A Phase I Study Investigating the Safety, Tolerability, Pharmacokinetics and Pharmacodynamic Activity of the Hepcidin Antagonist PRS-080#022. Results from a Randomized, Placebo Controlled, Double-Blind Study Following Single Administration to Healthy Subjects

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Hepcidin is a 25 amino acid peptide hormone that serves as a **key regulator of iron metabolism** by inhibiting iron entry into plasma from the three main sources of iron:

- Dietary absorption in the duodenum
- Release of recycled iron from macrophages
- Release of stored iron from hepatocytes
Antagonizing Elevated Hepcidin Levels in Anemias of Chronic Disease (ACD)

- Hepcidin elevated in multiple chronic inflammatory conditions associated with anemia
  - Infections, cancer, RA, chronic kidney disease (CKD)

- Iron metabolism regulated by hepcidin/ferroportin
  - Hepcidin inhibits iron export from cells by blocking ferroportin
  - Excess hepcidin is the root cause of hypoferremia and iron-restricted reduction of erythropoiesis seen in ACD
  - Hepcidin inhibits erythroid colony formation at reduced erythropoietin concentrations

- Inhibition of hepcidin to treat functional iron deficient erythropoiesis and anemia expected to
  - Increase availability of internal iron sources
  - Increase ESA responsiveness allowing reduction of ESA doses
  - Prevent iron overload from exogenous administration
  - Increase and stabilize Hb levels

PRS-080: Pegylated Anticalin®
Hepcidin Antagonist
PRS-080 is a Highly Potent Anticalin®
Hepcidin Antagonist

- **Anticalins®** – derived from human lipocalins – are a **novel class of therapeutic binding proteins** (MW 16-20kD), that demonstrate high target affinity and exquisite specificity

- **PRS-080 is a pegylated Anticalin®** protein derived from the human lipocalin NGAL, that acts as a **potent hepcidin antagonist**
  - 50 pM affinity for hepcidin
  - Produced by bacterial expression in E. coli
  - Inhibits hepcidin-induced ferroportin internalization
  - Optimized plasma half life by conjugation to PEG 30
  - No adverse effects in non-human primate toxicity studies
PRS-080 Has Been Investigated in Phase I in Healthy Subjects

- Single dose escalating study in healthy male subjects, n=48
  - Randomized, double blinded, placebo controlled study

- 6 dose cohorts
  - 0.08, 0.4, 1.2, 4.0, 8.0, 16.0 mg/kg (based on API without PEG)
  - I.V. infusion over 2 hours
  - 6 subjects receiving PRS-080, 2 subjects receiving placebo per cohort

- Endpoints
  - Safety and maximal tolerated dose
  - Pharmacokinetics
  - Pharmacodynamics (serum iron, transferrin saturation)
  - Hepcidin plasma concentrations
  - Immunogenicity
PRS-080 Was Well Tolerated in Healthy Subjects

- No serious Adverse Events (AE)
- 39 treatment emergent AEs (TEAE) in 22 subjects
  - 30 mild TEAEs
  - 9 moderate TEAEs
- Headache was most common TEAE (10 subjects)
- Otherwise, no association of AEs to specific organs
- No apparent correlation between dose and number of TEAEs
- No hypersensitivity, no infusion reactions
- Vital signs and ECG without changes
- No cytokine release upon PRS-080 administration
  - IFN-γ, IL-1β, IL-6 and TNF-α
PRS-080 Shows Dose Proportional Pharmacokinetics

- **Total PRS-080**
  - Dose proportional $C_{\text{max}}$ and AUC
  - $T_{\text{max}}$ at around 1h after end of infusion
  - $T_{1/2}$ at 64 to 81 hours (geometric mean)
    - 71 to 81 hours, arithmetic mean
  - Small volume of distribution (48 - 65 ml/kg)
    - Consistent with distribution to blood volume

- **Free PRS-080**
  - $C_{\text{max}}$ and AUC lower compared to total PRS-080
  - $T_{1/2}$ at 40 to 62 hours
    - Consistent with “consumption” of free PRS-080 by hepcidin binding

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### Plasma Concentration, µg/ml

<table>
<thead>
<tr>
<th>Dose administration</th>
<th>Time after start of infusion [hours]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.08 mg/kg</td>
<td>0  24  48  72  120  240</td>
</tr>
<tr>
<td>0.4 mg/kg</td>
<td></td>
</tr>
<tr>
<td>1.2 mg/kg</td>
<td></td>
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<tr>
<td>4.0 mg/kg</td>
<td></td>
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<tr>
<td>8.0 mg/kg</td>
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<tr>
<td>16.0 mg/kg</td>
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</tbody>
</table>
PRS-080 Shows Dose-Dependent Effects
Increased Duration & AUC of Elevated Serum Iron Concentrations

Mean Iron Concentrations

Dose: 1.2  4.0  8.0  16.0 mg/kg (all subjects)
10h 48h 72h  >120h #

Area Under the Curve

Iron concentration over baseline
Individual subjects and mean*

* Mean placebo value subtracted

# Time point of peak iron conc. (hrs after start of infusion)
PRS-080 Induced Iron Response is Correlated to Baseline Ferritin & Hepcidin

- Serum iron response generally observed in subjects with normal ferritin (> 30 ng/ml) and detectable hepcidin (> 0.5 nM) at baseline
- Subjects of dose cohorts 1.2 to 16.0 mg/kg shown below
  - Subjects achieving iron response (> 34.5 µM = Fe↑)
  - Subjects without iron response (< 34.5 µM = Fe→)

<table>
<thead>
<tr>
<th></th>
<th>Ferritin &gt; 30 ng/ml</th>
<th>Hepcidin &gt;0.5 nM</th>
<th>No. of subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PRS-080</strong></td>
<td>Fe↑</td>
<td>Fe→</td>
<td></td>
</tr>
<tr>
<td>[1.2–16 mg/kg]</td>
<td>+</td>
<td></td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td><strong>Placebo</strong></td>
<td>+</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td></td>
<td>9</td>
</tr>
</tbody>
</table>

Lower limit of normal ferritin (30 ng/ml)
Lower limit of hepcidin quantification (0.5 nM)
Duration of response increases with dose

Individual peak serum iron concentrations are independent of dose

**Mean Iron Concentrations**
Subjects achieving iron response > 34.5 µM

**Time to Peak**
**Duration of Response**
**Peak Iron Concentration**

<table>
<thead>
<tr>
<th>Dose [mg/kg]</th>
<th>1.2</th>
<th>4.0</th>
<th>8.0</th>
<th>16.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to peak iron concentration</td>
<td>10h</td>
<td>48h</td>
<td>72h</td>
<td>120h</td>
</tr>
<tr>
<td>Duration of iron response*</td>
<td>25h</td>
<td>64h</td>
<td>94h</td>
<td>185h</td>
</tr>
<tr>
<td>Peak serum iron concentration [µM]</td>
<td>42.5</td>
<td>53.6</td>
<td>45.2</td>
<td>52.2</td>
</tr>
</tbody>
</table>

* Estimated time point where serum iron falls <34.5 µM
PRS-080 Shows Favorable Safety Profile/Confirms Mechanism of Action in Phase 1

- PRS-080 was **well tolerated** in healthy subjects
- **Pharmacokinetics as expected:** $T_{1/2} \sim 3$ days
- Immediate **dose-dependent decrease in circulating hepcidin**
- **Dose-dependent duration of serum iron and TSAT responses**
  - From 24 hours up to $>120$ hours
  - Predominantly observed in subjects with normal ferritin ($>30$ ng/ml) and detectable hepcidin ($>0.5$ nM) at baseline
    - Sufficient tissue iron stores and target expression
  - Robust responses at doses of 1.2 mg/kg and above, with **statistically significant increase in total serum iron** relative to placebo ($p = .005$)
- **No risk of immunogenicity observed**
- **Data support further investigation of PRS-080 in patients with ACD**
Next Steps: Phase Ib/IIa Study to Investigate PRS-080 in Anemic CKD5 Patients

Planned Phase Ib/IIa in CKD5 hemodialysis patients
- Ib: Single Ascending dose; Safety, PK and pharmacodynamic activity (iron, TSAT, hepcidin)
- IIa: MAD, 4 week repeated dosing; anemia (Hb) as primary outcome measure

VALIDATED BIOLOGY

Elevated hepcidin levels in CKD patients as cause for anemia
- Restricted iron utilization
- Impaired erythropoiesis
- Anemia despite i.v. iron and high ESA doses

PROMISING INVESTIGATIONAL DRUG

PRS-080 = hepcidin antagonist
- Increases iron mobilization
- Tailored half-life
- Aim to
  - Increase erythropoiesis
  - Reduce ESA and prevent iron overload
  - Reduce anemia

PROMISING CLINICAL ACTIVITY

Phase I study in healthy subjects
- Excellent safety
- Pharmacologic activity demonstrated
Thank you

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