S843
HEPCIDIN MIMETIC PTG-300 FOR TREATMENT OF INEFFECTIVE ERYTHROPOIESIS AND CHRONIC ANEMIA IN HEMOGLOBINOPATHY DISEASES

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Hepcidin: Regulator of Iron Homeostasis

PTG-300: New Class of Agent – Hepcidin Mimetic

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

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

**HEK-GFP-Fpn Internalization Assay**

- **Fluorescence (FU)**
  - **[Agonist] (nM)**
  - **Hepcidin (human)**
  - **PTG-300**
  - **Max**

Protagonist Therapeutics
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
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

<table>
<thead>
<tr>
<th>Time</th>
<th>Vehicle</th>
<th>Vehicle + iron</th>
<th>PTG300</th>
<th>PTG300 + iron</th>
</tr>
</thead>
<tbody>
<tr>
<td>13h (1h post Fe)</td>
<td>**</td>
<td>***</td>
<td>****</td>
<td>****</td>
</tr>
<tr>
<td>14h (2h post Fe)</td>
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</tr>
<tr>
<td>16h (4h post Fe)</td>
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</tr>
<tr>
<td>18h (6h post Fe)</td>
<td>ns</td>
<td>ns</td>
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<td>ns</td>
</tr>
<tr>
<td>20h (8h post Fe)</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

Stats are compared to vehicle at time point:
ns p>0.05, * p≤ 0.05, ** p≤ 0.01, ***p<0.001, **** p<0.0001
PTG-300: New Class of Erythropoietic Agent
Treatment of Ineffective Erythropoiesis in Thalassemia/MDS/MF

Progenitors $\rightarrow$ Erythroblasts progenitors $\rightarrow$ Erythrocytes

- BFU-E
- CFU-E
- Pro-E
- Baso-E
- Poly-E
- Ortho-E

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- BFU-E
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Proliferation and Expansion

Apoptosis in Thalassemia

Iron Use

Reticulocyte

Mature RBC

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

- PTG-300 1 mg/kg dosed Q2D for 6 weeks (Hbb<sup>th3/+</sup> 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

Group 1
WT Vehicle

Group 2
Hbb\(^{th3+}\) Vehicle

Group 3
Hbb\(^{th3+}\) DFP

Group 4
Hbb\(^{th3+}\) PTG300

Group 5
Hbb\(^{th3+}\) PTG300 + DFP

Bone Marrow

CD44

Spleen

FSC

9
PTG-300 Improves RBC Survival in β-Thalassemia

**Hbb\textsuperscript{th3/+} Mouse Model**

- **Dose PTG-300** (0.97 mg/kg, Q2D)
- **Biotinylation** NHS-PEG4-Biotin @ End of Week 4
- **4 weeks**
- **Flow cytometry analysis over 7 weeks to measure RBC survival**
- **Continue dosing with PTG-300 for the entire duration**

### RBC survival in Male Hbb\textsuperscript{th3/+} Mice

**Half Life (Days)**

- **WT Vehicle**: 17.3 days
- **WT PTG-300**: 13.6 days
- **Hbb\textsuperscript{th3/+} Vehicle**: 6.5 days
- **Hbb\textsuperscript{th3/+} PTG-300**: 16.9 days

![Graph showing RBC survival in Male Hbb\textsuperscript{th3/+} Mice](image-url)
PTG-300: Efficacy based POC in β-Thalassemia Mouse Model
Decreased Splenomegaly and Liver Iron Overload

- PTG-300 1 mg/kg dosed Q2D for 6 weeks (Hbbth3/+ 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: Efficacy based POC in β-Thalassemia Mouse Model
No Increase in Heart Iron and Some Iron Retention in Duodenum

- PTG-300 1 mg/kg dosed Q2D for 6 weeks (Hbb\textsuperscript{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 Efficacy with Potential for Weekly Dosing
Transient Iron Restriction Improves Erythropoiesis in Hbb\textsuperscript{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

**Chronic Hemoglobin**

**Chronic Study (Hbb\textsuperscript{th3+}) Mice: Reticulocyte Percentage**

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

Serum Iron Levels

Time (hr) Post dose

[Hb (g/dL)]

Vehicle Q2D Q3D Q4D Q5D

Vehicle 7 8 9 10

Q2D 11

Q3D 11

Q4D 11

Q5D 11

[Hb (g/dL)]

Vehicle Q2D Q3D Q4D Q5D

Vehicle 4 10 17 24

Q2D 0

Q3D 0

Q4D 0

Q5D 0

[Serum iron (µM)]

Vehicle Q2D Q3D Q4D Q5D

Vehicle 4 10 17 24

Q2D 0

Q3D 0

Q4D 0

Q5D 0

**ns p>0.05, * p<0.05, ** p<0.01, ***p<0.001, **** p<0.0001**
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.
PTG-300: Future

- 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