might be altering CLL cell biology. By testing the expression of several homolog isoforms of UGTs in CLL patient samples and in the CLL cell line MEC-1, UGT2B17 mRNA expression was the only isoform expressed to any significant level. UGT2B17 mRNA expression was also shown to directly correlate with its enzymatic activity. Extending the biology further, UGT2B17 RNA silencing in the MEC-1 cell line was pursued followed by analysis of comparative microarray that identified several important activation markers including CD38 and CD86. Further phenotype associated with UGT2B17 knockdown as related to proliferation, spontaneous apoptosis, or resistance to fludarabine or the active metabolite of cyclophosphamide (4-HC) was not reported.

The current study is provocative and identifies something entirely new and leaves several questions to be addressed. First, is the UGT2B17 protein expressed in CLL and what does its knockdown do to proliferation and cell survival? The expression of UGT2B17 is investigated only at mRNA level in this article and the MEC-1 cell phenotype after knockdown is not described. Second, what factors induce UGT2B17 overexpression in CLL and what are the direct versus indirect consequences of UGT2B17 overexpression? Although gene-expression profile in the UGT2B17 knockdown MEC-1 cells is described in the article that identify several important genes modified including CD38 and CD86, it is uncertain if this is a direct or indirect consequence of UGT2B17 expression. Finally, how do we translate these findings to clinical applications relevant to CLL patients? UGT2B17 expression may be used as a prognostic marker in CLL; however, therapeutic drugs targeting UGT2B17 have not been investigated. Catechins in green and white tea and flavonoids from red wine have been shown to inhibit UGT2B17. These findings may help to design drugs targeting UGT2B17 for CLL therapy. Furthermore, efforts to eliminate induction of UGT2B17 with treatment once the mechanism of this is understood might also be a relevant strategy. Gruber and colleagues are commended for this fine study that provides new leads to pursue progression of CLL and potential drug resistance associated with its treatment.

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Comment on Schmidt et al, page 1200

Inhibiting the hepcidin inhibitor for treatment of iron overload

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In this issue of Blood, Schmidt et al report a lipid nanoparticle (LNP)–based pharmacologic treatment to induce liver–specific Timprss6 silencing and modulate hepcidin, the main regulator of iron homeostasis, in murine models of primary and secondary iron overload.1

Liver iron transcriptionally activates BMP6, which recruits BMP receptors (BMPRs) and HJV for SMAD1/5/8 binding. Binding of HFE to TFR2 positively modulates hepcidin expression through a still unclear mechanism (see figure).2 Hepcidin inhibition occurs in iron deficiency, hypoxia, and erythropoiesis expansion to meet the increased iron requests. TMPRSS6 is the only hepcidin inhibitor whose role has been clearly demonstrated in vivo. TMPRSS6 encodes for the hepatocyte-specific

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type II transmembrane serine protease, matriptase-2, that plays an essential role in erythropoiesis because its inactivation causes iron refractory iron deficiency anemia (IRIDA), characterized by inappropriately high levels of hepcidin both in mice and in humans. In vitro, Tmprss6 cleaves HJV, thus decreasing the BMP-SMAD signaling and inhibiting hepcidin expression (see figure).

Manipulation of ferroportin levels by biomimetic minihepcidins and manipulation of endogenous hepcidin expression are promising approaches to control excess iron deposition.

Schmidt et al report that down-regulation of Tmprss6 by LNP-delivered siRNA efficiently increases hepcidin levels, thus decreasing transferrin saturation and body iron in animal models of iron overload. Using both Hfe<sup>−/−</sup> mice, a model of the most common form of hemochromatosis, and Hbb<sup>β<sub>−/−</sub></sub> mice, which develop a form of thalassemia closely resembling the human β-thalassemia intermediate, the authors show an efficient increase in hepcidin levels after Tmprss6 silencing, with a reduction of iron overload in both models and an improvement of erythropoiesis in β-thalassemia mice.

LNP-based treatment, already used in clinics, is particularly attractive to manage iron overload because the liver, which is central to the regulation of iron homeostasis, is a direct target of LNP due to its high vascularization, permeable endothelium, and high numbers of lipid particle receptors.

Both short- and long-term treatment with LNP-Tmprss6 siRNA efficiently target Tmprss6 and although the transcriptional effect is transient, the resulting hepcidin increase and transferrin saturation decrease are prolonged.

It was previously demonstrated that genetic loss of Tmprss6 reduces systemic iron overload in Hfe<sup>−/−</sup> mice by increasing hepcidin through activation of the BMP-SMAD signaling. Accordingly, Tmprss6 silencing in Hfe null adult mice modulates systemic iron, reducing liver iron concentration, serum iron, and transferrin saturation. However, as already reported in the double Hfe-Tmprss6 KO mice, the long-term (6 weeks) treatment with Tmprss6 siRNA had a cumulative effect on erythropoiesis causing a mild degree of hypochromic microcytic iron deficiency anemia.

Even more striking are the results of Tmprss6 silencing in β-thalassemia mice. β-thalassemias are severe recessive disorders characterized by defective globin chain synthesis, microcytic anemia, and secondary iron overload. In the transfusion-independent β-thalassemia intermediate, anemia results from both ineffective erythropoiesis and decreased red blood cell (RBC) survival. Iron overload is caused by increased iron absorption because ineffective erythropoiesis suppresses hepcidin production. Homozygous inactivation of Tmprss6 in thalassemic mice increases hepcidin and ameliorates iron overload, but surprisingly improves ineffective erythropoiesis.

These results provided a proof of principle for the therapeutic approach proposed by Schmidt et al. Accordingly, pharmacologic activation of hepcidin, achieved by Tmprss6 silencing in Hbb<sup>β<sub>−/−</sub></sub> adult mice, not only corrects iron overload but also ameliorates anemia, as demonstrated by the increased hemoglobin levels, RBC count, and RBC survival, and by the decreased spleen size and serum erythropoietin. Trying to explain the mechanism of improved erythropoiesis, Schmidt et al demonstrate that the amount of membrane-associated globin is decreased in the double mutant mice compared with the thalassemic mice relieving the erythroid damage and death, likely as a result of low iron availability for single erythrocyt.

The effects of Tmprss6 inactivation strengthen and extend previous findings on the beneficial role of limiting iron supply in thalassemia by increasing circulating transferrin or directly enhancing hepcidin levels by transplantation of β-thalassemia bone marrow in Hamp transgenic mice.

However, despite the advantage obtained in β-thalassemia, the LNP-Tmprss6 siRNA treatment impaired erythropoiesis, thus causing hypochromic microcytic iron deficiency anemia in hemochromatosis mice, as shown in Hfe<sup>−/−</sup> mice with genetic inactivation of Tmprss6.

Tmprss6 appears to be a key molecule in the negative regulation of hepcidin, attenuating the BMP6 signaling effect in mice; as such, it could become an important therapeutic target also in humans. In the perspective of applying a similar therapeutic approach in patients, the challenge will be to develop protocols that precisely titrate hepcidin expression to avoid the unwanted side-effect of iron deficiency, as observed in human and murine models with chronic hepcidin activation.

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Comment on Ophir et al, page 1220

Location, location, location: advancing veto cell therapies

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