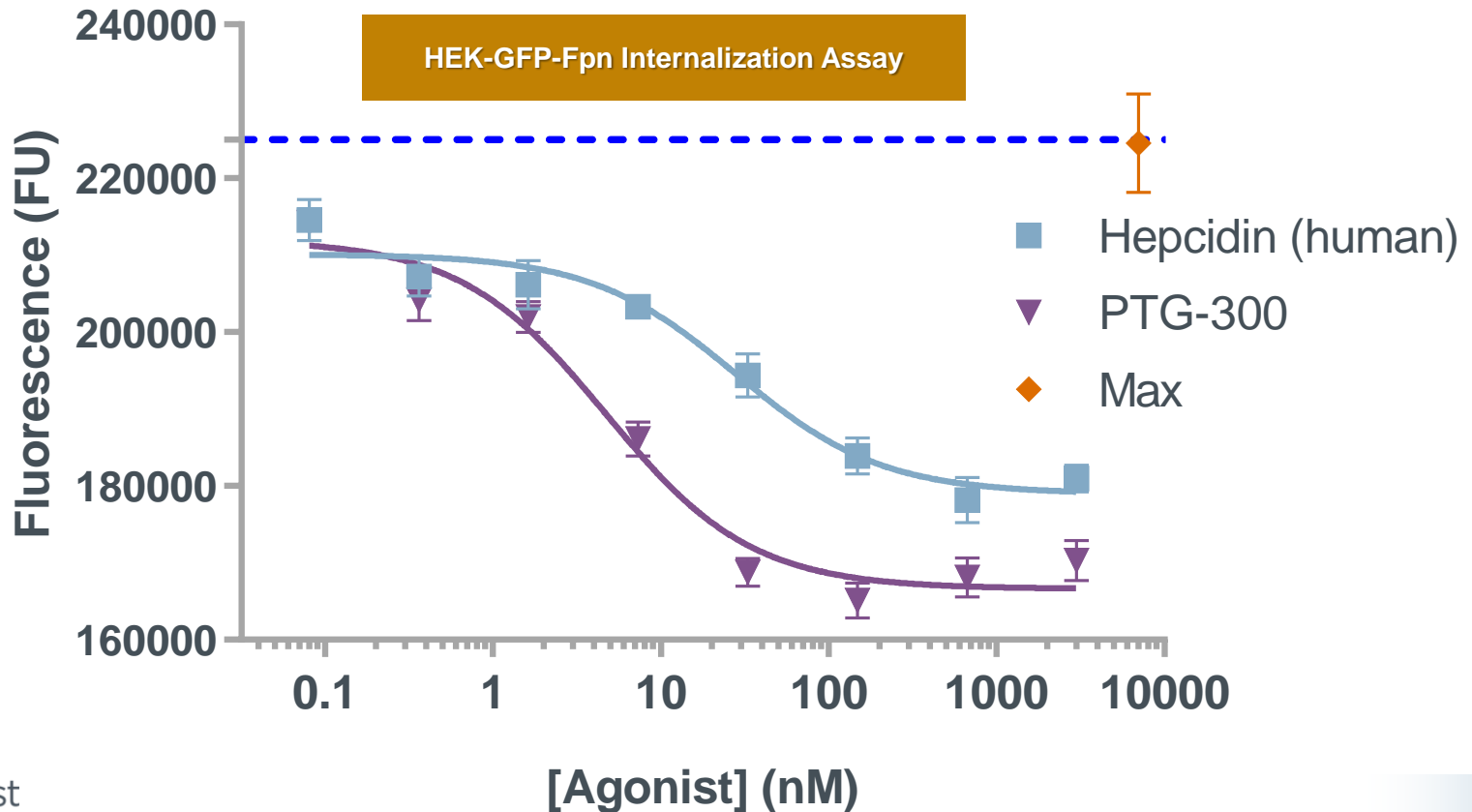
- Vectrix™ identifies a novel DSR peptide scaffold followed by phage display diversification and new chemistries for imparting drug-like properties



Discovery of PTG-300 Hepcidin Mimetic

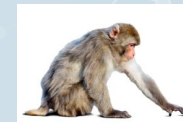
Potential Treatment for Iron Loading Anemia in β -Thalassemia

- Easier chemical synthesis, increased serum stability/aqueous solubility
- Increasing potency 6-fold compared to hepcidin
- 1 issued US patent and several pending applications

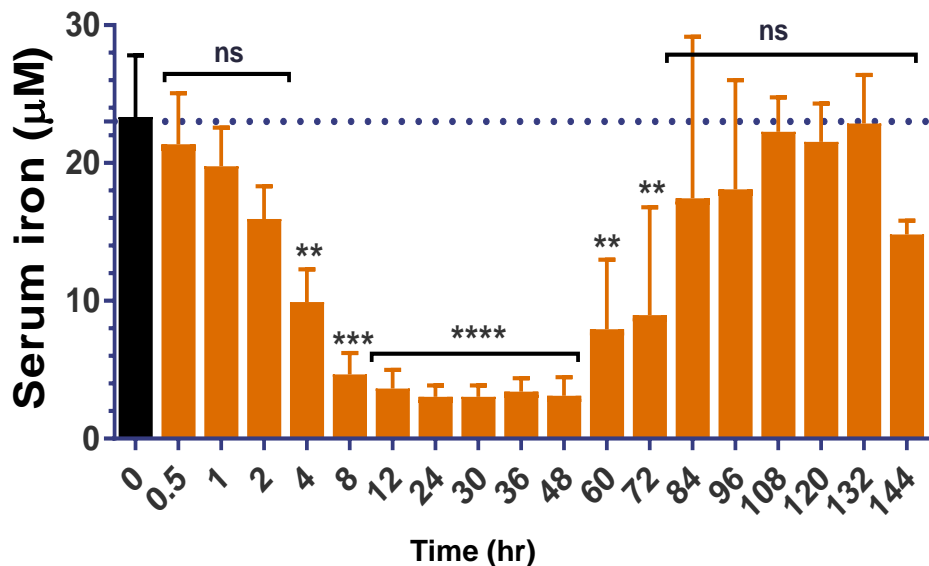


PTG-300 Reduces Serum Iron for >72 Hours

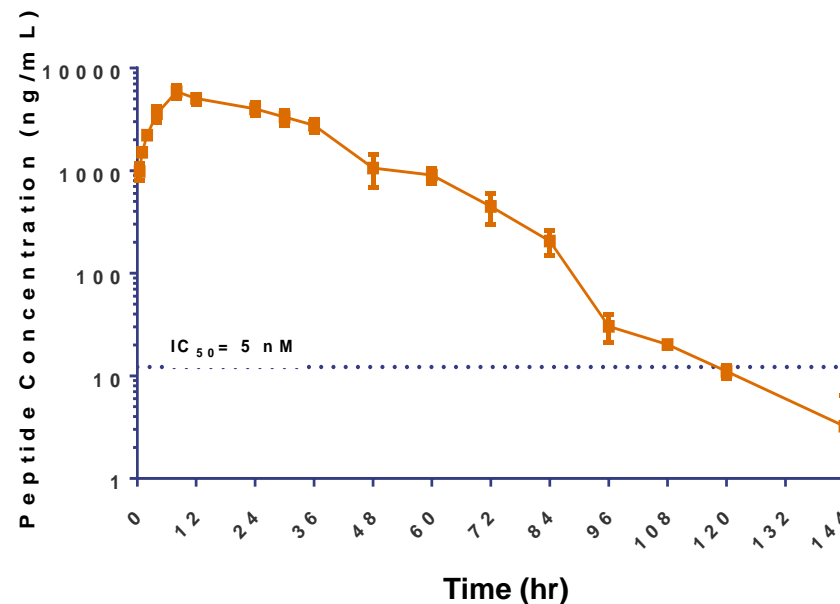
Serum PD Effect Consistent with PK in Cynomolgus Monkeys



PD – serum iron



PK – serum drug



Fsc% = 83% and $T_{1/2}$ 11.9 hours

ns $p > 0.05$, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$

PTG-300 Reduces Serum Iron After an Oral Iron Challenge

Blockade of Enterocyte Ferroportin



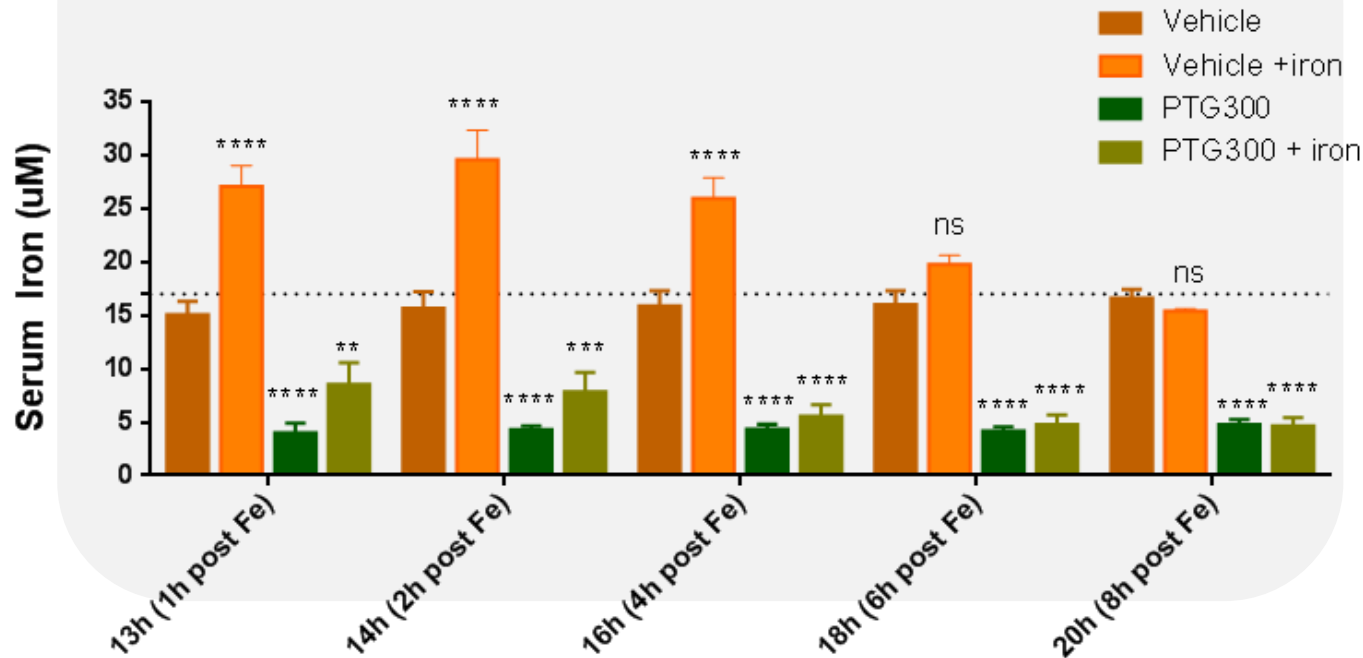
PTG-300
0.97 mg/kg s.c.
Wait 12 h



Oral Iron Gavage
2 mg/kg, iron

Measure “Serum Iron” after oral iron administration

Serum Iron Levels Post Fe Challenge

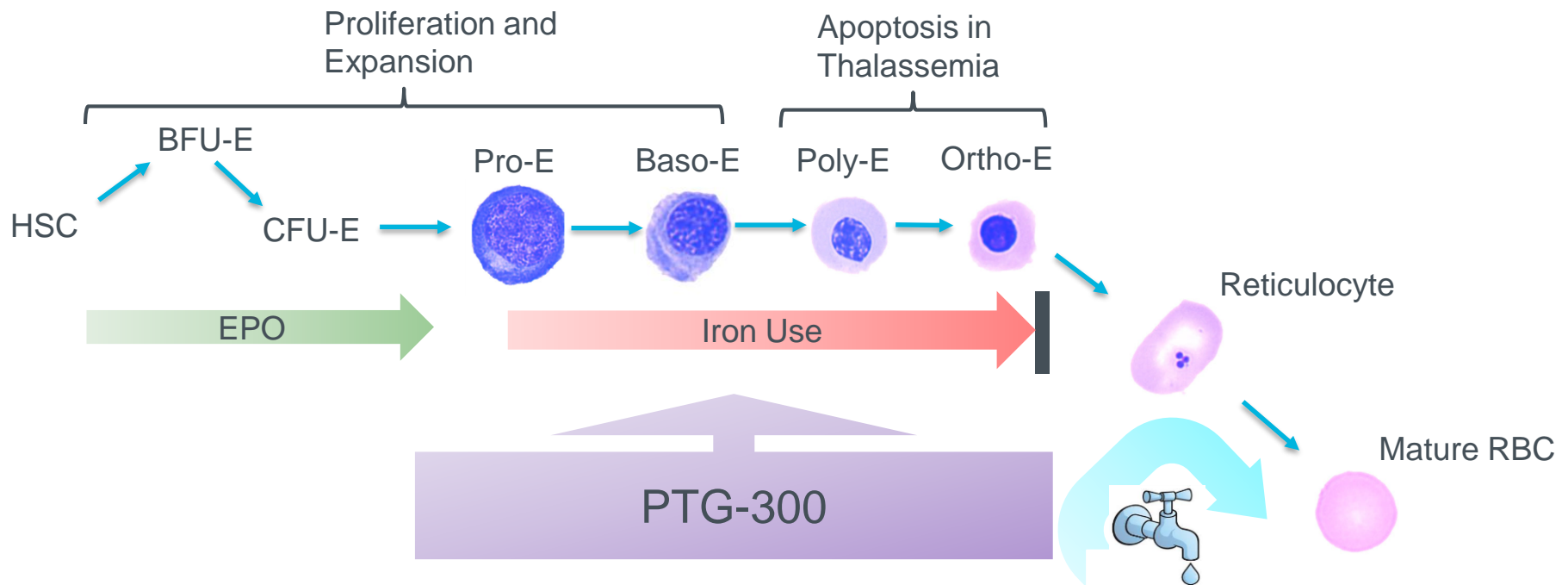


ns $p > 0.05$, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p < 0.001$, **** $p \leq 0.0001$
Stats are compared to vehicle at time point

PTG-300: New Class of Erythropoietic Agent

Treatment of Ineffective Erythropoiesis in Thalassemia/MDS/MF

Progenitors → Erythroblasts progenitors → Erythrocytes

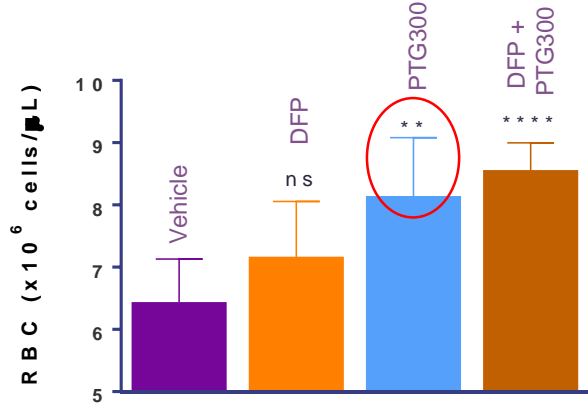


PTG-300: Efficacy based POC in β -Thalassemia Mouse Model

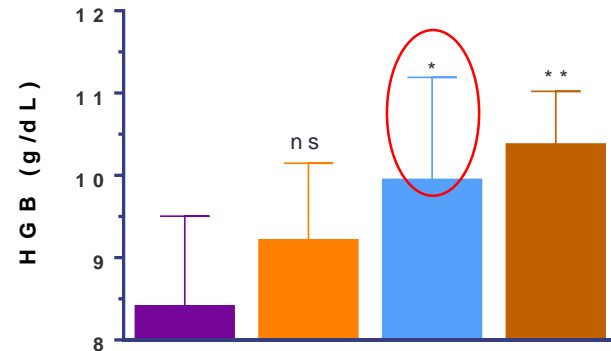
Improved Erythropoiesis



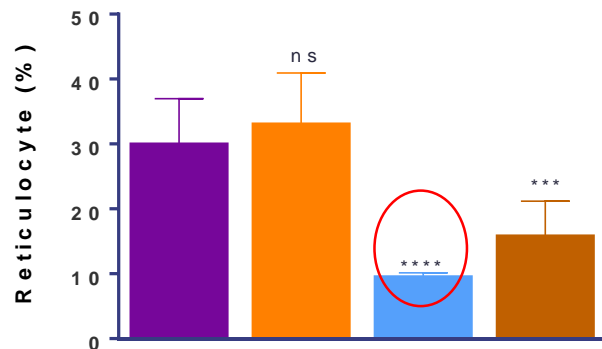
Red Blood Cells



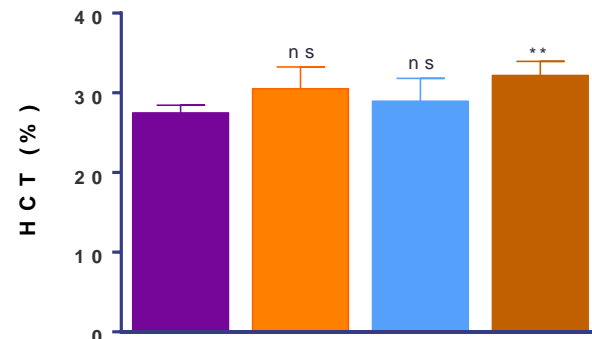
Hemoglobin



Reticulocyte (%)



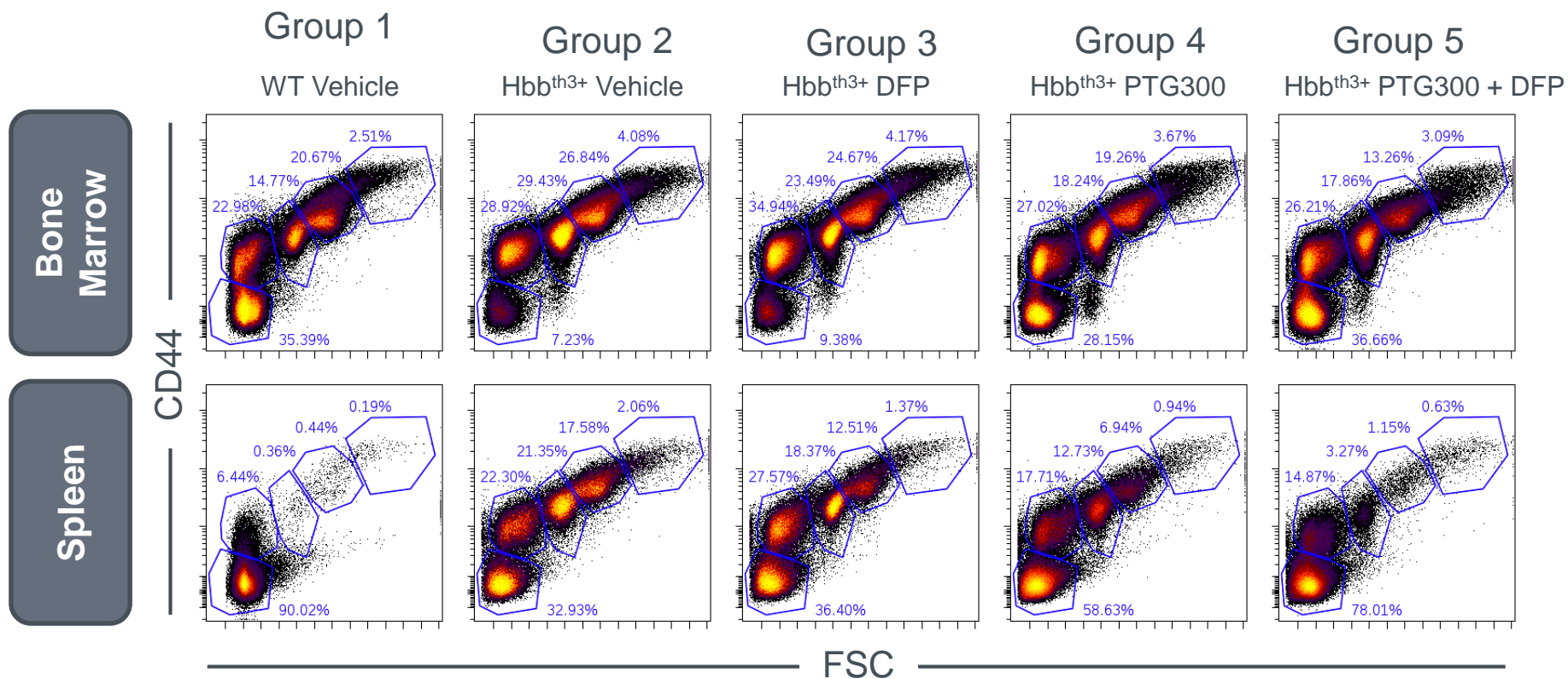
Hematocrit



■ th3 + Vehicle
 ■ th3 + DFP
 ■ th3 + PTG300
 ■ th3 + DFP + PTG300

- PTG-300 1 mg/kg dosed Q2D for 6 weeks (Hbb^{th3/+} Mice)
- DFP (deferiprone, oral iron chelator) dosed at 1.25mg/mL
- *p≤0.05; **p≤0.01; ***p≤0.001; ****p≤0.0001; ns- not significant

PTG-300 Improved RBC Maturation Redistribution and Normalization of Erythroid Subsets



PTG-300 Improves RBC Survival in β -Thalassemia $Hbb^{th3/+}$ Mouse Model

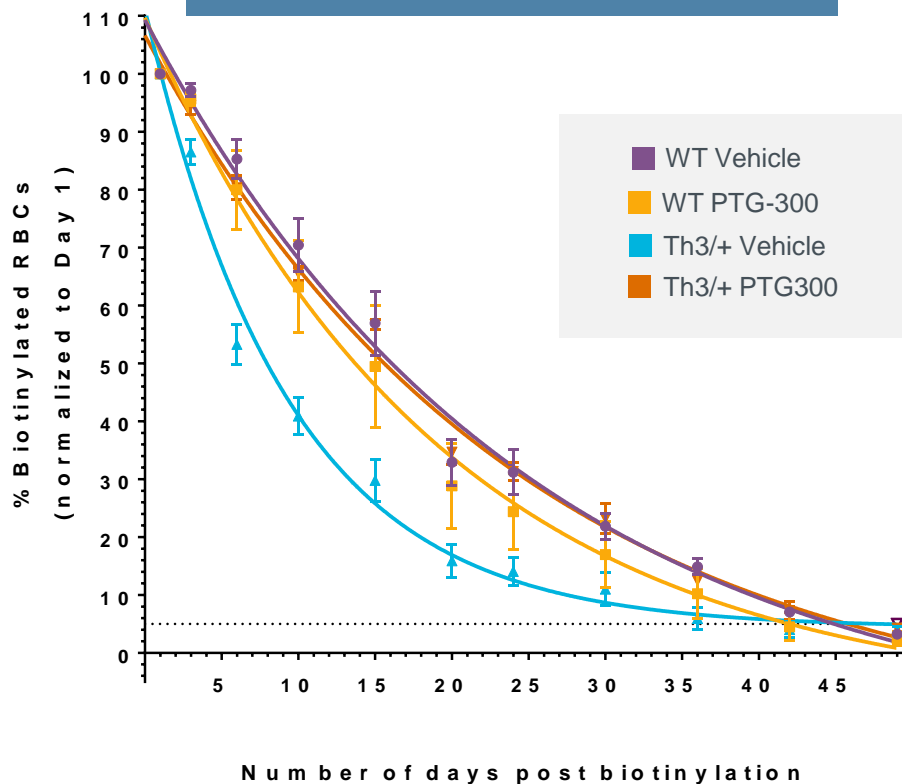
Dose PTG-300
(0.97 mg/kg, Q2D)

4 weeks

Biotinylation
NHS-PEG4-Biotin
@ End of Week 4

- Flow cytometry analysis over 7 weeks to measure RBC survival
- Continue dosing with PTG-300 for the entire duration

RBC survival in Male $Hbb^{th3/+}$ Mice



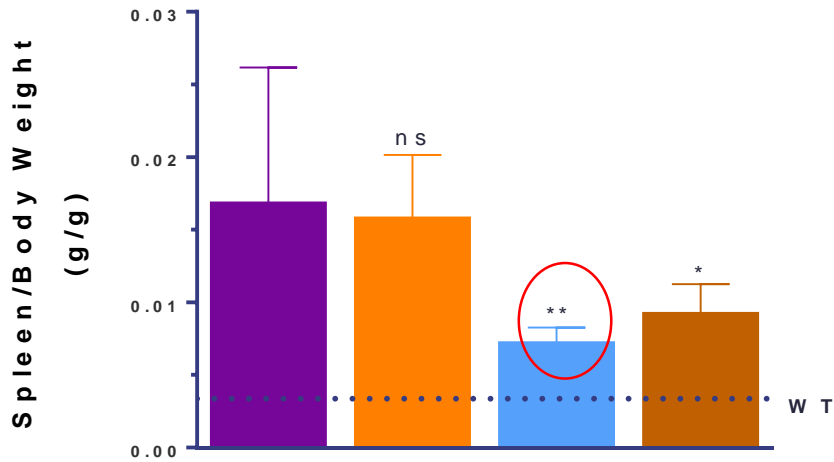
Half Life (Days)

WT Vehicle	17.3 days
WT PTG-300	13.6 days
$Hbb^{th3/+}$ Vehicle	6.5 days
$Hbb^{th3/+}$ PTG-300	16.9 days

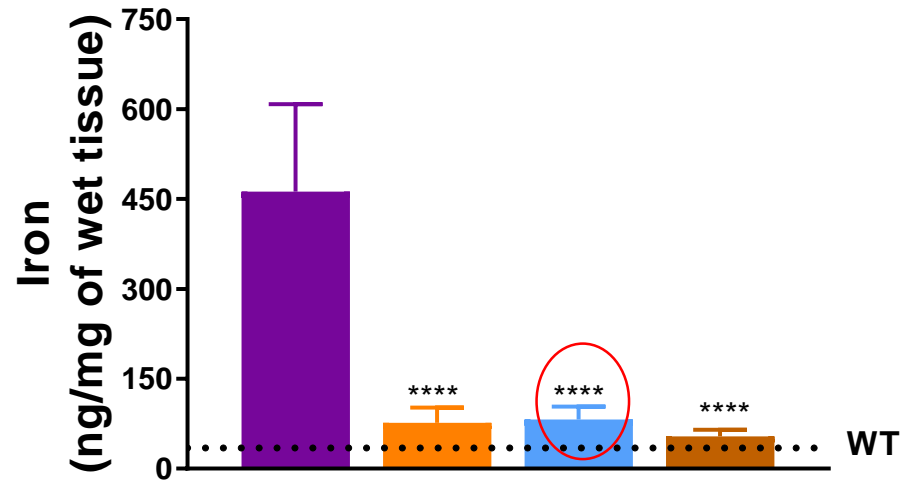
PTG-300: Efficacy based POC in β -Thalassemia Mouse Model

Decreased Splenomegaly and Liver Iron Overload

Spleen/Body Weight (g/g)



Non-heme Iron in Liver (ng/mg of wet tissue)



th3 + Vehicle

th3 + DFP

th3 + PTG300

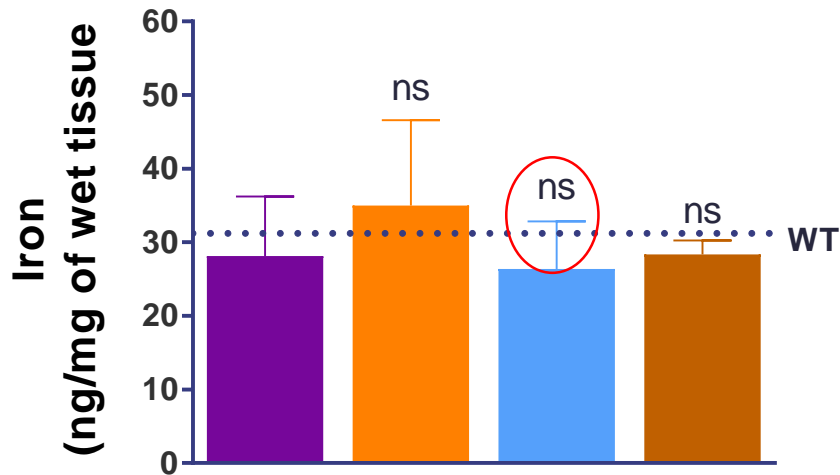
th3 + DFP + PTG300

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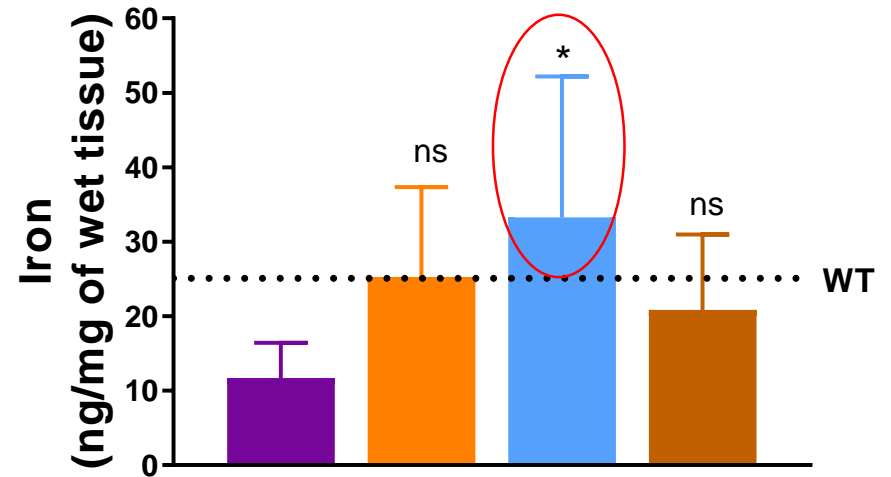
PTG-300: Efficacy based POC in β -Thalassemia Mouse Model

No Increase in Heart Iron and Some Iron Retention in Duodenum

Non-heme Iron in Heart



Non-heme Iron in Duodenum



th3 + Vehicle

th3 + DFP

th3 + PTG300

th3 + DFP + PTG300

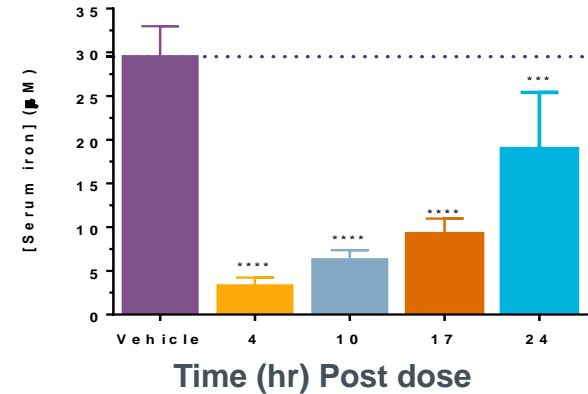
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PTG-300 Efficacy with Potential for Weekly Dosing

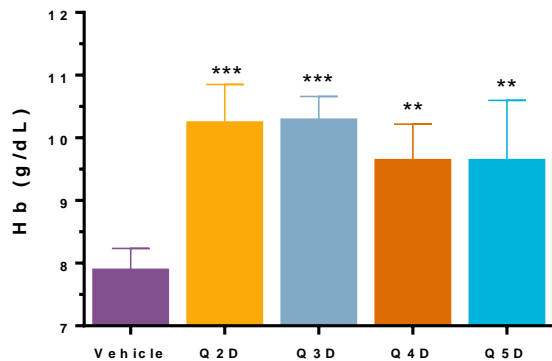
Transient Iron Restriction Improves Erythropoiesis in Hbb^{th3+} Mice

- PTG-300 decreases serum iron levels for 24h post dose
- Transient reduction in serum iron, during 24h post-dose, is sufficient to elicit efficacy with a Q4D or Q5D dosing regimen"

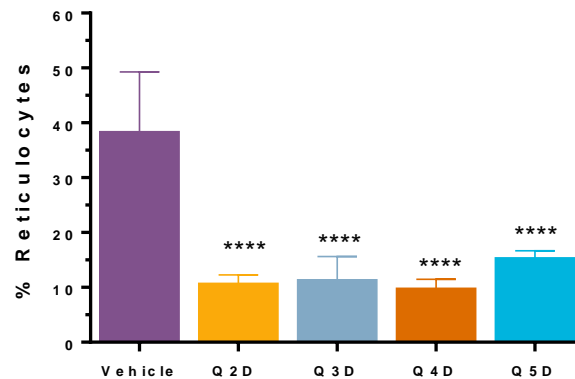
Serum Iron Levels



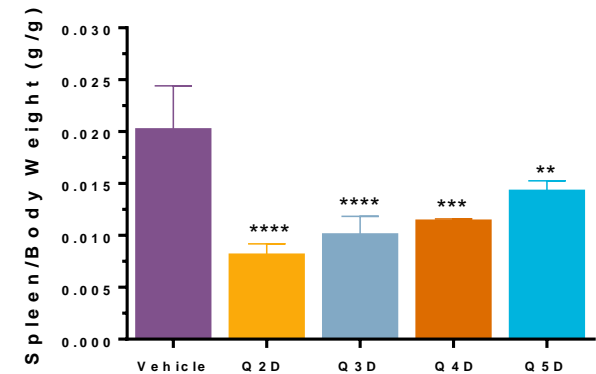
Chronic Hemoglobin



Chronic Study (Hbb^{th3+}) Mice: Reticulocyte Percentage



Chronic Study (Hbb^{th3+}) Mice: Spleen weight to Body Weight



PTG-300: Summary of Efficacy in β -Thalassemia Mouse

- PTG-300, discovered through the optimization of a rationally designed scaffold, is a potent hepcidin mimetic with excellent drug-like characteristics
- Well-behaved pharmacodynamics and pharmacokinetic profile
 - Preclinical behavior translatable to humans?
 - Phase 1 in healthy subjects (Abstract S695, Saturday 5:00 pm in Room A13)
- Stimulation of effective erythropoiesis
 - Correction in precursor cell distribution
 - Increased RBC survival
 - Decreased splenomegaly and disease biomarkers
 - Relative ineffectiveness of oral iron chelator
- Reduction of liver iron overload through retention in enterocytes and potential redistribution to macrophages
- Transient iron reduction is sufficient for the correction of anemia

PTG-300: Conclusions



- PTG-300 may potentially address pre-existing anemia *and* liver iron overload through iron restriction and redistribution to restore iron homeostasis
- Potential to address complications in β -thalassemia
 - Reduce primary iron overload
 - Reduce transfusions and subsequent secondary iron overload
 - Prevent splenomegaly and need for splenectomy with a reduction of thrombosis risk
- Potential to treat other diseases characterized by
 - Ineffective erythropoiesis, low hepcidin and iron overload, e.g. low risk MDS



- Excellent non-clinical safety profile with exaggerated pharmacology
- Global Phase 2 trial in β -thalassemic patients to start in 2018 Q4
 - Received orphan status in the US
- Potential for broad spectrum of diseases for human trials
 - Ineffective erythropoiesis, low hepcidin and iron overload, e.g. low risk MDS
 - Primary iron overload, low hepcidin, e.g. hereditary hemochromatosis
 - Exaggerated erythropoiesis, e.g. polycythemia vera
 - Chronic liver fibrosis, e.g. NASH