Conflict-of-interest disclosure: The author declares no competing financial interests. ■

REFERENCES

1. Kobe C, Goergen H, Baues C, et al. Outcome-based interpretation of early interim PET in advanced-stage Hodgkin lymphoma. *Blood*. 2018;132(21):2273-2279.
2. Eichenauer DA, Aleman BMP, André M, et al; ESMO Guidelines Committee. Hodgkin lymphoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2018;29(suppl 4):iv19-iv29.
3. Hutchings M, Loft A, Hansen M, et al. Position emission tomography with or without computed tomography in the primary staging of Hodgkin's lymphoma. *Haematologica*. 2006;91(4):482-489.
4. Gallamini A, Hutchings M, Rigacci L, et al. Early interim 2-[18F]fluoro-2-deoxy-D-glucose positron emission tomography is prognostically superior to international prognostic score in advanced-stage Hodgkin's lymphoma: a report from a joint Italian-Danish study. *J Clin Oncol*. 2007;25(24):3746-3752.
5. Aleman BM, van den Belt-Dusebout AW, Klokman WJ, Van't Veer MB, Bartelink H, van Leeuwen FE. Long-term cause-specific mortality of patients treated for Hodgkin's disease. *J Clin Oncol*. 2003;21(18):3431-3439.
6. Johnson PW. Response-adapted frontline therapy for Hodgkin lymphoma: are we there yet? *Hematology Am Soc Hematol Educ Program*. 2016;2016:316-322.
7. Borchmann P, Goergen H, Kobe C, et al. PET-guided treatment in patients with advanced-stage Hodgkin's lymphoma (HD18): final results of an open-label, international, randomised phase 3 trial by the German Hodgkin Study Group. *Lancet*. 2018;390(10114):2790-2802.
8. Barrington SF, Mikhaeel NG, Kostakoglu L, et al. Role of imaging in the staging and response assessment of lymphoma: consensus of the International Conference on Malignant Lymphomas Imaging Working Group. *J Clin Oncol*. 2014;32(27):3048-3058.
9. Gallamini A, Barrington SF, Biggi A, et al. The predictive role of interim positron emission tomography for Hodgkin lymphoma treatment outcome is confirmed using the interpretation criteria of the Deauville five-point scale. *Haematologica*. 2014;99(6):1107-1113.
10. Kluge R, Chavdarova L, Hoffmann M, et al. Inter-Reader Reliability of Early FDG-PET/CT Response Assessment Using the Deauville Scale after 2 Cycles of Intensive Chemotherapy (OEPA) in Hodgkin's Lymphoma. *PLoS One*. 2016;11(3):e0149072.

DOI 10.1182/blood-2018-09-873596

RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Artuso et al, page 2286

Tfr2 suppression benefits β-thalassemic erythropoiesis

Yelena Z. Ginzburg[1] and Robert E. Fleming[2] | [1]Icahn School of Medicine at Mount Sinai; [2]Saint Louis University School of Medicine

Multiple studies in murine model systems of β-thalassemia have demonstrated that iron restriction improves the ineffective erythropoiesis characteristic of this disorder,[1] the mechanisms of which have not yet been fully elucidated. In this issue of *Blood*, Artuso et al demonstrate that erythroid knockout of transferrin receptor 2 (*Tfr2*) also improves hematologic parameters in β-thalassemic mice.[2] The authors invoke changes in erythropoietin (Epo) sensitivity rather than erythroid iron delivery per se as the underlying mechanism.

Tfr2 is a transmembrane glycoprotein homologous to the classical transferrin receptor *Tfr1*. Whereas *Tfr1* is ubiquitously expressed and is the main mechanism for cellular iron uptake, *Tfr2* has more restricted expression and appears to function as a sensor of circulating iron. *Tfr2* is highly expressed in hepatocytes, where it participates in the regulation of hepcidin expression to modulate iron homeostasis. Loss-of-function mutations in the *Tfr2* gene result in inappropriately low hepcidin production, excess circulating iron, and hemochromatosis (type 3).[3,4] A role for *Tfr2* in erythroid cells was not initially apparent because murine models and human patients with hemochromatosis type 3 have no obvious abnormalities in erythroid parameters. It was later discovered that *Tfr2* complexes with and stabilizes cell-surface Epo receptor (EpoR).[5] In these studies, in vitro *Tfr2* silencing in human erythroid progenitors resulted in a significant decrease in erythroid lineage commitment. Elevated Epo levels in *Tfr2* knockout mice further supported a role for *Tfr2* in upregulating EpoR-mediated signaling. As such, one might predict that loss of erythroid *Tfr2* in vivo would lead to decreased Epo sensitivity and erythroid differentiation. However, subsequent observations in iron-deficient *Tfr2* knockout mice suggest the contrary.

Tmprss6 knockout mice with ubiquitous loss of *Tfr2* have higher red blood cell count, more severe microcytosis, and greater iron deficiency than Tmprss6 knockout mice with hepatocellular-specific *Tfr2* knockout.[6,7] These findings reveal that the additional loss of erythroid *Tfr2* is associated with increased erythropoiesis and suggest a role for erythroid *Tfr2* that is particularly relevant during iron restriction to prevent excess erythropoiesis when hemoglobinization is limited by limited iron. To specifically examine the role of erythroid (independent of hepatocellular) *Tfr2*, Nai et al transplanted *Tfr2* knockout bone marrow into wild-type recipient mice.[8] Bone marrow–specific loss of *Tfr2* resulted in more red blood cells, microcytosis, reduced apoptosis of erythroblasts, and evidence for increased Epo-mediated signaling, particularly in the setting of iron deficiency.[8] In another model system, floxed *Tfr2* mice crossed with Vav-Cre mice demonstrate an apparent block in erythroid differentiation during iron deficiency.[9] The authors suggest that a greater severity of iron deficiency in the different model systems may account for their findings.[8,9]

In the current work, the authors propose that removing *Tfr2* from erythroblasts would enhance Epo sensitivity, decrease erythroid precursor apoptosis, and improve erythropoiesis in β-thalassemia. At the same time, however, erythroferrone (ERFE), an erythroblast-derived regulator of hepcidin, is among the Epo-responsive genes upregulated in mice with erythroid loss of *Tfr2*.[8,9] Because suppression of ERFE appears to be an important contributor to the improvements in iron status in β-thalassemic mice,[10] it is unclear whether enhancing Epo sensitivity in this setting would be beneficial. Theoretically, loss of *Tfr2* could enhance ERFE expression,

further suppress hepcidin, and potentially worsen iron overload in β-thalassemia.

The authors examine the consequences of knockout of erythroid *Tfr2* on erythropoiesis in β-thalassemia by performing a bone marrow transplant of *Tfr2*$^{-/-}$ thalassemic (*Hbb*$^{th3/+}$) cells into *Hbb*$^{th3/+}$ mice.[2] The results demonstrate significantly elevated hemoglobin in *Tfr2*$^{-/-}$ *Hbb*$^{th3/+}$ relative to *Hbb*$^{th3/+}$ mice between 9 and 22 weeks following bone marrow transplant, with a decrease in serum Epo, fewer reticulocytes, and an increased proportion of mature erythroid precursors in the bone marrow. The increased hemoglobin is associated with a decrease in circulating Epo and modestly decreased expression of Epo-responsive genes (including ERFE). Spleen size is unchanged. Furthermore, the authors iron-restrict *Tfr2*$^{-/-}$ *Hbb*$^{th3/+}$ bone marrow–transplanted mice to inquire whether the mechanism of improved hematologic parameters is iron deficiency–driven or whether *Tfr2* loss works by an alternative mechanism. The authors propose that the improvement in hematologic parameters in *Tfr2*$^{-/-}$ *Hbb*$^{th3/+}$ bone marrow–transplanted mice is not the result of limitated available iron.

There are inherent complexities in the relationship between *Tfr2* and EpoR that require accounting for the circulating ligand for *Tfr2* (ie, transferrin isoforms) and for EpoR (ie, Epo concentration). Assessing Epo responsiveness in this setting is challenging, given the change in circulating Epo levels in the *Tfr2*$^{-/-}$ *Hbb*$^{th3/+}$ bone marrow–transplanted mice. Additional experiments are required to fully clarify the expected proportionality between circulating Epo levels and Epo-responsive gene expression. Although RNAseq analysis from spleen identify changes that might be expected with Epo-mediated increased erythropoietic activity, as pointed out by the authors, the analysis is confounded by differences in spleen iron. As such, the conclusion that erythroid parameter improvements in β-thalassemic mice with loss of erythroid *Tfr2* are entirely the result of enhanced Epo-sensitivity will likely require further study.

Based on these interesting findings, the authors suggest a potentially translatable approach by manipulating *Tfr2* in β-thalassemic erythroblasts. However, the therapeutic application of decreased *Tfr2* in erythroblasts may prove to be challenging. The beneficial effect on erythropoiesis in β-thalassemic mice dissipates at 37 weeks posttransplant, possibly as a consequence of critical iron deficiency for erythropoiesis. A better understanding of the basis for this effect, and the effect of transferrin and Epo on the functional properties of erythroid *Tfr2* are needed. Nonetheless, results of *Tfr2* haplo-insufficient *Hbb*$^{th3/+}$ mice suggest the possibility of partial *Tfr2* inhibition using antisense oligonucleotide or small interfering RNA technology. Last, investigating the consequences of *Tfr2* loss in mouse models of β-thalassemia major, rather than intermedia, would be informative.

Conflict-of-interest disclosure: Y.Z.G. serves as a consultant for La Jolla Pharmaceutical Company and has received funding from ApoPharma; R.E.F. serves on the Medical Advisory Board of Protagonist Therapeutics. ■

REFERENCES

1. Gardenghi S, Ramos P, Marongiu MF, et al. Hepcidin as a therapeutic tool to limit iron overload and improve anemia in β-thalassemic mice. *J Clin Invest*. 2010; 120(12):4466-4477.
2. Artuso I, Lidonnici MR, Altamura S, et al. Transferrin receptor 2 is a potential novel therapeutic target for β-thalassemia: evidence from a murine model. *Blood*. 2018; 132(21):2286-2297.
3. Camaschella C, Roetto A, Calì A, et al. The gene TFR2 is mutated in a new type of haemochromatosis mapping to 7q22. *Nat Genet*. 2000;25(1):14-15.
4. Nemeth E, Roetto A, Garozzo G, Ganz T, Camaschella C. Hepcidin is decreased in TFR2 hemochromatosis. *Blood*. 2005;105(4): 1803-1806.
5. Forejtnikovà H, Vieillevoye M, Zermati Y, et al. Transferrin receptor 2 is a component of the erythropoietin receptor complex and is required for efficient erythropoiesis. *Blood*. 2010;116(24):5357-5367.
6. Nai A, Pellegrino RM, Rausa M, et al. The erythroid function of transferrin receptor 2 revealed by Tmprss6 inactivation in different models of transferrin receptor 2 knockout mice. *Haematologica*. 2014;99(6): 1016-1021.
7. Wallace DF, Secondes ES, Rishi G, et al. A critical role for murine transferrin receptor 2 in erythropoiesis during iron restriction. *Br J Haematol*. 2015;168(6):891-901.
8. Nai A, Lidonnici MR, Rausa M, et al. The second transferrin receptor regulates red blood cell production in mice. *Blood*. 2015; 125(7):1170-1179.
9. Rishi G, Secondes ES, Wallace DF, Subramaniam VN. Hematopoietic deletion of transferrin receptor 2 in mice leads to a block in erythroid differentiation during iron-deficient anemia. *Am J Hematol*. 2016; 91(8):812-818.
10. Casu C, Oikonomidou PR, Chen H, et al. Minihepcidin peptides as disease modifiers in mice affected by β-thalassemia and polycythemia vera. *Blood*. 2016;128(2):265-276.

DOI 10.1182/blood-2018-10-876110

THROMBOSIS AND HEMOSTASIS

Comment on Amin et al, page 2298

Postthrombotic syndrome: simple prevention

Susan Solymoss | The McGill University Health Center

In this issue of *Blood*, Amin et al provide data showing early compression therapy post–venous thromboembolism (VTE) to be effective in reducing the incidence of postthrombotic syndrome (PTS) by achieving reduced residual vein obstruction (RVO) on follow-up ultrasound.[1]

PTS is a significant, disabling,[2] and costly[3] complication in up to 50%[4] of patients with VTE. Given that other PTS treatment interventions, such as the use of elastic compression stockings,[5] or early thrombolysis[6] provide limited benefit or are controversial in reducing the incidence of this morbidity, the current findings are potentially of great clinical interest. The scale of benefit appears comparable to the effect of well-controlled anticoagulation therapy on risk reduction of the incidence of PTS.[7]

Variable in incidence, and in severity, PTS is a common and potentially disabling