KY1066: Generation and characterisation of a fully human antibody targeting the enzymatic activity of matriptase-2 for the treatment of iron overload in β thalassemia

Poster 3532

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Introduction

Beta thalassemia is an inherited hemoglobinopathy caused by a genetic defect in the beta-globin gene and characterised by ineffective erythropoiesis, iron overload, splenomegaly and anemia. The role of matriptase-2 (MTP-2) in iron regulation is already well established and reducing *Tmprss6,* the gene encoding MTP-2 has shown to increase hepcidin expression and correct iron overload, splenomegaly and anemia in *Hbb*$^{th3/+}$ mice, a mouse disease model of beta thalassemia.

Here we describe a first-in-class antibody targeting MTP-2 for the treatment of iron overload diseases, such as beta thalassemia. In *Hbb*$^{th3/+}$ mice, a single dose of CL-286425 decreased serum iron and transferrin saturation (TSAT) and, with repeat dosing, was able to consistently restrict iron supply thus improving haemoglobin levels and splenomegaly. Furthermore, in combination with erythropoietin (epo), haemoglobin levels were further improved whilst also reducing splenomegaly, usually associated with epo treatment. Together this provides evidence that treatment with an anti-MTP-2 therapy has the potential to treat iron overloaded patients, such as in beta thalassemia, to improve the anemia and reduce iron overload resulting potentially in a lower transfusion burden and no requirement for iron chelation.

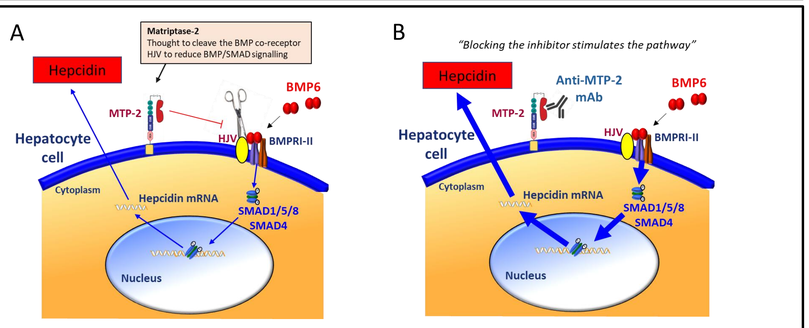

Figure 1. Blocking the inhibitor to stimulate the pathway. Cartoon diagram showing (A) normal BMP/SMAD/hepcidin signalling in hepatocyte cells and (B) the effect of MTP-2 mAb inhibition on the BMP/SMAD/hepcidin pathway and the associated increase in hepcidin expression.

Methods

Wildtype (WT) extracellular domain (ECD) proteins of human, cynomolgus monkey, mouse and rat MTP-2 and human MTP-2 missing the serine protease (SP) domain were expressed and purified in-house. *In vitro* studies: human MTP-2 was used in proteolytic assay using BOC-Gln-Ala-Arg-AMC peptide substrate (Bachem). *In vivo* studies: 10 mg/kg of antibodies were dosed by i.p. injection into C57BL/6J mice or *Hbbth-3/+* mice bred at the Animal Research Centre (Toulouse).

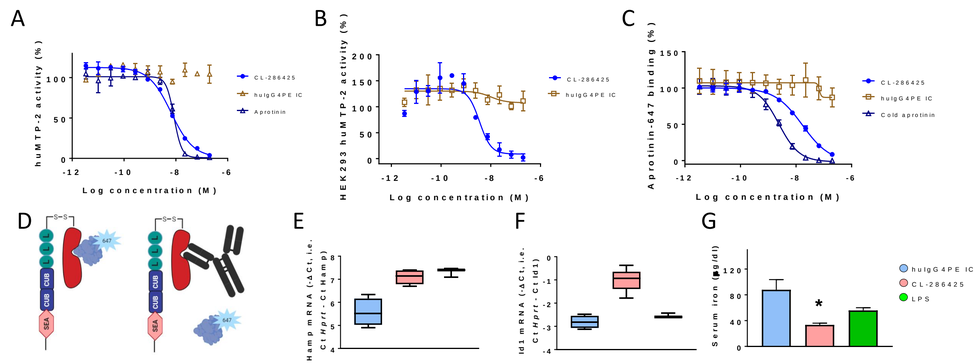

Figure 2. Anti-MTP-2 mAb blocks MTP-2 enzymatic function via binding to the active site to disrupt function. CL-286425 titration starting at 200nM was tested for inhibition against a fixed concentration of (A) huMTP-2 protein or (B) HEK293 huMTP-2 cells using 50 µM of BOC-Gln-Ala-Arg-AMC peptide. (C) Competition of antibody titration starting at 200nM for binding to huMTP-2 protein against 647-labelled Aprotinin (D) Cartoon of competition assay. (E-G) CL-285425 enhances BMP/SMAD signalling to suppress serum iron in WT C57BL/6J mice following ip injection. Mice were treated with either CL-286425 or isotype control (10 mg/kg) for 24 hours or LPS for 4 hours. Results are shown as box and whiskers (min to max) plot or as mean ± SEM. 1 way ANOVA statistical test was used applied comparing each group for comparison to the control group (isotype control antibody treated animals) was applied *p <0.05.

	Human	Mouse	Cyno	Rat	Human no SP
KD (M)	5.61E-09	9.00E-09	2.17E-09	3.01E-09	no significant binding

Table 1. Binding affinity analysis CL-450087 to various MTP-2 ECD proteins. Cross-reactivity and epitope specificity of CL-286425 was confirmed by testing a sequence modified version of CL-286425 to WT ECD proteins of human, cynomolgus monkey, mouse, rat MTP-2 and human MTP-2 missing the SP domain by SPR**.**

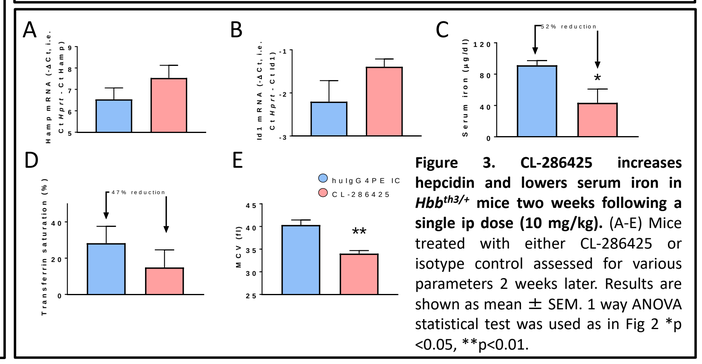

Figure 3. CL-286425 increases hepcidin and lowers serum iron in *Hbb*$^{th3/+}$ mice two weeks following a single ip dose (10 mg/kg). (A-E) Mice treated with either CL-286425 or isotype control assessed for various parameters 2 weeks later. Results are shown as mean ± SEM. 1 way ANOVA statistical test was used as in Fig 2 *p <0.05, **p<0.01.

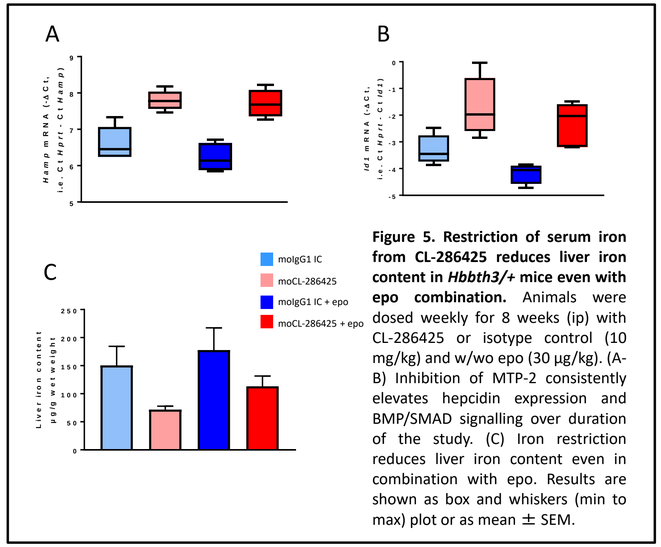

Figure 5. Restriction of serum iron from CL-286425 reduces liver iron content in *Hbbth3/+* mice even with epo combination. Animals were dosed weekly for 8 weeks (ip) with CL-286425 or isotype control (10 mg/kg) and w/wo epo (30 µg/kg). (A-B) Inhibition of MTP-2 consistently elevates hepcidin expression and BMP/SMAD signalling over duration of the study. (C) Iron restriction reduces liver iron content even in combination with epo. Results are shown as box and whiskers (min to max) plot or as mean ± SEM.

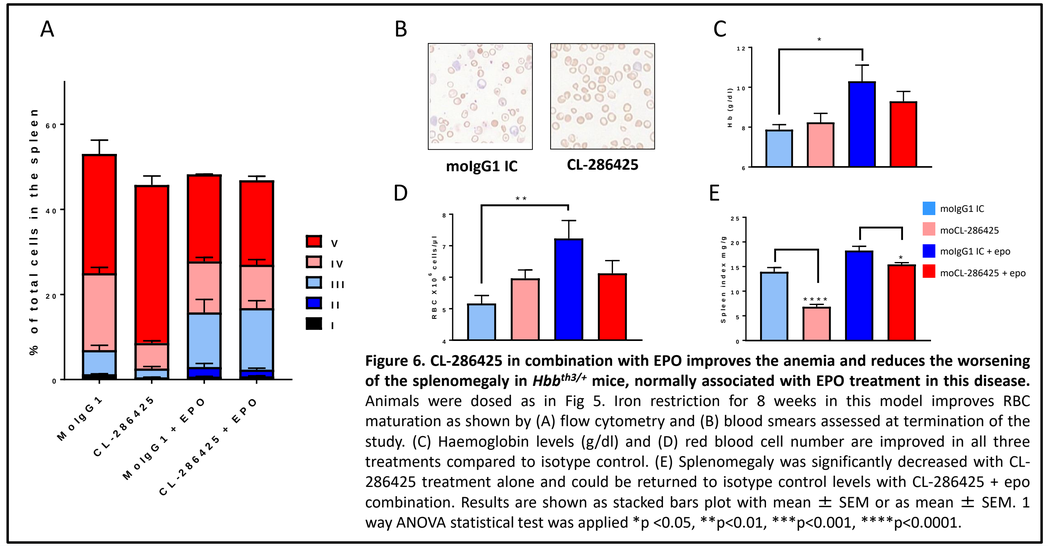

Figure 6. CL-286425 in combination with EPO improves the anemia and reduces the worsening of the splenomegaly in *Hbb*$^{th3/+}$ mice, normally associated with EPO treatment in this disease. Animals were dosed as in Fig 5. Iron restriction for 8 weeks in this model improves RBC maturation as shown by (A) flow cytometry and (B) blood smears assessed at termination of the study. (C) Haemoglobin levels (g/dl) and (D) red blood cell number are improved in all three treatments compared to isotype control. (E) Splenomegaly was significantly decreased with CL-286425 treatment alone and could be returned to isotype control levels with CL-286425 + epo combination. Results are shown as stacked bars plot with mean ± SEM or as mean ± SEM. 1 way ANOVA statistical test was applied *p <0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Conclusions

Following screening, multiple cross-reactive (human, cyno, mouse and rat) anti-MTP-2 neutralising mAbs were successfully identified. CL-286425, shown here, inhibits MTP-2 via binding to the serine protease active site. In vivo, hepcidin levels were increased to levels comparable to that seen in Tmprss6 knockout mice (data not shown) after just 24 hours post dosing. With repeat dosing in *Hbbth3/+* mice, there was a clear improvement in iron overload symptoms and erythropoiesis. In combination with EPO, we found that RBC number and hemoglobin levels could be improved whilst the splenomegaly induced by epo stimulation could be reduced. Together, these findings suggest that β thalassemia patients would benefit from anti-MTP-2 mAb therapy alone to improve iron overload symptoms and reduce transfusion need. Moreover, there is the possibility of an anti-MTP-2 mAb + epo combination therapy to simultaneously reduce iron overload, further improve the anemia but not worsen the splenomegaly associated with epo alone. Further studies will explore whether the positive effect on the anemia can be maintained whilst further reducing the splenomegaly induced by the epo co-treatment.