## Abstract: P744

# Title: TARGETING DYSREGULATION OF IRON HOMEOSTASIS IN MDS MICE WITH AN OPTIMISED GALNAC-CONJUGATED TMPRSS6 SIRNA

**Abstract Type: Poster Presentation**

**Topic: Myelodysplastic syndromes - Biology & translational research**

**Background:**

The Myelodysplastic syndromes (MDS) are characterized by ineffective erythropoiesis that in turn suppresses liver hepcidin production leading to unrestrained intestinal iron uptake. Iron overload has been increasingly recognised as an important player in MDS. Hepcidin, the main regulator of iron metabolism, is synthesized and released by hepatocytes in response to increased body iron concentration and inflammation. Considering hepcidin is under the negative control of TMPRSS6 via cleavage of hemojuvelin (HJV), a co-receptor for the BMP-SMAD signaling pathway, inhibition of TMPRSS6 expression represents a promising therapeutic strategy to increase hepcidin production and ameliorate anemia and iron overload in MDS.

**Aims:**

To target dysregulation of iron homeostasis in MDS mice using an optimized GalNAc-conjugated siRNA targeting TMPRSS6

**Methods:**

We investigated liver-specific delivery of an optimized GalNAc-conjugated siRNA targeting TMPRSS6 in MDS (NHD13) mice. N-acetylgalactosamine (GalNAc) ligand is a well-defined liver-targeting moiety benefiting from its high affinity to the asialoglycoprotein receptor (ASGPR). NHD13 and wild type (WT) mice were treated with monthly subcutaneous injections of GalNAc-TMPRSS6 siRNA (3mg/kg) or oral iron chelator deferiprone (1.25 mg/ml) in the drinking water. Mice were treated in Study 1 from 4 to 6 months and Study 2 from 6-15 months.

**Results:**

Using laser-ablation-inductively-coupled plasma-mass spectrometry (LA-ICP-MS) and Perls Prussian Blue Stain we demonstrated a ~3-fold higher iron levels in the bone marrow (BM) of NHD13 mice when compared to WT controls, highlighting a potential pathological impact of iron toxicity in the BM microenvironment. GalNAc-TMPRSS6 siRNA treatment reduced liver *Tmprss6* mRNA expression, which correlated with an increase in liver *Hamp* mRNA expression, and serum hepcidin levels in NHD13 mice. Increased serum hepcidin was associated with a significant reduction in serum iron levels and BM iron deposition following GalNAc-TMPRSS6 siRNA treatment. Moreover, long-term monthly treatment of NHD13 mice with GalNAc-TMPRSS6 siRNA, particularly into the later stages of disease development, significantly reduced ineffective erythropoiesis and extended the lifespan of NHD13 mice. Notably, 30% of mice were alive at 450 days, whereas 100% of the nonspecific siRNA control and DFP treatment groups succumbed to disease by 400 days.

**Summary/Conclusion:**

GalNAc-TMPRSS6 siRNA treatment reduced *Tmprss6* expression, induced hepcidin serum levels and suppressed BM iron deposition in NHD13 mice. Notably, long-term GalNAc-TMPRSS6 siRNA treatment of NHD13 mice suppressed emergence of disease emphasising the need for further investigations into mechanisms underlying the pathological impact of iron toxicity as well as the effects of iron restriction approaches in MDS.

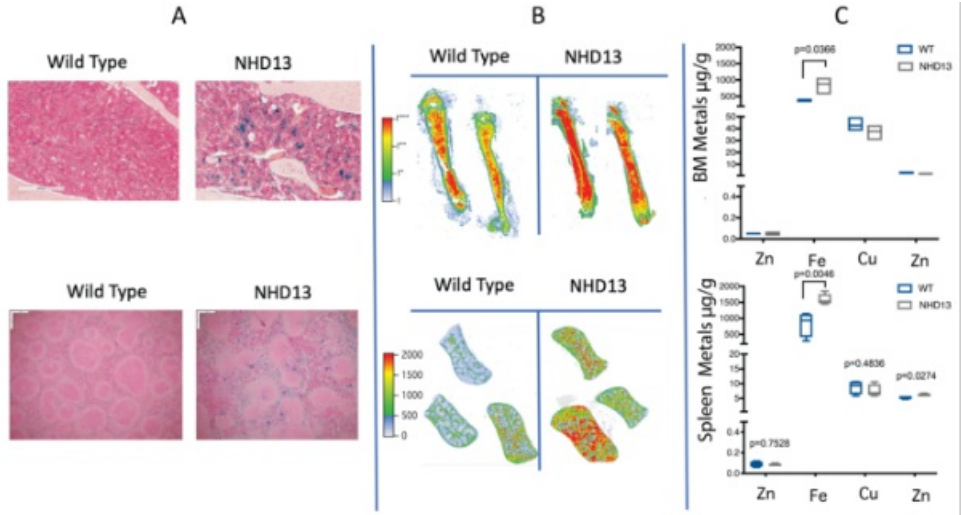


**Figure 1. NHD13 mice display increased iron deposition in the bone marrow and spleen.**
A) Representative images of Perls' Prussian Blue-stained bone marrow and spleen sections from WT and NHD13 mice. B) Fixed tissue sections were analysed by Laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) to assess anatomically defined elemental. Representative images of tissue iron levels in bone marrow (top) and spleen (bottom) from WT and NHD13 mice. C) Elemental and quantitative analysis of Mn, Fe, Cu, and Zn in the bone marrow (top) and spleen (bottom) of WT and NHD13 mice (n=5).

**Keywords:** MDS