Iron Through the Prism of Haematology

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Summary

Since the inception of the British Society for Haematology (BSH) 60 years ago, our increased scientific understanding of iron metabolism, together with clinical developments, have changed the way we diagnose and treat its disorders. In the UK, perhaps the most notable contributions relate to iron overload, some of which I will outline from personal experience. Diagnostically, this began with the identification of serum ferritin as a marker of iron overload and continued later with the application of MRI-based imaging techniques for iron and its distribution. Therapeutically, the first trials of both parenteral and oral chelation, which have radically changed the outcomes of transfusional iron-overloaded patients, took place in the UK and are now part of standard clinical practice. During this time, our scientific understanding of iron metabolism at a cellular and systemic level have advanced the diagnosis and treatment of inherited disorders of iron metabolism. There are potential novel applications related to our recent understanding of hepcidin metabolism and manipulation.

Keywords: iron, hepcidin, non-transferrin-bound iron, chelator, haemochromatosis.

I will outline the impact of interactions between clinical haematology and scientific advances in iron metabolism over the last 60 years, since the inception of the BSH. This is not intended as an exhaustive scientific critique but a personal reflection, with highlights of the changes in haematology and research in this area. This will broadly be divided into what came before the 1980s and what has developed since that time.

Reflections on iron metabolism and UK haematology over time

In the 1970s, UK haematologists were beginning to emerge from the laboratory to establish a more clinically based specialty, but the full thrust of malignant haematology as the predominant preoccupation for haematologists had yet to be realised. Clinical resources and academically funded research at this time reflected a continuing interest in red cell haematology, which was then largely independent of big Pharma. By the 1980s, although Sir John Dacie had recently retired from the Royal Postgraduate Medical School, Prof. Lucio Luzatto was maintaining a keen and productive interest in red cell haematology. In Cardiff, Prof. Alan Jacobs had assembled an impressive group of clinical and laboratory haematologists to work on iron metabolism. Prof. Sir David Weatherall, in addition to his other seminal contributions to thalassaemias, was working with Dr (later Prof.) Martin Pippard at Oxford on iron absorption in iron-loading anaemias,[1] while Martin Pippard had also worked with Clem Finch in the USA on the mechanisms of actions of desferrioxamine.[2]

Fundamental and clinical research on iron in the UK had hitherto been carried out mainly in Cardiff, Oxford and London. Ferrokinetic studies, sadly no longer practicable in the UK, were offered by several labs within the National Health Service setting, based on pioneering work from Prof. Clem Finch in Seattle.[3] His centre also acted as a major conduit for the scientific training of future haematologists, both in the UK and worldwide. My original mentor, Prof. Ernie Huehns, had spent time with Clem Finch, as had several of Alan Jacobs' Cardiff group. Prof. Chaim Hersko in Jerusalem had also worked in Clem Finch's lab, among other things on mechanisms of desferrioxamine action. It was Hershko who later developed the concept of plasma non-transferrin bound iron (NTBI).[4] At the time this was under-recognised and this later enticed me into several decades of work on this important concept.[5]

Prof. Ernie Huehns, who was the academic head of the haematology department at University College London, was also keenly interested in theoretical and practical aspects of iron metabolism. This included for example how iron was delivered to cells by the two different iron-binding sites on transferrin – the so called 'Fletcher-Huehns' hypothesis[6] – but he was also interested in iron overload, partly because of the relatively large number of iron-overloaded thalassaemia patients who attended UCH from north London. Desferrioxamine was in use for these patients, initially as an intramuscular bolus after it had first been shown to induce urinary iron excretion by Septhon-Smith in London[7] and later shown

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to be effective in improving survival and preventing liver fibrosis in thalassaemia patents,[8] even when given as short acting intramuscular boluses. Although longer duration subcutaneous infusions were in wide usage by the early 1980s, following the initial lead of Propper from David Nathan's Boston group, followed by evidence from Victor Hoffbrand's group at the Royal Free Hospital, compliance was still a big issue and mortality from myocardial siderosis was common. An orally active chelator was clearly desirable. Ernie was already collaborating with Bob Hider, a medicinal chemist then working at Essex University (now Professor at King's), and had obtained funding from British Technology Group to investigate bidentate 3-hydroxypyridin-4-ones as orally active iron chelators, and for which Bob had obtained a patent.[9] The PhD student assigned to this project, George Kontoghiorghes, a chemistry graduate from Essex University, was working in Ernie's lab at UCL and had identified that several of these compounds were orally effective in mice. George was keen for thalassaemia patients to begin treatment immediately with one of these (1,2-dimethyl-3-hydroxypyridin-4-one, or 'L1' (deferiprone), later known as deferiprone) but before the detailed toxicology that would be required today had been undertaken. Ernie and Bob felt that toxicology was either lacking or too preliminary and that 'L1' might not be the best hydroxypyridinone for clinical development. However George Kontoghiorghes obtained funding from the UK thalassaemia society and took the ongoing work from University College London, to the Royal Free Hospital under Prof Victor Hoffbrand with the intention of starting clinical trials.

By this time in the early 1980s, I was working in Ernie's lab under a MRC fellowship to study the role of lactoferrin as a putative mediator of the hypoferraemia of inflammation (there was none as it turned out). I became involved in iron chelation, firstly because someone was needed to run the thalassaemia clinic at University College London Hospitals (UCLH), and secondly because the cell culture model I had developed for studying iron mobilisation by lactoferrin turned out to be useful for studying hydroxypyridinone chelators[10] as well. When I then looked at the toxicology of hydroxypyridinones in mice, I found dose-dependent depression of the bone marrow by L1, a feature largely absent with other compounds in this series.[11,12] This caused some controversy as George Kontoghiorghes had already started clinical trials with Victor Hoffbrand and indeed found agranulocytosis in some patients.[13] Controversies later developed new dimensions in Canada where initially academically funded trials under Dr. Nancy Olivieri (Canadian MRC) had evolved into pharma-backed trials (Apotex). These issues are beyond the scope of this article but have had a lasting impact throughout the field of red cell haematology. The unconventional and uncomfortable birth of this imperfect but ultimately useful drug would possibly be less likely today, because the regulatory fault-lines between clinician-driven research and pharma are now more clearly defined. I went on to work closely with Ciba-Geigy, which became Novartis, on a novel tridentate chelator, deferasirox, which became known as Exjade. The way in which this, now first-line, orally active chelator was developed contrasted with that of L1, with more controlled data in larger numbers of patients and with the dose-response effects described both therapeutically and toxicologically.[14–16] The scope and thoroughness of this work has led to a knowledge base that has been invaluable in optimising the current clinical use and monitoring of this drug.

Global developments in iron metabolism and treatment

Scientific advances in iron metabolism

While a detailed chronology of developments in the last 60 years is not possible here, I will attempt to outline what I think have been important scientific advances. Prior to the 1980s, understanding of iron homeostasis was, in retrospect, sketchy. The main iron storage protein (ferritin) was well described structurally and functionally, as was the key iron transport protein (transferrin). The uptake mechanisms of iron from transferrin into cells bearing the transferrin receptor (now known as TfR1) by receptor-mediated endocytosis was also reasonably clear, as were changes in transferrin saturation in different disease states. However, the mechanisms regulating iron uptake into the body from diet and the mechanisms modulating transferrin saturation under different disease states were largely unknown. The mechanisms underlying genetic haemochromatosis were also obscure. I shall describe three areas where advances have been most impressive.

Cellular iron homeostasis. Understanding of iron homeostatic regulation at a cellular level was limited but began to accelerate in the 1980s. A key prior concept was the existence of a labile cellular iron pool with regulatory functions on iron metabolism. This concept was known to those of us working on iron chelation from studies on transient chelateable iron pools in Chang cells by Alan Jacob's group.[17] Elegant fluorimetric methods later allowed the tracking of changes in this iron pool under conditions of iron deficiency and excess.[18] The regulatory role of changes in labile cellular iron were shown with the identification of iron-responsive element-binding proteins (IRE-BP) for regulating ferritin synthesis[19] and other aspects of iron metabolism, such as transferrin receptor 1 mRNA (messenger ribonucleic acid) stability and erythroid 5-aminolevulinate synthase mRNA translation.[20] The magnitude of the labile iron pool is sensed by the iron regulatory proteins (IRPs), allowing cellular iron homeostatic mechanisms to come into play. IRP1, now known to be cytoplasmic aconitase, binds to iron-responsive elements (IREs) on RNA in its iron-free state, whereas it functions as cytoplasmic aconitase in its iron-bound state. A

second IRP (IRP2) is a homologue of IRP1 but has no aconitase activity and its regulatory behaviour is controlled by iron-dependent degradation in proteosomes. For the regulation of transferrin receptor 1, under iron-deficient conditions IRP binds to an IRE, 3' to the transferrin receptor-coding region. This stabilises TFR1 mRNA, and thus increases membrane TFR1 expression, correcting cellular iron deficiency. In the case of translational regulation of ferritin synthesis, IRPs bind to IREs on mRNA, 5' to the translated region, thus blocking the translation of ferritin mRNA. IRP-IRE interaction has now been described for other systems where iron availability within cells needs to be adjusted, for example ALA synthase.[21]

Explaining genetic haemochromatosis. While the above advances explained how individual cells regulated their iron requirement, a 'conductor' capable of orchestrating iron uptake and distribution in the body as a whole was still sought. In the 1980s, genetic haemochromatosis was well described clinically but the underlying genetic and molecular causes of the iron overload were largely a mystery. Unraveling of the molecular pathways responsible for the inherited disorders of iron overload progressed alongside and complemented fundamental understanding of iron homeostasis of body iron as a whole. An early, important advance came from the work of Marcel Simon in Rennes who identified the link between the HLA type and generic haemochromatosis.[22] This later acted as a spur for the localisation and identification of the HFE gene by Feder[23] and the subsequent unraveling of the underlying mechanisms for HFE haemochromatosis. Later, the mechanisms of other forms of haemochromatosis such as mutations of transferrin receptor 2, haemojuvelin and ferroportin were explained.[24,25] These can now be identified by genetic diagnosis in iron-overloaded patients who lack the classic HFE mutations, thus fundamentally impacting on how we approach the diagnosis and management of iron-overloaded patients.

The hepcidin ferroportin axis. Remarkably, a conductor has now been found for the disparate players involved in iron-binding, iron-transport, functional-iron and iron-regulatory factors. This not only explains how the body responds to conditions of iron paucity or excess but also provides an underlying explanation for genetically determined disorders of iron metabolism. This is the hepcidin-ferroportin system.[26] In the 1980s, how iron exited from cells was essentially unknown. In my own area of research, the way in which iron exited cells was important to the design of iron chelators, because iron egress from most cell types was typically slow and we found that acceleration of release by chelation required chelators to enter cells, to then bind to labile intracellular iron, before exiting as neutrally charged and relatively lipophilic-chelating molecules.[10] High concentrations of chelators outside such cells therefore had little impact on cellular iron release.

We now know that iron exits from macrophages, hepatocytes and other cells only as reduced Fe^{2+}, through ferroportin channels, and that cell-surface expression of ferroportin is regulated by hepcidin. Hepcidin – a 25-amino acid peptide synthesised mainly in hepatocytes – circulates, binds and causes internalisation with degradation through ubiquitination of membrane ferroportin. The control of hepcidin synthesis is complex but clinically important, through several independent pathways. A cluster of proteins exists on hepatocyte membranes which senses plasma iron status and signals to upregulate hepcidin synthesis. Hepdicin synthesis can be initiated by interactions of transferrin receptor 1 and HFE protein as well as with transferrin receptor 2 on hepatocyte membranes through SMAD signalling. This system acts to sense plasma iron status, possibly responding to transferrin iron saturation, by displacing HFE from the HFE–TFR1 complex.[27]

Transcriptional upregulation of hepcidin occurs mainly through bone morphogenetic proteins (BMPs), of which BMP6 appears to be a key BMP ligand. HFE mutations are associated with deficiency in BMP-SMAD signalling, leading to hepcidin deficiency, consistent with the phenotype of C282Y homozygous HFE haemochromatosis. Unmutated HFE can interact with BMP type 1 receptor (ALK3) at the cell surface to enhance BMP signalling.[28] Another breakthrough occurred when hemojuvelin (HJV), the protein typically mutated in juvenile haemochromatosis, was shown to act as a BMP co-receptor,[29] acting through the SMAD pathway, so that mutations result in inappropriately low hepcidin synthesis and iron overload. Upregulation of hepcidin synthesis can also occur through iron and SMAD-independent pathways, such as IL6-associated inflammation though a STAT3 pathway.

Downregulation of hepcidin synthesis occurs under conditions of hypoxia and iron deficiency. Iron deficiency downregulates hepcidin synthesis though a soluble form of plasma HJV (s-HJV), which is thought to act as a decoy receptor, attenuating BMP signalling to hepcidin. Furthermore, sHJV appears to be negatively regulated by both transferrin-bound iron (holo-Tf) and non-transferrin-bound iron (NTBI). Downregulation of hepcidin synthesis is also responsive to liver Tmprss6-expression (transmembrane protease, serine 6), acting upstream of the BMP–SMAD pathway.[30] The function of Tmprss6 is to cleave HJV, thereby suppressing BMP-SMAD-signalling and decreasing the transcription of hepcidin. Tmprss6 levels are increased under conditions of low cellular iron, thus indicating an iron-sensing role. Mutations of this protease enzyme result in dis-inhibition of hepcidin synthesis, leading to the clinical phenotype of IRIDA (iron-refractory iron deficiency anaemia), hitherto clinically recognised but unexplained. Another key downregulator of hepcidin is the bone marrow-derived erythroferrone which, along with possible other erythron-derived factors such as GDF15, inhibit hepcidin synthesis.[31] This system explains why those marrow disorders most associated with erythron

expansion, and therefore with the highest erythroferrone, are those most associated with excess iron absorption, secondary to hepcidin synthesis inhibition. The clinical applications of these discoveries both diagnostically and therapeutically are yet to be fully realised (see below).

Clinical advances in managing disorders of iron metabolism

As with the previous section, three key areas of change will be highlighted, not as a 'How I Treat', but to give context to evolution of the field with time.

Iron monitoring. Clinical advances have been both diagnostic and therapeutic. Sixty years ago, the determination of iron status relied largely on red cell morphology, transferrin saturation and biopsies of marrow and/or liver. Today these are performed less frequently, due to the development of non-invasive markers, the first of which was serum ferritin. This, developed by Prof. Mark Worwood from the Cardiff group as a marker of iron storage status, was key because iron deficiency is the only cause of low serum ferritin (other than ascorbate deficiency).[32] It also emerged that although less specific, a high serum ferritin was a useful marker of iron overload and could be used to monitor the treatment of iron overload. The development of reliable MRI platforms to measure myocardial iron[33] and liver iron concentration[34] has also allowed better targeting of chelator regimens to fit an individual patient's needs and has led to decreased cardiac complications. A clear link between the risk of heart failure in the subsequent 6–12 months and cardiac T2* has been established, which has allowed a focused prioritisation of chelation intensification.[35]

Managing severe iron overload. Advances in the clinical management of iron overload have been impressive. When I began the thalassaemia clinic at UCLH, barely a month went by without a thalassaemia patient dying of heart failure from iron overload. At that time, we found that regular quantitative monitoring of the ejection fraction [using a multigated acquisition scan (MUGA) at that time], could identify small sequential changes in the left ventricular ejection fraction (LVEF), which were often precursors to heart failure, allowing early intensification.[36] The advent of cardiac MRI monitoring, as described above, allowed a further refinement to identify those patients at highest risk of heart failure.[35] For those patients who developed heart failure, either through under-dosing or under-compliance, the initiation of immediate intravenous desferrioxamine 24 h infusion, followed by long-term DFO infusions though indwelling lines, led to long-term recovery and survival in over 60% of patients at 13 years.[37] This regimen was chosen because we had found that even transient interruption of infusion lead to rebound levels of non-transferrin-bound iron[5] with its potential to directly damage the myocardium. The oral chelator deferiprone was authorised by the EMA in 1999 and Exjade in 2005, which has helped compliance and further improved long-term outcomes in patients as a whole.[38] We performed a randomised study to determine if addition of deferiprone to intravenous desferrioxamine during heart failure improved outcomes. Although we found no difference in short-term outcomes with and without deferiprone under the conditions of the study, we generally recommend addition of deferiprone under these circumstances if there are no contraindications.[39]

Parenteral treatment of iron deficiency. At the time of the inception of the BSH, iron deficiency was treated orally and parenteral treatment was rarely used because of the frequency of 'anaphylaxis' with iron dextran preparations such as imferon. The development of safer intravenous iron preparations, such as Venofer® (iron sucrose) and (Ferinject®) ferric carboxymaltose, has led to a proliferation of research on how anaemias other than classic iron deficiency can be treated. The concept of 'functional iron deficiency' has become fashionable, where the body has sufficient iron stores but cannot utilise them adequately. This concept overlaps with that of the anaemia of chronic disease first described extensively by Cartwright.[40] High hepcidin levels also lead to a decreased response to oral iron therapy[41] and it is conceivable that hepcidin antagonism might also be effective in such circumstances.[42]

Future directions and conclusions

The future in iron metabolism is exciting, both diagnostically and therapeutically, and a key area for both of these is the measurement and therapeutic manipulation of hepcidin. How and when can plasma hepcidin measurement be incorporated into the haematologist's clinical arsenal? Low plasma hepcidin is a feature of common forms of haemochromatosis and could be an important component of the diagnostic pathway, particularly in atypical cases. Informed management of the anaemia of chronic disease or inflammation would also be aided by access to measurement of plasma hepcidin for the National Health Service (NHS), provided a validated standardised test can be agree upon. The therapeutic use of hepcidin manipulation to improve ineffective erythropoieis in thalassaemias or myelodysplastic syndrome (MDS) by increasing hepcidin levels, so restricting iron delivery to the erythron, or conversely to treat the anaemia of chronic disease by decreasing hepcidin levels are exciting therapeutic possibilities.[43] For example, administration of short peptides that mimic the activity of hepcidin have improved ineffective erythropoiesis, anaemia and iron overload in thalassaemic mice.[44] This effect can also be achieved in thalassaemic mice by targeting Tmprss6 mRNA with Tmprss6 antisense oligonucleotides alone or combined with other modalities.[45] Clinical trials have started where exogenous hepcidin mimetics, administered to humans, have been shown to lower

transferrin saturation,[46] although the effect in thalassaemia and MDS is not yet known.

In the field of iron overload and chelation, we need to understand more about how safely to combine chelators to achieve the ferritin values at the low or normal levels and closer to those recommended for maintenance management of HFE haemochromatosis. This is because with modern treatment and monitoring, thalassaemia patients in the UK are now living into their 50s and 60s, but the risk of cirrhosis and hepatocellular carcinoma becomes greater with advancing age. There is still a place for an orally active chelator which requires less close monitoring than currently used chelators, for example of the white count or of renal function. Such compounds are under investigation and it is hoped they may soon become a reality. For conditions such as thalassaemia, the longer-term relevance of advances in these iron-related fields will depend to some extent on advances not directly related to iron metabolism, such as allogeneic bone marrow transplantation and autologous transplantation with gene therapy.

On a still wider organisational perspective, it is gratifying that specialist networks have recently been set up in the NHS for red cell disorders and rare anaemias, which it is hoped will improve the dissemination and application of expertise and new therapies throughout the UK. How the management of haemochromatosis, which has hitherto spanned haematology and hepatology with considerable regional variability, would fit into a network structure will need consideration.

Conflicts of interest

Dr Porter serves on advisory boards for Celgene, Vifor, Silence Therapeutics, Cerus, Bluebird Bio.

References

1. Pippard MJ, Callender ST, Warner GT, Weatherall DJ. Iron absorption and loading in beta-thalassaemia intermedia. *Lancet*. 1979;**2**:819–21.
2. Pippard MJ, Callender ST, Finch CA. Ferrioxamine excretion in iron-loaded man. *Blood*. 1982;**60**:288–94.
3. Finch CA, Deubelbeiss K, Cook JD, Eschbach JW, Harker LA, Funk DD, et al. Ferrokinetics in man. *Medicine*. 1970;**49**:17–53.
4. Hershko C, Peto TE. Non-transferrin plasma iron. *Br J Haematol*. 1987;**66**:149–51.
5. Porter JB, Abeysinghe RD, Marshall L, Hider RC, Singh S. Kinetics of removal and reappearance of non-transferrin-bound plasma iron with deferoxamine therapy. *Blood*. 1996;**88**:705–13.
6. Fletcher J, Huehns ER. Significance of the binding of iron by transferrin. *Nature*. 1967;**215**:584–6.
7. Sephton-Smith R. Iron excretion in thalassaemia major after administration of chelating agents. *Br Med J*. 1962;**2**:1577–80.
8. Modell B, Letsky EA, Flynn DM, Peto R, Weatherall DJ. Survival and desferrioxamine in thalassaemia major. *Br Med J*. 1982;**284**:1081–4.
9. Hider R, Kontoghiorghes G, Silver J. Pharmaceutical Compositions. *UK Patent GB 2118176A*. 1982.
10. Porter JB, Gyparaki M, Burke LC, Huehns ER, Sarpong P, Saez V, et al. Iron mobilization from hepatocyte monolayer cultures by chelators: the importance of membrane permeability and the iron-binding constant. *Blood*. 1988;**72**:1497–503.
11. Porter JB, Hoyes KP, Abeysinghe R, Huehns ER, Hider RC. Animal toxicology of iron chelator L1. *Lancet*. 1989;**2**:156.
12. Porter JB, Hoyes KP, Abeysinghe RD, Brooks PN, Huehns ER, Hider RC. Comparison of the subacute toxicity and efficacy of 3-hydroxypyridin-4-one iron chelators in overloaded and nonoverloaded mice. *Blood*. 1991;**78**:2727–34.
13. Hoffbrand AV, Bartlett AN, Veys PA, O'Connor NTJ, Kontoghiorghes GJ. Agranulocytosis and thrombocytopenia in patients with Blackfan-Diamond anaemia during oral chelator trial. *Lancet*. 1989;**2**:457. (letter)
14. Cappellini MD, Porter J, El-Beshlawy A, Li CK, Seymour JF, Elalfy M, et al. Tailoring iron chelation by iron intake and serum ferritin: the prospective EPIC study of deferasirox in 1744 patients with transfusion-dependent anemias. *Haematologica*. 2010;**95**:557–66.
15. Cohen AR, Glimm E, Porter JB. Effect of transfusional iron intake on response to chelation therapy in beta-thalassemia major. *Blood*. 2008;**111**:583–7.
16. Porter J, Galanello R, Saglio G, Neufeld EJ, Vichinsky E, Cappellini MD, et al. Relative response of patients with myelodysplastic syndromes and other transfusion-dependent anaemias to deferasirox (ICL670): a 1-yr prospective study. *Eur J Haematol*. 2008;**80**:168–76.
17. Bailey-Wood R, White G, Jacobs A. The use of chang cells in votro for the investigation of cellular iron metabolism. *Br J Exp Pathol*. 1975;**56**:358–62.
18. Breuer W, Epsztejn S, Cabantchik ZI. Iron acquired from transferrin by k562 cells is delivered into a cytoplasmic pool of chelatable iron (II). *J Biol Chem*. 1995;**270**:24209–15.
19. Rouault TA, Hentze MW, Dancis A, Caughman W, Harford JB, Klausner RD. Influence of altered transcription on the translational control of human ferritin expression. *Proc Natl Acad Sci USA*. 1987;**84**:6335–9.
20. Constable A, Quick S, Gray NK, Hentze MW. Modulation of the RNA-binding activity of a regulatory protein by iron in vitro: switching between enzymatic and genetic function? *Proc Natl Acad Sci USA*. 1992;**89** (10):4554–8.
21. Cox T, Bawden M, Martin A, May B. Human erythroid 5-aminolevulinate symthase: promotor analysis and identification of an iron responsive element in the mRNA. *EMBO J*. 1991;**10**:1891–902.
22. Simon M, Alexandre JL, Bourel M, Le Marec B, Scordia C. Heredity of idiopathic haemochromatosis: a study of 106 families. *Clin Genet*. 1977;**11**:327–41.
23. Feder JN, Gnirke A, Thomas W, Tsuchihashi Z, Ruddy DA, Basava A, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. *Nat Genet*. 1996;**13**:399–408.
24. Camaschella C, Roetto A, De Gobbi M. Genetic haemochromatosis: genes and mutations associated with iron loading. *Best Pract Res Clin Haematol*. 2002;**15**:261–76.
25. Griffiths W, Cox T. Haemochromatosis: novel gene discovery and the molecular pathophysiology of iron metabolism. *Hum Mol Genet*. 2000;**9**:2377–82.
26. Nemeth E, Tuttle MS, Powelson J, Vaughn MB, Donovan A, Ward DM, et al. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science*. 2004;**306**:2090–3.
27. Schmidt PJ, Toran PT, Giannetti AM, Bjorkman PJ, Andrews NC. The transferrin receptor modulates Hfe-dependent regulation of hepcidin expression. *Cell Metab*. 2008;**7**:205–14.
28. Wu X-G, Wang Y, Wu Q, Cheng W-H, Liu W, Zhao Y, et al. HFE interacts with the BMP type I receptor ALK3 to regulate hepcidin expression. *Bood*. 2014;**124**:1335–43.
29. Babitt JL, Lin HY. The molecular pathogenesis of hereditary hemochromatosis. *Semin Liver Dis*. 2011;**31**:280–92.
30. Poggiali E, Andreozzi F, Nava I, Consonni D, Graziadei G, Cappellini MD. The role of TMPRSS6 polymorphisms in iron deficiency anemia partially responsive to oral iron treatment. *Am J Hematol*. 2015;**90**(4): 306–9.

31. Kautz L, Jung G, Valore EV, Rivella S, Nemeth E, Ganz T. Identification of erythroferrone as an erythroid regulator of iron metabolism. *Nat Genet*. 2014;**46**(7):678–84.
32. Jacobs A, Miller F, Worwood M, Beamish MR, Wardrop CA. Ferritin in the serum of normal subjects and patients with iron deficiency and iron overload. *Br Med J*. 1972;**4**:206–8.
33. Anderson LJ, Holden S, Davis B, Prescott E, Charrier CC, Bunce NH, et al. Cardiovascular T2-star (T2*) magnetic resonance for the early diagnosis of myocardial iron overload. *Eur Heart J*. 2001;**22**:2171–9.
34. St Pierre TG, Clark PR, Chua-anusorn W, Fleming AJ, Jeffrey GP, Olynyk JK, et al. Noninvasive measurement and imaging of liver iron concentrations using proton magnetic resonance. *Blood*. 2005;**105**:855–61.
35. Carpenter JP, He T, Kirk P, Roughton M, Anderson LJ, de Noronha SV, et al. On T2* magnetic resonance and cardiac iron. *Circulation*. 2011;**123**:1519–28.
36. Davis BA, O'Sullivan C, Jarritt PH, Porter JB. Value of sequential monitoring of left ventricular ejection fraction in the management of thalassemia major. *Blood*. 2004;**104**:263–9.
37. Davis BA, Porter JB. Long-term outcome of continuous 24-hour deferoxamine infusion via indwelling intravenous catheters in high-risk beta-thalassemia. *Blood*. 2000;**95**:1229–36.
38. Thomas A, Garbowski M, Ang AL, Shah F, Walker M, Moon J, et al. A decade follow-up of a thalassemia major (TM) cohort monitored by cardiac magnetic resonance imaging (CMR): significant reduction in patients with cardiac iron and in total mortality. *ASH*. 2011;**116**: Abstract 1011.
39. Porter JB, Wood J, Olivieri N, Vichinsky EP, Taher A, Neufeld E, et al. Treatment of heart failure in adults with thalassemia major: response in patients randomised to deferoxamine with or without deferiprone. *J Cardiovasc Magn Reson*. 2013;**15**:38.
40. Cartwright GE. The anemia of chronic disorders. *Semin Hematol*. 1966;**3**:351–75.
41. Camaschella C, Girelli D. The changing landscape of iron deficiency. *Mol Aspects Med*. 2020;**75**:100861. https://doi.org/10.1016/j.mam.2020.100861
42. Wang CY, Babitt JL. Hepcidin regulation in the anemia of inflammation. *Curr Opin Hematol*. 2016;**23**(3):189–97.
43. Parrow NL, Gardenghi S, Rivella S. Prospects for a hepcidin mimic to treat beta-thalassemia and hemochromatosis. *Exp Rev Hematol*. 2011;**4**:233–5.
44. Casu C, Oikonomidou PR, Chen H, Nandi V, Ginzburg Y, Prasad P, et al. Minihepcidin peptides as disease modifiers in mice affected by beta-thalassemia and polycythemia vera. *Blood*. 2016;**128**:265–76.
45. Casu C, Pettinato M, Liu A, Aghajan M, Lo Presti V, Lidonnici MR, et al. Correcting beta-thalassemia by combined therapies that restrict iron and modulate erythropoietin activity. *Blood*. 2020; Online ahead of print. https://doi.org/10.1182/blood.2019004719
46. Richard F, van Lier JJ, Roubert B, Haboubi T, Gohring UM, Durrenberger F. Oral ferroportin inhibitor VIT-2763: First-in-human, phase 1 study in healthy volunteers. *Am J Hematol*. 2020;**95**:68–77.

