Disclosure

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

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Mechanism of Systemic Iron Regulation and Hematocrit Control By Hepcidin Peptidomimetics in Pre-Clinical Models

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Systemic Iron Modulation has Potential Disease Modifying Effects in Polycythemia Vera

- In polycythemia vera (PV), point mutation in JAK2 kinase (V617F) confers constitutive activity to JAK2 leading to excessive erythropoiesis that is independent of erythropoietin.
- Polycythemia vera presents elevated hematocrit, bone marrow erythroid hyperplasia, consequent dysregulated iron homeostasis, and iron deficiency due to chronic phlebotomy.
- Elevated hematocrit (HCT) and hyper-viscosity in the blood are risk factors for thrombosis and other symptoms. (Ref: Stein BL, J Clin Oncol, 2015)
- Iron restriction from erythropoiesis provides a mechanism for hematocrit control in PV, along with potential improvement in iron deficiency related to phlebotomy (Ref: Ginzburg YZ, Leukemia 2018).

PTG-300 is a subcutaneous injectable hepcidin mimetic, currently in clinical Phase2 studies in polycythemia vera

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

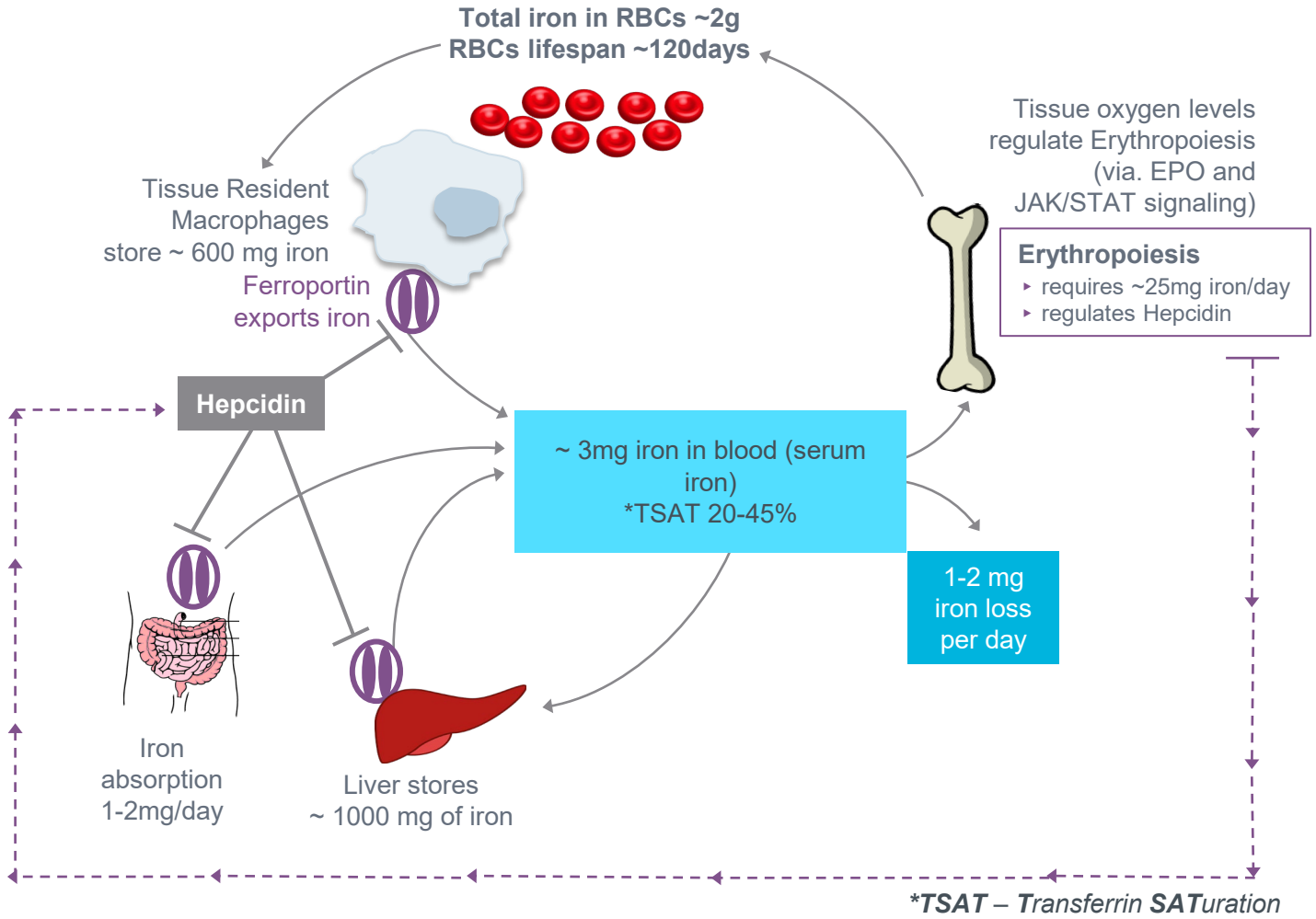
Poster: Abstract #1689
Hepcidin Mimetic (PTG-300) Reverses Iron Deficiency While Controlling Hematocrit in Polycythemia Vera Patients

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

Poster: Abstract #2592
Hepcidin Peptidomimetics – Oral Efficacy in Pre-Clinical Disease Model of Iron Overload

Systemic Iron Regulates Erythropoiesis

- Hepcidin targets iron exporter ferroportin, causing its internalization and subsequent degradation
  - Macrophages which recycle iron from senescent RBCs are the primary source of iron for erythropoiesis
- PTG-300, a hepcidin mimetic, targets ferroportin
  - thereby, restricting iron availability for erythropoiesis

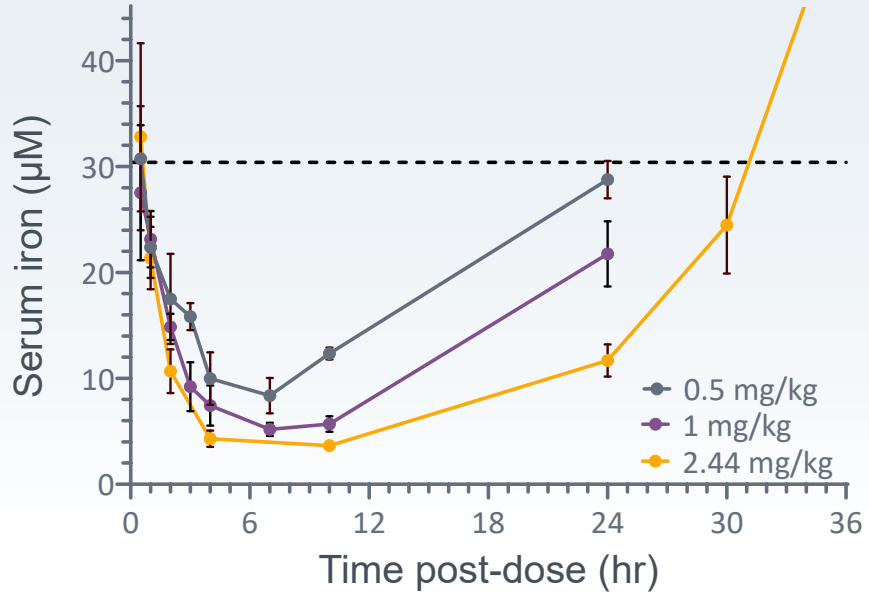


Pharmacodynamics of PTG-300

Rapid, Reversible and Dose-Dependent Reductions in Serum Iron

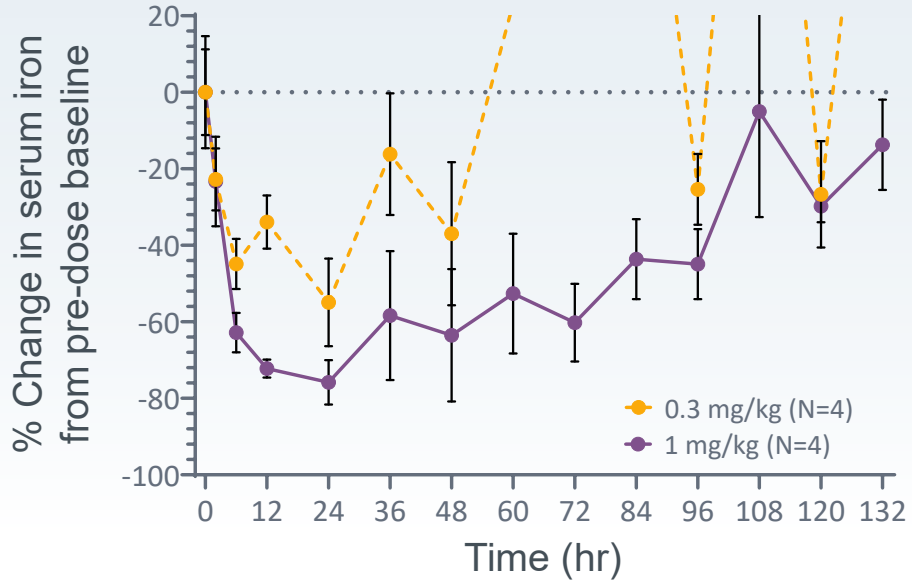
PTG-300 subcutaneous injection in healthy mouse:

- ~ 85% reduction in serum iron by 7 hr for doses ≥ 1 mg/kg
- Longer sustained reductions at higher doses, over ~ 24 hr for 1 mg/kg and ~ 32 hr for 2.44 mg/kg



PTG-300 subcutaneous injection in healthy cynomolgus monkey‡:

- 80% reduction in serum iron by 24 hr after 1 mg/kg dose
- Sustained reduction up to ~ 96 hr for 1 mg/kg



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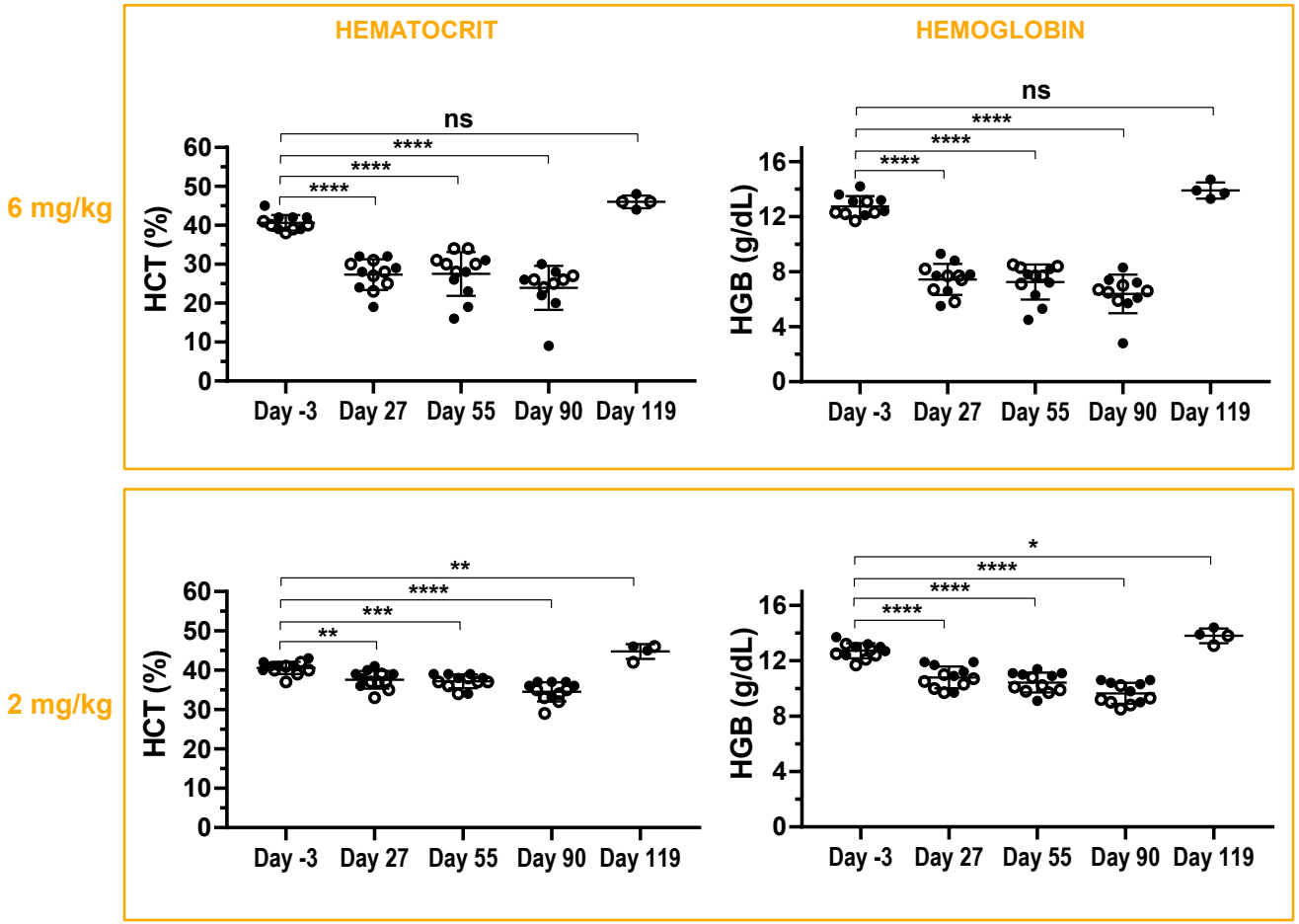
‡ Baseline serum iron in healthy cynomolgus monkey ~30 µM

Chronic PTG-300 Treatment in Healthy Cynomolgus Monkey Induced Dose-Related Anemia

- In 6 mg/kg groups, hematocrit reduced by >10 % and hemoglobin by ~6 g/dL by Day 27 i.e. after 4 injections
  - no further reductions until end of study
- In 2 mg/kg groups, hematocrit reduced by ~6% and hemoglobin by ~4 g/dL by Day 90
  - slower reductions in this low dose group
- Rapid recovery of all parameters back to baseline after a 30-day recovery period.

PTG-300 treatment in healthy monkey, weekly SC injections



*Statistical analysis: One-way ANOVA with Dunnet's Comparisons vs. Day -3; **** p<0.0001, *** p<0.001, , ** p<0.01, , *p<0.05, ns non-significant*

Closed circles: Male; Open circles: Female

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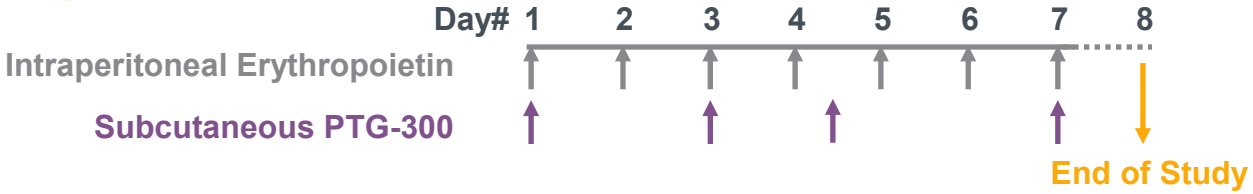
Justification for Selecting Polycythemia Vera as Clinical Target

- Pharmacodynamic properties of PTG-300 translated well from rodent models to healthy monkey.
- Exaggerated pharmacological effects of PTG-300 in healthy monkeys were dose-related reductions in hematocrit and hemoglobin.
  - fast onset of anemia by Day 27 in the higher dose group treated with 6 mg/kg.
- PTG-300 induced anemia in monkeys was reversible, all hematology parameters returned to baseline after a 30-day recovery period.
- “Minihepcidin” has previously been shown to be efficacious in mouse model for polycythemia vera (model carries the same JAK2 mutation found in human PV).
  - Ref: Casu C, Blood 2016
- Above data provided rationale for testing PTG-300 in human polycythemia vera for potential benefit in lowering hematocrit and thereby reducing the need for phlebotomy.

PTG-300 is Efficacious in Suppressing Aberrant Erythropoiesis in Mouse Model for Secondary Erythrocytosis

EPO driven mouse model for secondary erythrocytosis

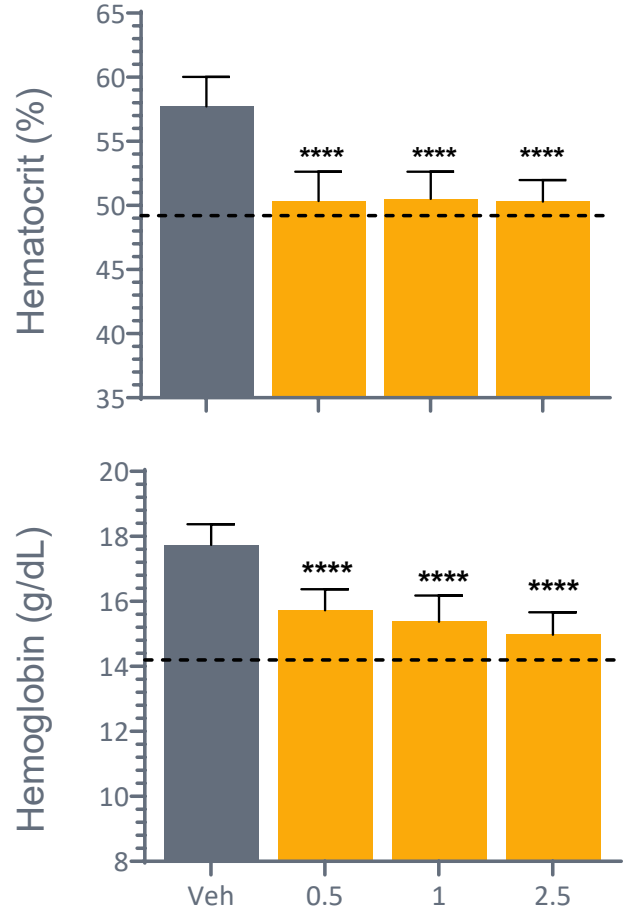
(Ref: Wang J, Haematologica 2018)



- EPO model mimics the hematological parameters of polycythemia vera mouse model where JAK2 mutation drives the erythrocytosis.
- Reductions in hematocrit and hemoglobin in PTG-300 treated groups
  - Hematocrit was normalized at all doses tested: 0.5, 1, and 2.5 mg/kg
  - Higher dose of 2.5 mg/kg of PTG-300 was required to normalize hemoglobin

(data for equipotent Peptide #A is described in the abstract)

PTG-300



Veh: Vehicle control in EPO treated mice ------ Normal values in mice **not** treated with EPO

*Statistical analysis: One-way ANOVA with Dunnet's Comparisons; **** p<0.0001*

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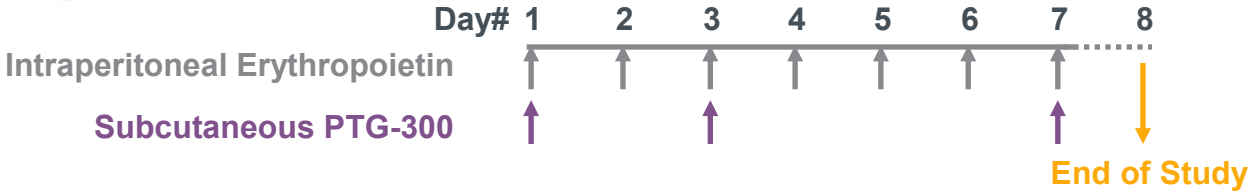
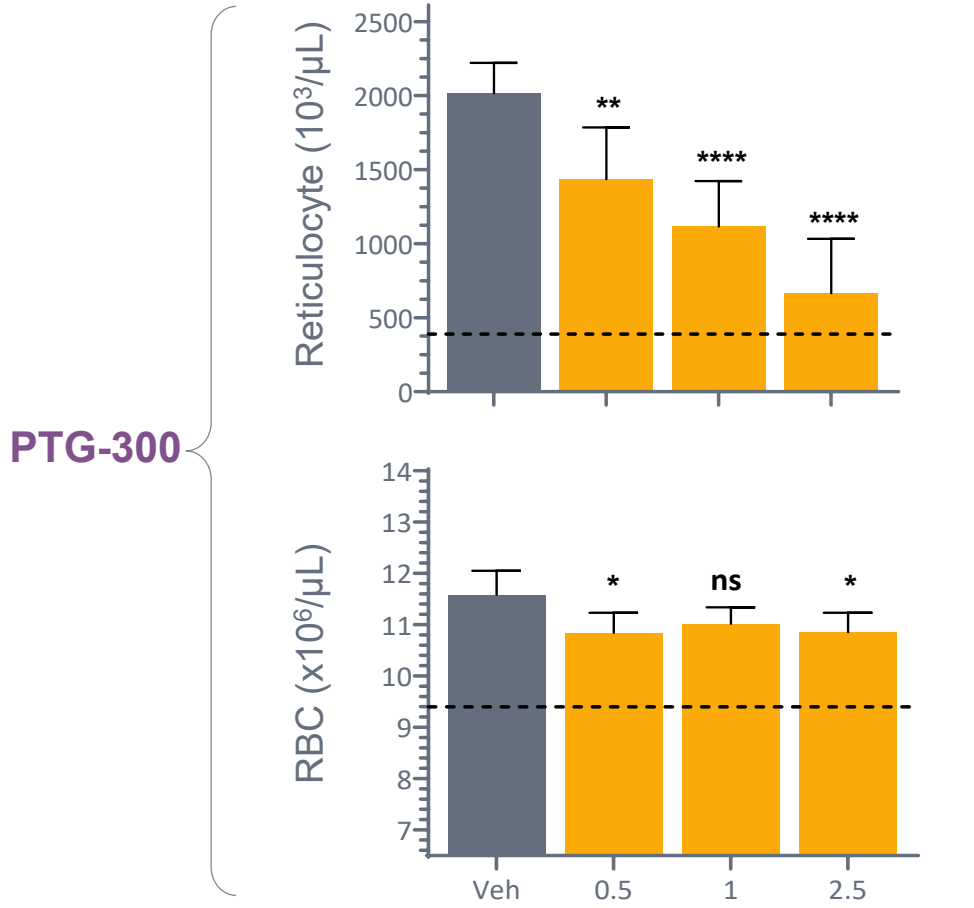
Higher Dose of 2.5 mg/kg Required to Reduce Reticulocytes under Iron Replete Conditions in EPO Mouse Model

EPO driven mouse model for secondary erythrocytosis

(Ref: Wang J, Haematologica 2018)



- Group treated with highest dose of 2.5 mg/kg PTG-300 showed reductions in Reticulocytes to near normal levels but very moderate changes in RBCs.
  - Indicates that longer sustained iron reduction is needed for erythrocytosis control.
  - Longer that one week of dosing may be needed for normalizing RBCs.
- For the highest dose of Peptide #A, at 5 mg/kg, RBCs were also reduced to near baseline levels.
  - Data for Peptide #A is presented in the abstract and not shown here



Veh: Vehicle control in EPO treated mice ------ Normal values in mice **not** treated with EPO

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Conclusions

- PTG-300 and Peptide #A were both efficacious in reducing hematocrit in pre-clinical models.
- EPO expression is suppressed in PV due to hyperoxia conditions
  - however, EPO treatment mimics the unregulated erythropoiesis in JAK2 mutant PV
- PV subjects present iron deficiency due to frequent phlebotomy
  - Efficacious dose of PTG-300 required to sustain serum iron reduction would be lower under iron deficient conditions
- EPO Model data is supportive of potential efficacy of PTG-300 in human secondary erythrocytosis conditions that are EPO driven, including certain Congenital Erythrocytosis indications (e.g. HIF stabilizing mutations).