A Fully Human Anti-BMP6 Antibody Reduces the Need for Erythropoietin Stimulating Agent in Two Rodent Anemia of Chronic Disease Models

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Background: Anemia of chronic disease (ACD) is the most common cause of anemia in hospitalized patients. Despite causative treatment of the underlying disease, anemia management remains a major challenge to many chronic diseases such as chronic kidney disease, rheumatoid arthritis, inflammatory bowel disease, cancer, and infections. Especially Erythropoietin as well as intravenous iron replacement therapy are integral parts of ACD therapy at present. However, both have been associated with possible negative side effects. Hepcidin, a liver-derived hormone, is the master regulator of systemic iron homeostasis and is central to the iron-restrictive phenotype in ACD patients. As BMPs, mainly BMP2 and BMP6, have been reported to be involved in hepcidin control, a fully human anti-BMP6 antibody (KY1070) has been developed to suppress hepcidin expression. We here report the effects of such an anti BMP6-antibody therapy on anemia, iron metabolism, erythropoiesis and Erythropoietin dosing in two different, well established rodent models of ACD.

Results

1. In the murine CKD-animal model (IIb), KY1070 reduces hepcidin levels in a dose-dependent manner and leads to increased erythropoiesis.

2. In the murine arthritis model (ABP-Arth), KY1070 reverses anemia and reduces hepcidin levels.

Conclusions: anti-BMP6 targeted therapy works synergistically with Erythropoietin treatment in two different models of anemia of chronic disease leading to significantly increased hemoglobin levels, a reduced Erythropoietin need as well as reduced iron overload in organs of the mononuclear phagocyte system. Furthermore, these experiments clearly show that treatment of anemia of chronic disease, being a complex multifactorial disease, benefits from using a combination of diversified approaches to overcome anemia and significantly reduce the dose of each therapeutic.

Figure 1: Specificity and activity of KY1070 in vitro. (A) Cross-reactivity of KY1070 against human, rat and mouse BMPs was tested using a HepG2 cell line. A fixed concentration of recombinant human, rat or mouse BMPs of 100 ng/mL was used. KY1070 inhibition curves were generated by titration starting at a final concentration of 600 nM. (B) KY1070 specificity was tested by ELISA for BMP5 and BMP7. HepG2 cell line was stimulated with a fixed concentration of recombinant human BMPs or BMP7 of 100 ng/mL; a human IgG isotype control was included in each assay.

Figure 2: KY1070 in combination with Darbepoetin alpha (EPO) effectively reverses anemia in a rat arthritis anemia of chronic disease (ACD) model and impacts on iron metabolism during inflammation. (A) ACD in female Lewis rats was induced by intraperitoneal administration of PG-AFP. ACD rats were treated with IgG isotype control, KY1070, EPO or both. (B) Hematological parameters (hemoglobin, Red Blood Cells, Mean Corpuscular Volume and Mean Corpuscular Hemoglobin), were determined in ACD rats over the time of treatment. (C) Kinetic analysis of plasma hepcidin and transferrin saturation one week after treatment start in ACD rats. (D) Transferrin Saturation and plasma hepcidin levels at the end of treatment (Week 6). Multiple linear regression and Dunnert corrected post hoc paired t-test (pairing by values belonging to the same animal) for comparisons with the control group (ACD or AKD) was applied. Results are shown as mean ± SEM; *p<0.05, **p<0.01, ***p<0.001

Figure 3: KY1070 mobilizes iron for erythropoiesis in a murine chronic kidney disease (CKD) model and has a synergistic effect on anemia compared with Darbepoetin alpha. (A) CKD in male C57BL/6N mice was induced by a diet containing 0.2% phytic acids. CKD mice were treated with KY1070 (3 mg/kg; 3x/w), Darbepoetin alpha (EPO) (200 µg/kg; 3x/w), Darbepoetin alpha + KY1070 (3 mg/kg; 3x/w) or saline. (B) Correlation parameters (hepcidin, Red Blood Cells, Mean Corpuscular Volume) were determined in CKD mice at Week 4. (C, D) Transferrin saturation and plasma hepcidin levels at the end of treatment (Week 4). (E) Representative Western blot of Fpn1 and Ftb1 of whole spleen lysates. (F) Flow cytomteric analysis of bone marrow erythropoiesis at the end of treatment in mice of all different treatment groups. Representative dot plots for Lineage- cells with percentages of the different erythropoietic populations (0-9) per femur are shown. Two-way ANOVA with Dunnett corrected post hoc test for comparisons with the control group (ACD or CKD) was applied. Results are shown as mean ± SEM; *p<0.05, **p<0.01, ***p<0.001

Figure 4: KY1070 increases the sensitivity of EPO in the murine CKD-associated anemia model. Data from experiments addressing ESA sensitivity were fed into this first order linear model. Curves depict concentrations of KY1070 and Darbepoetin alpha calculated from the models, which are required to reach the given increase in the particular hematological parameter (Hemoglobin, Mean Corpuscular Volume, Red Blood Cells). Dots indicate the KY1070 and EPO dose used in our experiments. Model estimations and 95% confidence intervals were calculated with the least square method.

Figure 5: KY1070 leads to an EPO-sparing effect in ACD rats. (A) Experimental setup for assessment of EPO-sparing in ACD rats is shown. (B) Hemoglobin levels in ACD rats over the time of treatment. (C) Number of total possible vs. index consumed EPO doses needed for anemia correction in ACD rats with a EPO monotherapy and KY1070 plus EPO combination protocol is shown. Statistical significance was assessed using Fisher’s exact test; *p<0.01.

Conclusions: anti-BMP6 targeted therapy works synergistically with Erythropoietin treatment in two different models of anemia of chronic disease leading to significantly increased hemoglobin levels, a reduced Erythropoietin need as well as reduced iron overload in organs of the mononuclear phagocyte system. Furthermore, these experiments clearly show that treatment of anemia of chronic disease, being a complex multifactorial disease, benefits from using a combination of diversified approaches to overcome anemia and significantly reduce the dose of each therapeutic.

PosterCast