Pharmacodynamics and Chronic Toxicology Assessment in Monkeys of IONIS-TMPRSS6-L_{Rx}, a 2'-MOE Antisense Oligonucleotide Conjugate that Targets the Human Transmembrane Protease Serine 6 (TMPRSS6)

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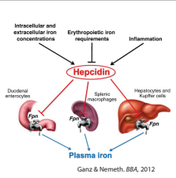
ABSTRACT

IONIS-TMPRSS6-L_{Rx} (ISIS 702843) is a triantennary N-acetyl galactosamine (GalNAc) ligand-conjugated, 2'- methoxyethyl (2' MOE) modified antisense oligonucleotide (ASO) with a mixed PS/PO backbone that targets human transmembrane protease, serine 6 (TMPRSS6). The drug is currently in clinical trials for the treatment of β-thalassemia. GalNAc (GN3), is a high-affinity ligand for the hepatocyte-specific asialoglycoprotein receptor (ASGPR). When conjugated to 2'-MOE antisense oligonucleotides (ASOs) the GN3 ligand has been previously shown to enhance the potency to multiple targets by 6- to 10-fold in mice and monkeys, and up to 30-fold in humans. Toxicity and pharmacologic activity of ISIS 702843 were evaluated in a chronic toxicology study in cynomolgus monkeys at doses up to 30 mg/kg/week for 13 weeks, or up to 12 mg/kg/wk for 39 weeks. A dose-dependent decrease in liver TMPRSS6 mRNA was observed, and was associated with expected decreases in serum iron and transferrin saturation. Secondary to the decreases in serum iron parameters were changes in hematology parameters such as decreases in hemoglobin and mean corpuscular volume and an increase in reticulocytes. Other effects observed were typical of those observed for ASOs of this chemical class, were correlated with high concentrations of the ASOs in tissues, and were unrelated to inhibition of TMPRSS6. There were no adverse toxicities associated with chronic ISIS 702843 administration to monkeys, including no effect on platelet count. Based on the dose-response relationships for pharmacology and tolerability, the therapeutic index for ISIS 702843 is significantly greater than for unconjugated MOE ASOs. In a Phase 1 clinical trial in healthy volunteers, evidence of a pharmacodynamic effect was also observed, as there was a dose-dependent reduction of serum iron and transferrin saturation. The NOAEL in the monkey of 12 mg/kg/wk (Human Equivalent Dose = 840 mg/week) provides a good safety margin for human patients. The overall tolerability of ISIS 702843 is representative of other 2'-MOE ASO-GalNAc conjugates in monkeys.

BACKGROUND

Hepcidin: the Master Regulator of Iron Homeostasis

- 25-aa peptide hormone, predominantly produced and secreted from liver
- Inhibits iron uptake in intestines and prevents iron release from macrophages, by inducing internalization and degradation of ferroportin (FPN), the sole iron exporter
- Hepcidin levels are inappropriately suppressed in thalassemia intermedia (TI) and hereditary hemochromatosis
- TMPRSS6 is a major negative regulator of hepcidin expression



Ganz & Nemeth. *BBA*, 2012

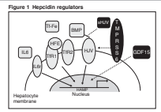


Figure 1 Hepcidin regulators

Identified hepcidin regulators are depicted as square-shaped (circulating regulators) or oval-shaped (membrane-associated regulators) items. Inhibitors, black; activators, light gray. BMP, bone morphogenic protein; BMPR, BMP receptor; erythroferrone, ERFE; GDF15, growth differentiation factor 15; HAMP, hepcidin gene; HJV, hemojuvelin; IL6, interleukin 6; IL6r, interleukin 6 receptor; sHJV, soluble hemojuvelin; TF-Fe, circulating iron transferrin; TfR1, receptor 1 for transferrin; TFR2, receptor 2 for transferrin; TMPRSS6, transmembrane serine protease 6 (matriptase-2)

Pietrangelo, A., *Current Opinion in Gastroenterology*, 2009

INTRODUCTION

TMPRSS6 ASO Treatment Reduced Iron Overload and Ameliorated Anemia in *th3*/+ Mice

- Tmprss6 ASO treatment in *th3*/+ mice resulted in significant reduction of Tmprss6 at the mRNA and protein level and significant increases in hepcidin expression in the liver, which leads to
  - Reduction in serum iron, transferrin saturation, and liver iron accumulation
  - Ameliorated anemia (Guo, Casu, et al. *JCI*, 2013)
- Similar results were obtained with GalNAc conjugated ASO at 10-fold lower dose (Aghajan, et al. *ASH*, 2016)
- Tmprss6 ASO treatment can be effectively combined with iron chelators for the management of iron overload and anemia in NTDT (Casu, et al. *Haematologica*, 2016)
- Targeting TMPRSS6 provides a potential therapy for hereditary hemochromatosis and β-thalassemia in humans

IONIS-TMPRSS6-L_{Rx}

- TMPRSS6 development candidate (IONIS-TMPRSS6-L_{Rx} ; a.k.a. ISIS 702843)
  - 5'-THA $GalNAc_3$ 5-10-5 MOE w/mixed backbone

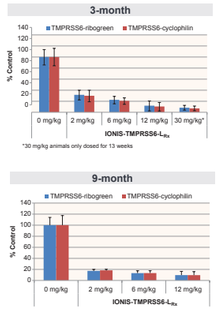
  - Showed significantly improved (~10-fold) activity in human TMPRSS6 transgenic mice compared to the unconjugated parent ASO
  - Completed 3- and 6-month GLP mouse tox studies
  - Completed 3/9-month GLP monkey tox study
    - Including a 3-month treatment arm at a high dose
  - Phase I Clinical Trial is ongoing

FINDINGS: MONKEY DATA

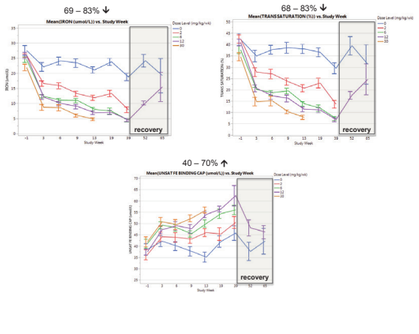
Significant TMPRSS6 mRNA Reduction in Monkeys

- Animals were dosed twice a week for the first two weeks and weekly thereafter by subcutaneous administration
- >90% target mRNA reduction achieved after 13 weeks

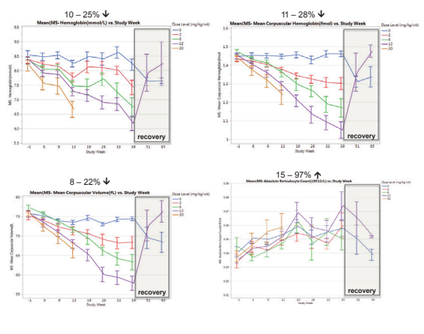
Cyno Liver TMPRSS6 mRNA Expression



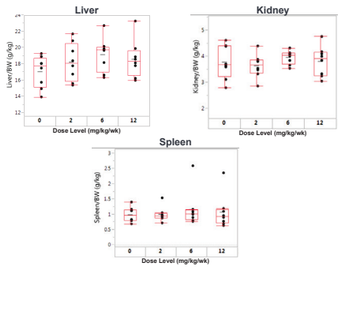
Dose-Dependent Changes in Serum Iron Parameters in Monkeys



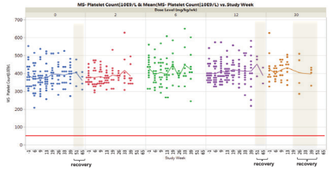
Reduction of Hematology Parameters Secondary to Pharmacologic Activity



No Changes in Organ Weights in Monkeys after 9 Months of Treatment



No Effect on Platelet Count in Monkeys through 9 Months of Treatment

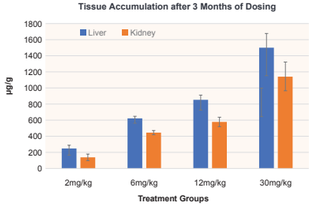


No Adverse Histologic Changes in Monkeys after 9 Months of Treatment

No adverse findings at any dose level (up to 30 mg/kg/wk for 13 weeks and up to 12 mg/kg/wk for 39 weeks)

- Findings observed were due to ASO accumulation in tissues
- Liver
  - Basophilic granules (minimal) in Kupffer cells at ≥ 6 mg/kg/wk after 13 and 39 weeks
  - Minimal to marked hepatocellular hypertrophy at ≥ 6 mg/kg/wk after 13 and 39 weeks
- Kidney
  - Basophilic Granules in proximal tubules (minimal) at 30 mg/kg/wk after 13 weeks and at 12 mg/kg/wk after 39 weeks
- Lymph Nodes
  - Minimal to marked granular macrophages at ≥ 2 mg/kg/wk after 13 and 39 weeks

Monkey Tissue Concentrations of IONIS-TMPRSS6-L_{Rx}



- Tissue half-life in monkey liver is 17.5 days, supporting monthly subcutaneous dosing regimen in the clinic

Estimated Safety Margins Based on NOAEL from Chronic Monkey Study and Projected Clinical Doses

NOAEL Dose (mg/kg/wk)	Margin based on 40 mg/month clinical dose (equivalent to 10 mg/wk; 0.14 mg/kg/wk)	Margin based on 60 mg/month clinical dose (equivalent to 15 mg/wk; 0.21 mg/kg/wk)	Margin based on 80 mg/month clinical dose (equivalent to 20 mg/wk; 0.29 mg/kg/wk)
2 (HED = 140 mg/wk)	14	9	7
6 (HED = 420 mg/wk)	42	28	21
12 (HED = 840 mg/wk)	84	56	42

HED = Human Equivalent Dose (assumes a 70 kg subject)

FINDINGS: IONIS-TMPRSS6-L_{Rx} CLINICAL DATA

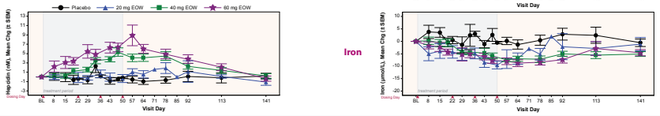
IONIS-TMPRSS6-L_{Rx} Clinical Program

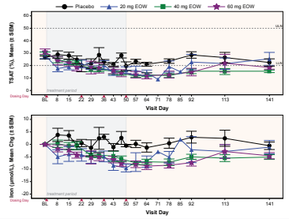
- Current Status
  - 20 mg (Cohort A): Completed dosing and follow up
  - 40 mg (Cohort B): Completed dosing and follow up
  - 60 mg (Cohort C): Dosing completed, follow-up ongoing

- Early evidence of pharmacodynamic response
  - *Reduction of plasma iron, TSAT and reticulocyte Hgb levels*
  - No treatment-related safety signals of concern

ISIS 702843-CS1: Early Evidence of PD Response in Healthy Volunteers

- Evidence of dose-dependent induction of hepcidin
- Evidence of dose-dependent reduction of:
  - Plasma Iron
  - Plasma Transferrin Saturation (TSAT)
  - Reticulocyte Hgb





ISIS 702843-CS1: Safety Summary

- IONIS-TMPRSS6-L_{Rx} was well-tolerated in healthy subjects during both the single-dose and entire study periods
- For both periods, there were no deaths or serious adverse events (SAEs) reported
- No adverse events of thrombocytopenia reported or any clinically meaningful reductions in platelet count reported
- No clinically meaningful dose dependent renal or hepatic abnormalities, nor complement or ECG findings reported
- No reported AEs that were indicative of constitutional symptoms
- No local cutaneous reactions at the injection site

No Effect on Platelets in Phase 1 Trial

Platelet Count (x10^9/L)	Placebo (N=9)	ISIS 702843 20 mg (N=9)	ISIS 702843 40 mg (N=9)	ISIS 702843 60 mg (N=9)
Day 57 (absolute values)				
N	7	7	9	8
Mean (SD, SEM)	236 (54, 20)	239 (70, 26)	260 (45, 15)	245 (73, 26)
Median (P25, P75)	217 (197, 299)	237 (188, 286)	246 (234, 251)	223 (190, 295)
Min - Max	176 - 321	163 - 367	223 - 349	170 - 373
Day 57 (% change from Baseline)				
N	7	7	9	8
Mean (SD, SEM)	4.0 (6.4, 2.4)	-2.7 (11.0, 4.2)	7.6 (11.3, 3.8)	10.8 (11.2, 3.9)
Median (P25, P75)	5.6 (0.0, 8.8)	-5.6 (-10.4, 2.1)	7.6 (1.7, 14.1)	9.9 (4.8, 18.1)
Min - Max	-7.4 – 11.4	-13.8 – 19.5	-9.7 – 30.1	-7.3 – 28.2

CONCLUSIONS & PATH FORWARD

- TMPRSS6 ASO treatment in thalassemia mice demonstrated significant benefit in iron overload and anemia
- IONIS-TMPRSS6-L_{Rx} is a GalNAc-conjugated ASO that is potent and well tolerated
  - 6-month mouse and 9-month monkey toxicology studies were completed with no major tox findings
  - In the monkey study, expected changes in iron and RBC parameters upon TMPRSS6 reduction were observed, which were reversed after cessation of dosing
- Phase 1 clinical trial has completed
  - Evidence of pharmacology in healthy volunteers
  - Excellent tolerability profile
  - Phase 2 Trial to start in late 2019
  - Low dose, low volume, self-administered subcutaneous injection monthly or less frequent

DISCLOSURES

Author(s) of this presentation are full-time employees of [1]Ionis Pharmaceuticals, Inc. or [2]Covance Preclinical Services and have the following to disclose concerning possible financial or personal relationships with commercial entities that may have a direct or indirect interest in the subject matter of this presentation:

- T Zanardi[1]: Nothing to disclose
- S Guo[1]: Nothing to disclose
- M. Aghajan[1]: Nothing to disclose
- B. Kornbacher[2]: Nothing to disclose
- L. Boone[2]: Nothing to disclose
- J. Kaspareit[2]: Nothing to disclose
- S. Henry[1]: Nothing to disclose

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