Treating iron overload disorders with a novel therapeutic antibody targeting TMPRSS6

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Abstract

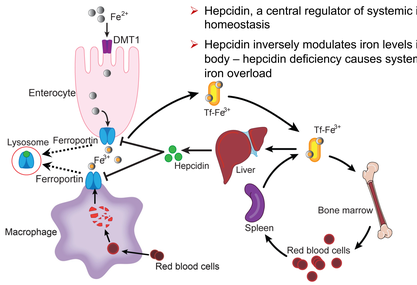
Iron is an essential element for almost all living organisms as it participates in a wide variety of metabolic processes. Disorders of iron metabolism are among the most prevalent human diseases, ranging from anemia to hemochromatosis. Excessive iron accumulations in major organs of iron overload patients can lead to high mortality. Hepcidin, a *HAMP*-encoded liver hormone, is the master regulator of iron homeostasis. By binding to the sole iron exporter ferroportin and causing internalization and degradation of the complex, hepcidin inhibits cellular iron efflux, thereby lowers plasma iron levels. Inappropriately suppressed/low hepcidin production is central to iron overload. Transmembrane protease serine-6 (TMPRSS6), a type II transmembrane serine protease primarily expressed in liver, downregulates hepcidin expression through BMP-SMAD pathway. TMPRSS6 deficiencies have been shown to cause hepcidin overexpression in both TMPRSS6-mutant mice and in patients with iron-refractory iron deficiency anemia (IRIDA). Therefore, TMPRSS6 is a viable therapeutic target for iron overload disorders.

Here we report the generation of an anti-TMPRSS6 antibody through a hybridoma campaign using a DNA-based immunization approach, followed by humanization and sequence optimization. Lead antibody, hzMWTx-003 selectively binds human TMPRSS6 with low nanomolar affinity (KD: 7.6nM), and is cross-reactive to rodent (mouse and rat) and monkey (cynomolgus and rhesus) TMPRSS6. Single-dose injection of hzMWTx-003 was able to significantly elevate serum hepcidin and liver *HAMP* RNA levels in wildtype mice, resulting in significantly reduced serum iron level. The *Hbb*$^{th3/+}$ mouse model of β-thalassemia, like its human counterpart, is characterized by iron overload, ineffective erythropoiesis and splenomegaly. Treatment of *Hbb*$^{th3/+}$ mice with MWTx-003 effectively increased hepcidin expression at both protein and RNA levels, leading to significantly reduced serum iron and liver non-heme iron content. MWTx-003 also dramatically improved anemia and ineffective erythropoiesis, and alleviated splenomegaly in these mice.

CMC development of hzMWTx-003 confirms outstanding biophysical properties. Preliminary studies in cynomolgus monkey using GLP-grade material demonstrated good pharmacokinetics of hzMWTx-003 and expected pharmacodynamic response where reduction of serum iron could be sustained for 21 days after single dose administration. A single dose toxicology study in cynomolgus monkey revealed no safety concerns, and no production of anti-idiotype antibodies was detected. In summary, anti-TMPRSS6 antibody MWTx-003 represents a promising therapy for iron overload disorders such as β-thalassemia, and potentially other diseases where iron restriction is beneficial.

Introduction (1)

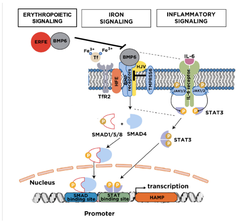
Systemic iron homeostasis



- Hepcidin, a central regulator of systemic iron homeostasis
- Hepcidin inversely modulates iron levels in the body – hepcidin deficiency causes systemic iron overload

Introduction (2)

Regulation of hepcidin



- Major physiological hepcidin inducers:
  - BMP6 (iron)
  - IL-6 (inflammation)
- Major physiological hepcidin suppressors:
  - ERFE (erythropoiesis)
  - **TMPRSS6**
    - TMPRSS6, a type II transmembrane serine protease produced primarily in the liver.
    - *Tmprss6* knockout mouse and patients with *TMPRSS6* mutations confirm that ***TMPRSS6 deficiency leads to elevated hepcidin levels and subsequent iron restriction***.

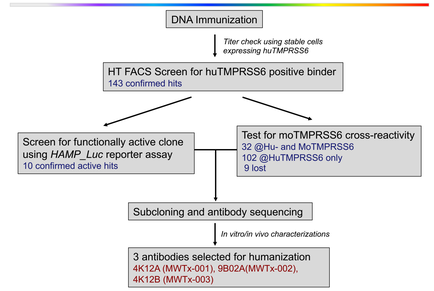
Adapted from Katsarou A *et al.*, Pharmaceuticals 2018, 11:127

Rationale of TMPRSS6 targeting therapy

- ***siRNA inhibition of TMPRSS6 in mouse model of β-thalassemia*** results in increased production of hepcidin, leading to ***more effective red blood cell production in the bone marrow and reduced iron toxicity in the liver***.
- **Inhibition of TMPRSS6 represents an unique strategy to control iron availability,** improve liver iron toxicity, and increase effective erythropoiesis **under conditions of β-thalassemia, or more broadly, of iron-loading anemias and hemochromatosis.**
- Anti-TMPRSS6 mAb could be **monotherapy for non-transfusion dependent** *β-thalassemia or* **combination therapy for transfusion dependent** *patients.*

Antibody discovery

Hybridoma campaign

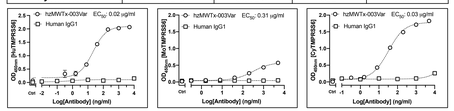


Characterizations of antibody lead (1)

Affinity and Cross-reactivity

- **Based on the totality of results obtained from bioanalytical and functional assessments, hzMWTx-003Var was selected as anti-TMPRSS6 antibody lead.**

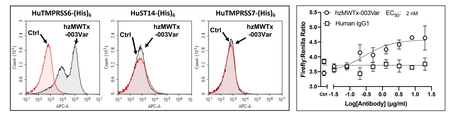
	hzMWTx-003Var (sequence optimized)			
Antigen	**K_D (M)**	**k_{on} (1/Ms)**	**k_{off} (1/s)**	**R^2**
HuTMPRSS6	1.37E-8	4.79E+3	6.58E-5	0.9996
MoTMPRSS6	1.24E-7	9.55E+3	1.18E-3	0.9975
CyTMPRSS6	1.15E-9	8.86E+4	1.02E-4	0.9993



- **The antibody lead, hzMWTx-003Var, has a high affinity towards human TMPRSS6, and is cross-reactive with TMPRSS6 of rodent and monkey.**

Characterizations of antibody lead (2)

Specificity and *in vitro* activity

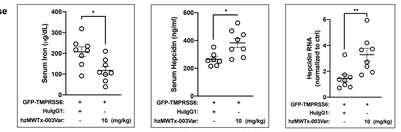


- **The antibody lead, hzMWTx-003Var, specifically recognizes TMPRSS6, but not Matriptase or Matriptase-3 homologs.**
- **The antibody lead, hzMWTx-003Var, inhibits TMPRSS6-mediated suppression on *HAMP* promoter in a dose-dependent manner.**

Developability assessment of antibody lead

In vivo pharmacodynamic activity (1)

Mouse



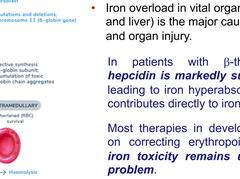
- hzMWTx-003Var was IP injected.
- huTMPRSS6 plasmid was delivered by hydrodynamic injection.

- **Treatment of hzMWTx-003Var effectively increased serum hepcidin and liver *HAMP* RNA, leading to a significant reduction in serum iron.**

Developability assessment of antibody lead

In vivo pharmacodynamic activity (2)

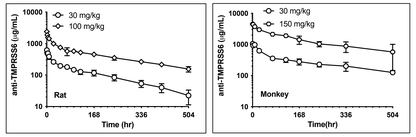
Monkey



- **A single dosing of hzMWTx-003Var effectively reduced serum iron in cynomolgus monkey, and the reduction was sustained for over 3 weeks.**

Developability assessment of antibody lead

In vivo pharmacokinetics

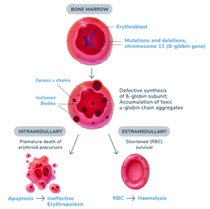


- **hzMWTx-003Var showed a good pharmacokinetics profile, which is similar to human IgG1.**
- **A single dose toxicology study in cynomolgus monkey revealed no safety concerns, and no production of anti-idiotype antibodies was detected either.**

Introduction (3)

Iron-loading anemia - β-thalassemia

Pathology of β-thalassemia



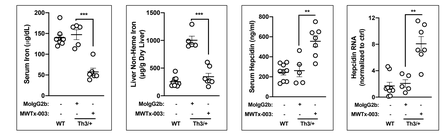
- Anemia characterized by ineffective erythropoiesis
- Systemic iron overload
- Iron overload in vital organs (e.g. heart and liver) is the major cause of death and organ injury.

In patients with β-thalassemia, ***hepcidin is markedly suppressed***, leading to iron hyperabsorption, and contributes directly to iron toxicity.

Most therapies in developing focus on correcting erythropoiesis, while ***iron toxicity remains unresolved problem***.

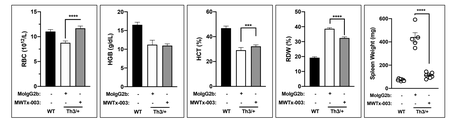
In vivo efficacy of anti-TMPRSS6 antibody in a mouse model of β-thalassemia (1)

- Mice with both *Hbb-b1* and *Hbb-b2* gene alleles deleted (aka. Th3 mouse) were chosen as the mouse model of β-thalassemia.
- Th3 mouse was treated with 10mg/kg MWTx-003 once every 3 days for 4 weeks.



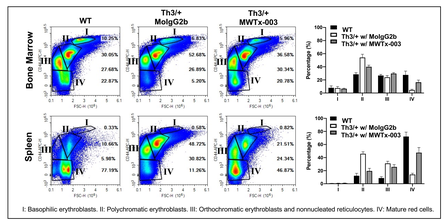
- **MWTx-003 treatment effectively increased serum hepcidin and liver *HAMP* RNA, decreased serum iron and liver non-heme iron in β-thalassemia mice.**

In vivo efficacy of anti-TMPRSS6 antibody in a mouse model of β-thalassemia (2)



- **MWTx-003 treatment effectively improved anemia, reduced splenomegaly in β-thalassemia mice.**

In vivo efficacy of anti-TMPRSS6 antibody in a mouse model of β-thalassemia (3)



I: Basophilic erythroblasts. II: Polychromatic erythroblasts. III: Orthochromatic erythroblasts and nonnucleated reticulocytes. IV: Mature red cells.

- **MWTx-003 treatment effectively improved ineffective erythropoiesis, promoting erythroblasts differentiation into mature red blood cells in β-thalassemia mice.**

Summary

1. **Anti-TMPRSS6 monoclonal antibodies were identified through hybridoma campaign, using TMPRSS6 cDNA as immunogen.**
2. **3 hybridoma hits were selected for antibody lead development, and hzMWTx-003Var was selected as antibody lead, which demonstrated good affinity, specificity, *in vitro* functional activities.**
3. **hzMWTx-003Var showed outstanding biophysical properties, expected pharmacodynamic responses and good pharmacokinetics profile. Toxicology study in cynomolgus monkey revealed no safety concern, and no production of anti-idiotype antibodies was detected.**
4. **Anti-TMPRSS6 antibody is efficacious in preventing systemic iron overload and improving ineffective erythropoiesis and anemia in a mouse model of β-thalassemia.**
5. **Anti-TMPRSS6 antibody represents a promising therapy for β-thalassemia, in particular, for intermediate or non-transfusion dependent thalassemia patients, and potentially other diseases where iron restriction is beneficial.**
6. **More details: WO 2021/2027072 'ANTI-TRMPRSS6 ANTIBODIES AND USES THEREOF', published on October 14th, 2021.**