SLN124, a GalNAc siRNA Conjugate Targeting TMPRSS6, for the Treatment of Iron Overload and Ineffective Erythropoiesis, such as in β-Thalassemia

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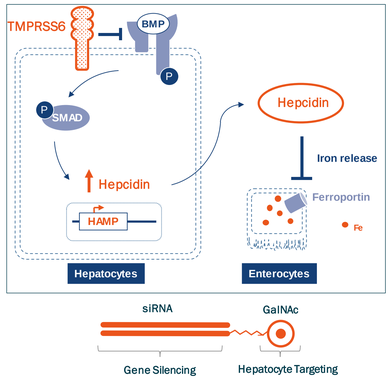
1. Introduction

- **Iron overload** (IO) is a serious health condition. It leads to organ damage and dysfunction and serious clinical consequences including liver cirrhosis, heart failure, diabetes and growth retardation.
- **In β-thalassemia** IO is caused by frequent blood transfusions and by ineffective erythropoiesis and low Hepcidin levels, which mediate gastrointestinal iron hyperabsorption.
- **In hereditary hemochromatosis** iron overload is caused by a genetic defect in the hepcidin-ferroportin axis, which leads also to enhanced absorption of dietary iron and tissue iron accumulation.
- **TMPRSS6** is a target gene involved in the regulation of Hepcidin expression and iron homeostasis.
- Here, we report on the pharmacological characterization of **SLN124**, a GalNAc siRNA conjugate targeting TMPRSS6, in preclinical models.

10. Summary & Conclusions

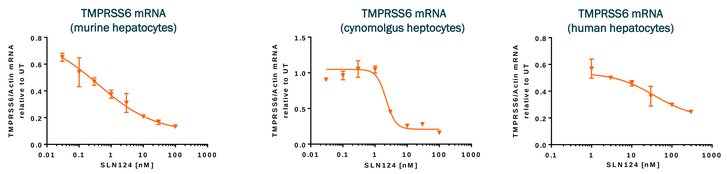
- SLN124 is a highly potent siRNA conjugate for inhibition of TMPRSS6 expression and for modulation of iron homeostasis.
- Single s.c. injection of SLN124 is sufficient to lower TMPRSS6 expression and reduce systemic iron levels in vivo for several weeks.
- Demonstrated therapeutic efficacy in clinically relevant animal models for hereditary hemochromatosis type 1 and for β–thalassemia intermedia.
- Currently in preclinical development with plans to enter clinical development in 2019.

2. Rationale for targeting TMPRSS6

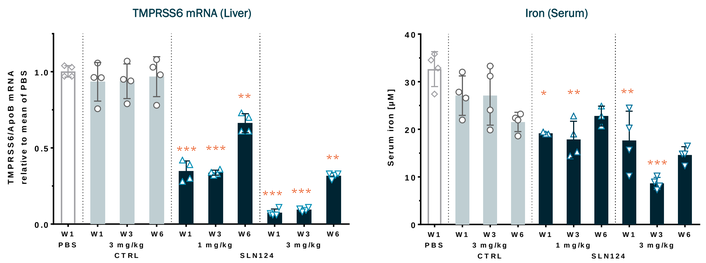


- **TMPRSS6** is a negative regulator of the BMP/SMAD signaling pathway inducing Hepcidin expression.
- **Hepcidin** reduces uptake of dietary iron and efflux of iron from storage cells.
- Hepcidin levels are low in patients with iron loading anemias.
- Inhibition of **TMPRSS6** expression in the liver will raise Hepcidin and reduce iron absorption.
- **GalNAc siRNA approach** for gene silencing in the liver.

3. SLN124 reduces TMPRSS6 mRNA expression in 1° hepatocytes from different species by receptor mediated uptake

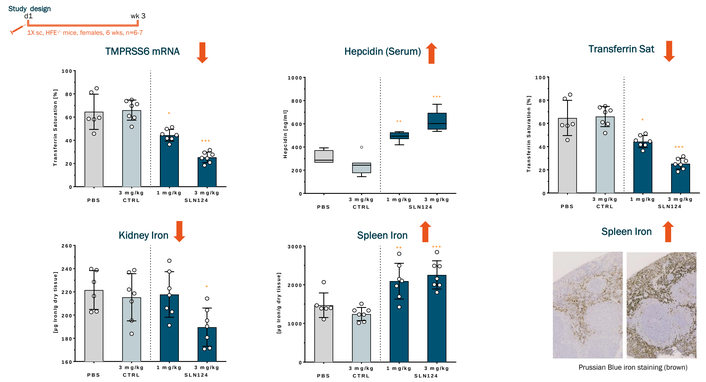


4. Reduction of TMPRSS6 mRNA in the liver and serum iron levels in mice by single s.c. application



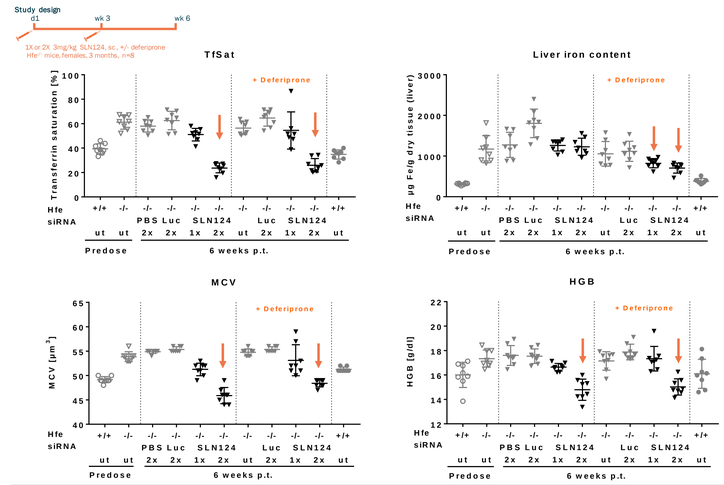
C57BL/6 mice were treated with single s.c. dose of TMPRSS6 siRNA conjugate (SLN124). Shown are individual mice and group mean ± SD. W = week; CTRL = Luciferase GalNAc-siRNA. Two-way ANOVA with multiple comparisons. Depicted are comparisons to CTRL groups from the same time point: *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001.

5. Therapeutic efficacy by SLN124 in rodent model for hereditary hemochromatosis type 1 (HFE$^{-/-}$ mice)



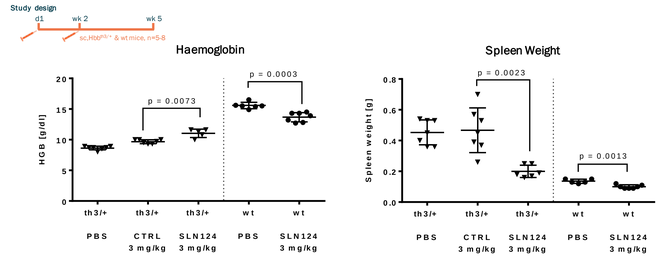
Female HFE$^{-/-}$ mice (6w), D1 s.c. injection with SLN124, D22 blood collection and termination; CTRL = GalNAc-Luc siRNA. Kruskal-Wallis Test with uncorrected Dunn's test against CTRL. Bar diagrams (mean values ± SD). Box and Whiskers (Tukey, median values): *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001.

6. Treatment of mice with established IO and in combination with Deferiprone

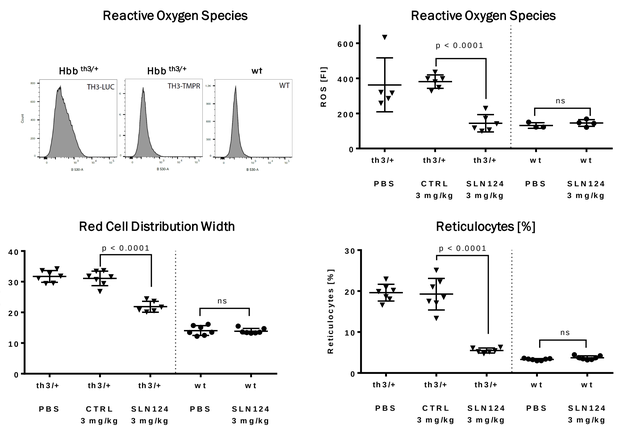


Female HFE $^{-/-}$ mice (12w), D1 s.c. injection with SLN124 (1X) or on D1 + D22 (2X), D43 blood collection and termination, CTRL = GalNAc Luci siRNA. Where indicated, Deferiprone was supplied in drinking water (0.5 mg/ml). Wildtype mice (+/+) were used for comparisons. Tissue samples of Predose cohorts were collected on D1.

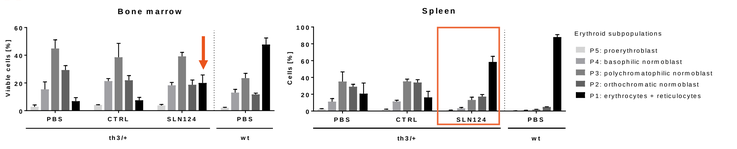
7. Therapeutic efficacy by SLN124 in rodent model for β-thalassemia intermedia (Hbb$^{th3/+}$ mice)



8. SLN124 reduces ROS and normalizes RBC maturation in Hbb$^{th3/+}$ mice



9. SLN124 improves RBC maturation in bone marrow and spleen of Hbb$^{th3/+}$ mice



Hbb$^{th3/+}$ mice (2-3 months), s.c. injection of GalNAc-siRNAs D1 & D15, blood collection D36, blood and tissue collection for FACS analysis D39. CTRL=GalNAc Luc siRNA. Welch's t-test uncorrected for multiple comparisons (mean values ±SD).

Disclosures: U Schaeper, S Dames, C Frauendorf, M Aleku and S Schubert are employed by Silence Therapeutics GmbH. M Muckenthaler received research funding from Roche.

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