Targeting the Hepcidin Pathway with RNAi Therapeutics for the Treatment of Anemia

December 12, 2011
Hepcidin is Central Regulator of Iron Homeostasis

- Hepcidin is liver-expressed, secreted peptide hormone that regulates iron.
- Hepcidin exerts action through regulation of ferroportin:
  - Binds ferroportin, causing ubiquitination, internalization, and degradation in lysosomes.
- Ferroportin is only known cellular iron exporter in vertebrates:
  - Found on hepatocytes, macrophages, and enterocytes.

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<th>Fe Release from Cellular Stores</th>
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Nemeth, Adv Hematol., 2010

Fpn = Ferroportin
Fe-Tf = Transferrin-bound iron
Anemia of Chronic Disease (ACD) and Hepcidin

- ACD often diagnosed in 3 settings, each associated with elevated serum hepcidin levels
  - Chronic kidney disease
  - Cancer
  - Chronic inflammatory states (e.g. rheumatoid arthritis)
- Currently managed with ESAs and IV iron to achieve target Hb levels
- Inappropriately elevated hepcidin results in impaired mobilization of iron needed for efficient erythropoiesis

Targeting the hepcidin pathway represents a novel, physiological, and potentially safer approach to managing ACD
RNA Interference (RNAi)
A New Class of Innovative Medicines

RNAi Therapeutics

- Harness natural pathway
  - Catalytic mechanism
  - Mediated by small interfering RNAs or “siRNAs”
- Treat disease with therapeutic gene silencing
  - Any gene in genome
- Major breakthroughs in delivery achieved
  - Includes systemic RNAi with formulations and chemistries
  - Enable advancement of RNAi products to clinic and market
Silencing hepcidin (HAMP1) mRNA increases serum iron in mouse model

- >80% silencing of liver hepcidin mRNA after single dose
- Results in ~2-fold increase in serum iron levels
- Phenocopies genetics

**HAMP1 mRNA**

**Serum Iron**

ANOVA, Dunnet's t post-hoc, compared to Control siRNA
* p < 0.05, *** p < 0.001

C57BL6 mice, 48 hr post-administration, mean ± sd
Proof of Concept in Nonhuman Primates

- Animals: male cynomolgus monkeys
- Dose: 1 mg/kg of LNP-formulated *HAMP*-targeting siRNA via 15 min IV infusion

Single administration of LNP-siRNA leads to rapid reduction of hepcidin mRNA and protein, resulting in elevation of serum iron levels in NHPs.
Exploring Additional Targets in Hepcidin Pathway

Rationale

- Number of additional targets have human genetic validation
  - HFE: HH type 1
  - HJV: HH type 2A
  - TFR2: HH type 3
- Use RNAi technology to improve our understanding of hepcidin signaling pathway

Targets

- HJV, TFR2, HFE, BMPRI, BMPRII, BMP6, Neogenin, IL6R, SMAD4

TFR2 is Attractive Hepcidin Pathway Target

...Results in HAMP1 Silencing

C57BL6 mice, 48 hr post-administration, mean ± sd
TFR2 Targeting Results in Sustained Elevation of Transferrin Saturation

...Results in HAMP1 Silencing

...And Increases Transferrin Saturation

C57BL6 mice, single IV 0.3 mg/kg dose, mean ± sd
Efficacy in a Rat Anemia of Chronic Disease Model

**Model**
- Lewis rats, male
- Initiation with single IP injection of PG-APS (polymers form Group A Streptococci)
- Treat 3X weekly starting D21
- Measure serum and hematology parameters biweekly and at 48 hr post final treatment
- Liver mRNA measurement 48 hr post final treatment

**Results**
- Effective silencing of TFR2 and HAMP mRNA
- Approximately 2X increase in serum iron upon treatment
- Increase in Hb to 11-12 g/dL with treatment

*following Coccia et al., Exp Hematology, 2001*
Summary

• ACD represents area of unmet need and hepcidin thought to play an important role in disease pathology
• Using RNAi to target hepcidin pathway represents novel, physiological, and potentially safer approach to managing ACD
• Silencing of hepcidin has been achieved in rodents and NHPs using siRNA, resulting in concomitant increases in serum iron
• Evaluation of hepcidin pathway reveals TFR2 as particularly attractive target for RNAi
• TFR2 silencing leads to potent and durable reduction in hepcidin, persistent elevation in serum iron, and amelioration of anemia in rodent model of ACD