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1 **Too much iron: a masked foe for leukemias**

2

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ABSTRACT

39 The role of iron in non-erythroid hematopoietic lineages and its implication in hemato-oncogenesis are
40 still debated. Iron exerts an important role on hematopoietic stem cell transformation and on mature
41 white blood cell differentiation. Iron acts experimentally as an oncogenic cofactor but its exact role in the
42 transformation of the myelodysplastic syndrome into leukemia continues to be discussed. Body iron
43 overload frequently develops mainly as the result of multiple erythrocyte transfusions in patients with
44 leukemia or myelodysplastic syndrome, and, in the latter, as a result of increased ineffective
45 erythropoiesis. Iron overload, especially through the deleterious effects of reactive oxygen species, leads
46 to organ damage that likely impacts the global outcome of patients, especially after hematopoietic stem
47 cell transplantation (HSCT). In these pathological settings (before and after HSCT), oral iron chelation
48 should be considered whenever body iron overload has been firmly established, ideally by magnetic
49 resonance imaging.

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51

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KEYWORDS

53 Iron; leukemia; myelodysplastic syndrome; hematopoietic stem cell transplantation; oral chelation;
54 phlebotomy.

55

ABBREVIATIONS

57 ALL: acute lymphoblastic leukemia

58 AML: acute myeloid leukemia

59 DFX: deferasirox

60 HSC: hematopoietic stem cell

61 HSCT: hematopoietic stem cell transplantation

62 LIC: liver iron content

63 LPI: labile plasma iron

- 64 LPS: lipopolysaccharide
- 65 MDS: myelodysplastic syndromes
- 66 MDS-RS: myelodysplastic syndrome with ring sideroblasts
- 67 MSC: mesenchymal stromal cell
- 68 MRI: magnetic resonance imaging
- 69 NTBI: non transferrin-bound iron
- 70 ROS: reactive oxygen species

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71 **INTRODUCTION**

72 The crosstalk between iron and hematology is illustrated by the well-known relationship between
73 iron and erythrocytes where iron is required in order to sustain erythrocyte production within the
74 bone marrow. Iron is abundantly found in the blood within these erythrocytes, and released during
75 erythrocyte degradation mainly within the spleen (1). The goal of the present review, beyond
76 examining the role of iron in the erythroid lineage, is to focus on the relationship between iron and
77 hematopoietic cells, with a special emphasis on iron overload and leukemias. Systemic iron overload
78 frequently develops in leukemias, mainly due to transfusion therapy (2-4). Another mechanism,
79 involved in myelodysplastic syndromes (MDS) in particular (hematologic malignancies characterized
80 by a risk of progression to acute myeloid leukemia (AML)), is ineffective erythropoiesis (5). Ineffective
81 erythropoiesis, notably through increased bone marrow production of the hormone erythroferrone
82 (6), reduces hepcidin expression (7), leading to increased digestive absorption of iron and increased
83 splenic release of the iron originating from erythrophagocytosis (8). After a reminder on the
84 physiological role of iron in bone marrow stem cells and mature white blood cells, the following
85 pathological aspects will be considered: the body iron load status in leukemia patients both at
86 diagnosis and during medical care, the negative consequences of iron excess on patient outcome,
87 especially after hematopoietic stem cell transplantation (HSCT), and the clinical interest of removing
88 excessive iron in leukemia patients.

89

90 1. IRON: HOMEOSTASIS AND FUNCTIONAL LINKS BETWEEN IRON AND BONE MARROW

91

92 1.1. General iron homeostasis

93 Iron is critical for life due to its major roles in oxygen transport, key enzymatic reactions (such
94 as DNA synthesis or detoxification) and the growth of microorganisms. In vertebrates, iron is
95 abundantly present within red blood cells and in a tiny but functionally essential amount in

96 the plasma; this plasma iron ensures the delivery of iron to the bone marrow primarily for
97 erythrocyte synthesis and to all living cells (9-11) (Fig. 1). Iron is stored in parenchymal cells
98 (essentially hepatocytes where it is sequestered within ferritin molecules) and in
99 macrophages (mainly splenic macrophages). The only physiological source of iron for the
100 body is alimentary, explaining the risk of iron deficiency when this input is limited. In the case
101 of excessive iron input, either by increased intestinal absorption (such as in
102 hemochromatosis) or by excessive parenteral entry (due to multiple transfusions or excessive
103 iron supplementation), there is no effective possibility for the human body to adapt its
104 excretory pathways, which explains the risk of iron overload. This dual vulnerability to iron
105 deprivation and to iron excess explains that iron homeostasis is very finely regulated, both at
106 the systemic and cellular levels. At the systemic level, the key role belongs to hepcidin, the
107 major hormone regulating iron metabolism. Hepcidin is a small peptide of 25 amino acids,
108 essentially produced by hepatocytes, that decreases the plasma iron concentration. The
109 decreasing effect exerted by hepcidin on the plasma iron level is mediated by the degradation
110 of ferroportin, which is the only known protein to export cellular iron into the plasma. This
111 hepcidin-ferroportin duo acts at two main sites, duodenal enterocytes and the spleen. At the
112 digestive level, through the combined action of ferroportin export and hephaestin oxidation,
113 iron is delivered to plasma transferrin which principally targets the bone marrow in order to
114 produce new red blood cells (see Table 1 for names and symbols of the genes). In the spleen,
115 iron originating from erythrophagocytosis is exported into the plasma through ferroportin,
116 oxidized by ceruloplasmin, bound to transferrin and recycled toward the bone marrow. Many
117 factors can regulate hepcidin expression. Body iron load status is a major regulator: a
118 decrease in iron levels (either plasma transferrin saturation level and/or intrahepatocyte iron
119 load level) leads to lower hepcidin production in order to counteract iron deficiency. The
120 reverse phenomenon occurs in the case of iron overload. Inflammation is also an important
121 regulatory factor, in which the IL6-STAT3 pathway is involved to increase hepcidin production.

122 The erythropoietin hormone, mainly produced by the bone marrow, is another important
123 regulatory factor (12, 13). Its increased production in the case of dyserythropoiesis leads to
124 the downregulation of hepcidin expression. This decrease in hepcidin production may
125 override the expected increased hepcidin expression in the presence of coexisting iron
126 excess. At the cellular level, iron homeostasis is mainly ensured at a post-transcriptional level
127 by the iron regulatory proteins (IRP). Schematically, in a context of cellular iron deficiency,
128 IRP1 and IRP2 bind to iron regulatory elements (IRE) located in the 5' or 3' untranslated
129 transcribed region (UTR) of the mRNA involved in the entry (TFR1), storage (ferritin) and
130 export of iron (ferroportin). This leads, through different mechanisms (inhibition of
131 translation or stabilization of transcripts), to increased cellular iron entry and decreased
132 cellular iron storage and egress. The reverse effects occur in the case of cellular iron overload,
133 where IRP1 and IRP2 lose their capacity to bind to IRE. Any acquired and/or genetic
134 abnormality in these regulatory processes may lead to iron-related disorders.

135 Altogether, many of the players involved in iron metabolism are now described at the
136 molecular level. They are transporters, receptors, oxido-reductases, sensors, chelators,
137 regulatory pathway players and hormones. Their interdependency and fine-tuning explain
138 the functional relationship between diverse tissues and iron.

139
140

141 1.2. The functional relationship between iron and bone marrow

142 There is a very close relationship between iron and bone marrow that can be considered through
143 two reciprocal perspectives. On the one hand, there is an iron flux toward the bone marrow that
144 is physiologically required to sustain crucial hematopoietic functions, and which, when excessive,
145 can exert deleterious effects on hematopoiesis (14-16). On the other hand, the bone marrow not
146 only ensures the delivery into the blood of the different hematological cells involved in iron

147 metabolism (the erythrocytes in first line), but also plays a key role in the systemic distribution of
148 iron.

149

150 1.2.1. From iron to the bone marrow: iron is used by all hematopoietic lineages

151

152 1.2.1.1. Iron and bone marrow stem cells: hematopoietic and mesenchymal stromal cells (HSC and
153 MSC)

154 Iron, through its propensity for producing reactive oxygen species (ROS), exerts a determining role
155 on the fate of HSC (14, 15) and its impact depends on the dose of iron. Low ROS levels are required
156 for HSC renewal. ROS levels also fine-tune HSC differentiation: very low levels can hamper HSC
157 differentiation, whereas increasing ROS levels favor their differentiation. On the contrary, very high
158 ROS levels promote stem cell exhaustion and death by causing cytotoxicity through the binding of
159 ROS to lipid membranes, proteins and DNA, promoting deleterious oxidation of these
160 macromolecules. Excessive iron impairs hematopoiesis by inducing apoptosis and cell cycle arrest
161 (17), and decreases the ratio and clonogenic function of hematopoietic stem and progenitor stem
162 cells (18). Iron also plays a role in other cells in the hematopoietic niche. Iron increases the
163 proliferation of human MSCs and accelerates their entry into S-phase (19). In contrast, excess iron
164 negatively impacts the hematopoietic microenvironment, as shown by the delayed hematopoietic
165 reconstitution observed in mice overloaded by iron-dextran and transplanted with bone marrow
166 from untreated mice (20). Moreover, in MDS patients, iron overload has been shown to promote
167 mitochondrial fragmentation of mesenchymal stromal cells (21).

168

169 1.2.1.2. Iron in white cell lineages

170 A short history... In 1952, CB Laurell wrote that “nobody has investigated whether the white blood
171 cells have any function in iron transportation or hemoglobin breakdown, in spite of the fact that
172 leucocytes have a relatively high iron content” in a seminal review entitled “Plasma iron and iron
173 transport in the organism” (22). Twenty-two years later, Summers and Jacobs (23, 24) showed that
174 monocyte ferritin content was approximately seven times higher than that in lymphocytes or
175 polymorphs in normal subjects. They also reported that the ferritin content increased during
176 anemia of chronic disease in lymphocytes, monocytes and polymorphs. Iron uptake by lymphocytes
177 was also greatly increased during iron deficiency anemia. At the same time, Worwood *et al.*
178 reported that leukemia cells had a markedly increased ferritin content (25). We will now consider
179 the present state of knowledge about iron content, function and impact in white blood cells.

180 **Iron and monocytes-macrophages.** Monocytes are known to correspond to circulating
181 macrophages. Iron is required for the differentiation of peripheral blood monocyte precursors into
182 functional macrophages; iron deprivation by desferrioxamine generates macrophages unable to
183 develop a mature phenotype, with an impaired capacity for phagocytosis (23). It is also well known
184 that iron sequestration occurs within macrophages during inflammation. Importantly, interleukin
185 6 (IL-6), which is produced by macrophages in response to pathogen-associated molecular patterns,
186 activates the STAT3 signaling pathway leading to hyperhepcidinemia, and therefore to the
187 degradation of ferroportin, and eventually to hyposideremia. It has also been shown that
188 inflammation can cause hyposideremia through a hepcidin-independent mechanism involving the
189 transcriptional downregulation of ferroportin (26). Depending on their microenvironment and
190 cooperation with lymphocytes, monocytes polarize into active macrophage populations that are
191 subdivided into activated M1 macrophages exerting proinflammatory and antitumor activities, and
192 into activated M2 macrophages that are immunosuppressive and promote tumor activity.
193 Importantly, iron modulates this macrophage polarization, increasing the M2 phenotype and
194 decreasing the M1 proinflammatory lipopolysaccharide (LPS)-induced response (27). In a mouse
195 model of hemochromatosis (a genetic iron overload disease), attenuated inflammatory responses

196 to *Salmonella* infection or LPS were observed compared to non-hemochromatosis control mice.
197 This study demonstrates a novel role of iron in the regulation of macrophage IL-6 cytokine mRNA
198 translation (28). This result is coherent with a previous report that showed that TNF α
199 concentrations were decreased in the supernatants of monocytes from hemochromatosis patients
200 (29). Youssef *et al.* (30) reported that, following increased erythrophagocytosis, splenic
201 macrophages undergo ferroptosis and are replaced by circulating monocytes and local cell division.
202 Haschka *et al.* (31) found that classical (CD14⁺, CD16⁻) and intermediate (CD14⁺, CD16⁺) subsets of
203 human monocytes are involved in clearing non-transferrin-bound iron (NTBI) and damaged red
204 blood cells. Therefore monocytes may play a role in limiting iron toxicity in iron overload conditions.

205 **Iron and dendritic cells.** Kramer *et al.* showed that differentiation from human peripheral blood
206 monocyte precursors into functional dendritic cells requires iron and is dependent on the
207 expression of the cyclin-dependent kinase inhibitor p21 (32). Iron depletion by the chelator
208 desferrioxamine produced undifferentiated dendritic cells unable to stimulate naive allogeneic T
209 lymphocytes. Olakanmi *et al.* showed that lung myeloid dendritic cells acquire iron from various
210 types of extracellular sources (iron-citrate, iron-transferrin and iron-lactoferrin) (33).

211 **Iron and neutrophils.** The positive role of iron on granulopoiesis is suggested by studies in anemic
212 Belgrade (b/b) rats. Indeed, an increased proliferation of granulocytic cells is observed in iron-
213 treated b/b rats (34). Importantly, neutrophils impact iron metabolism during inflammation by
214 secreting lactoferrin (35, 36). Lactoferrin is an iron-binding multifunctional glycoprotein secreted
215 by neutrophils and exocrine glands. During inflammation or infection, the plasma lactoferrin
216 concentration increases through the recruitment of neutrophils (37). The antibacterial and
217 antibiofilm activity of lactoferrin is dependent, although not exclusively, on its iron-binding affinity.
218 However, no evidence has been brought forward regarding a significant impact of lactoferrin on
219 human granulopoiesis (38).

220 **Iron and eosinophils.** To our knowledge, the sole interference of iron with the eosinophil lineage
221 involves the role of iron-loaded lactoferrin. Bovine lactoferrin regulates the pathway implicating
222 the CD11b and CD49d integrins and the MIP-1 α and MCP-1 chemokines in granulocyte-macrophage
223 colony-stimulating factor (GM-CSF)-treated human eosinophils (39).

224 **Iron and lymphocytes.** Transferrin receptor 1 (TFR1)-mediated iron uptake is essential for
225 lymphocyte proliferation (40). Iron also plays a key role in the differentiation of T lymphocytes. The
226 absence of TFR1 in genetically modified mice leads to a total arrest of T lymphopoiesis at a very
227 early stage of maturation, and the function and impact of iron on B lymphocytes is less pronounced
228 (41). A significant increase in lymphocyte number is observed in iron overload situations such as
229 human *HFE*-related hemochromatosis (42). The number of CD8⁺ T lymphocytes negatively
230 correlates with the severity of iron overload in *HFE*-related hemochromatosis (43). Lymphocytes
231 can also internalize iron through pathways involving non-transferrin-bound iron (NTBI) (44, 45),
232 supporting the view that T lymphocytes may play a protective role against the consequences of
233 iron excess. This result is in accordance with the proposal that T lymphocytes act as a first line
234 protective barrier against the deleterious effects of iron excess (46). The cellular localization of iron
235 was not addressed in this study but the authors suggest that it may be stored within ferritin, in
236 agreement with the demonstration that T lymphocytes are able to synthesize H-ferritin (47).

237 In summary, all hematopoietic lineages use iron for proliferation and/or differentiation functions,
238 including the white blood cell lineages. The physiological flux of iron toward the bone marrow exerts
239 proliferative and differentiating effects on HSCs and MSCs. An excess of iron within the bone
240 marrow impairs these processes, mainly through an excessive production of ROS. Regarding mature
241 white blood cells, iron is required for the maturation and phagocytotic activity of peripheral
242 monocytes, the differentiation of monocytes into functional dendritic cells, the proliferation of
243 granulocytic cells, and T lymphopoiesis.

244

245 1.2.2. From the bone marrow to iron: bone marrow is a prominent player in systemic iron
246 metabolism

247
248 Bone marrow, erythrocyte production, and spleen erythrocyte degradation participate in the
249 systemic distribution of iron. Once produced inside the bone marrow, erythrocytes are released into
250 the plasma in order to help oxygenate the body. Circulating red blood cells contain half the total
251 quantity of whole-body iron, *i.e.* approximately 1.5 to 2 g of iron. After 120 days, senescent
252 erythrocytes are degraded within macrophages that release iron from hemoglobin. Iron then re-
253 enters the iron cycle without being excreted.

254 Bone marrow also plays a critical role in systemic iron regulation by modulating the expression of
255 hepcidin (Fig. 2). The main bone marrow factors that may ultimately regulate hepcidin are GDF15
256 (growth and differentiation factor 15) (48), TWSG1 (twisted gastrulation BMP signaling modulator 1)
257 (49), and the hormone erythroferrone (encoded by the gene *ERFE*) (6, 7, 12). During
258 dyserythropoiesis, as seen for instance in thalassemia major or intermedia (50-52) or in MDS (5, 53),
259 those factors secreted by the bone marrow may negatively impact hepcidin synthesis, leading to
260 hepcidin deficiency and, in turn, to hypersideremia and subsequently body iron overload (54-56),
261 even prior to any transfusion. Bair *et al.* (57) showed that allogeneic T-replete stem cell
262 transplantation alters iron homeostasis in non-obese diabetic/severe combined immunodeficient
263 (NOD/SCID) mice, by downregulating liver hepcidin synthesis ahead of upregulating duodenal
264 ferroportin .

265 In summary, we have shown in this first section that iron is an essential player of bone marrow
266 functions through its implication in proliferation/apoptosis and differentiation of all hematopoietic
267 lineages. In return, the bone marrow impacts the systemic homeostasis of iron by the massive release
268 of iron after erythrophagocytosis, the major source of circulating iron, and by modulating two crucial
269 hormones of iron metabolism, hepcidin and erythroferrone. Having described the normal functional

270 relationships between iron and the bone marrow, we next explore pathological aspects of this
271 relationship. In the following sections, we report the causative links between iron and leukemia and
272 their clinical implications.

273

274

275 2. IRON STATUS IN LEUKEMIAS

276

277 2.1. The most suitable methods to assess body iron load in patients

278 Evaluating the total body iron load requires the use of reliable tools. In particular, it may not be
279 sufficient to rely exclusively on plasma ferritin levels in light of the numerous non-iron-related factors
280 susceptible to induce hyperferritinemia (58, 59). In leukemia patients, these factors include
281 inflammation (60), the metabolic syndrome (61) and a high ferritin content within leukemic cells (25).
282 It should also be noted that plasma ferritin levels not only depend on the amount of cellular iron
283 deposition but also on the cellular iron distribution. For equivalent cellular iron concentrations, the
284 corresponding plasma ferritin levels are higher for macrophagic (typically transfusional) iron than for
285 parenchymal (hepatocyte) iron, as typically observed in iron excess related to ineffective
286 erythropoiesis (62). The clinician must keep this difference in mind since it may impact the threshold
287 ferritin values used for clinical decision-making when treating iron overload. Combining plasma
288 ferritin and transferrin saturation levels is especially informative, widely available, and cost-effective
289 for the clinician, although both measurements can be affected by inflammation and must be
290 interpreted with caution in this setting. In addition, ferritin can be affected by liver damage, and
291 transferrin saturation fluctuates diurnally and is also affected by cytolysis (63). However useful these
292 iron-related blood parameters may be, the main message is that it is of utmost importance to rely on
293 the direct visualization and quantification of the tissue iron content (especially in the liver and spleen)
294 in order to rigorously assess the body iron load. Non-invasive approaches such as magnetic resonance

295 imaging (MRI) should be preferentially used (64-67). MRI to determine the liver iron concentration
296 (LIC) has now largely replaced histological assessment by liver biopsy. However, MRI is not widely
297 available nor uniformly done. Bone marrow iron evaluation using MRI, the interest of which has
298 recently been reported for Gaucher disease (68), has not yet been fully evaluated in hematologic
299 malignancies (69). As an illustration of the superiority of MRI evaluation as compared to serum
300 ferritin for body iron load assessment, no hepatic iron overload was found in 13 out of 39 patients
301 who had a serum ferritin level over 1000 ng/mL (70) (71). The bone marrow iron score could be an
302 interesting indicator of secondary iron overload in acute myeloid leukemia patients (72). Dual-energy
303 computed tomography (73) may be a promising technique for the precise assessment of intrahepatic
304 iron distribution in transfusion-dependent patients with hematological malignancies (74).

305 In summary, the assessment of body iron stores is a two-step process. First, together with the
306 hemoglobin level, the reticulocyte count, red blood cell morphology, serum ferritin and transferrin
307 saturation must be checked. If both the ferritin and transferrin saturation parameters are normal,
308 reflecting an absence of iron excess, no further investigations are required. If they are elevated, and
309 after having checked for possible confounding factors, a direct evaluation of the tissue iron load is
310 required. MRI has currently become the preferred approach given its non-invasive nature, provided
311 it is available and affordable. Among the various methods, the signal intensity ratio method (64, 75)
312 is quite promising as it enables the direct assessment of the hepatic and splenic iron load without
313 requiring specific MRI equipment and provides free interpretation.

314

315 2.2. Iron status at diagnosis

316 It remains difficult to obtain precise data on the body iron status at the time of diagnosis since the
317 iron load check-up is usually done during or after chemotherapy and often from the perspective of
318 HSCT. However, Vag *et al.* (76) reported that LIC measured by MRI was close to normal in eight
319 patients with acute leukemia for whom the determination was performed within 10 days after

320 therapy initiation. Moreover, the study by Moafi *et al.* (77), based on a histological evaluation of the
321 bone marrow iron stores, reported that, when 30 acute lymphoblastic leukemia (ALL) patients at
322 diagnosis were compared with 30 control subjects, the bone marrow iron score did not differ
323 significantly whereas a significant elevation appeared in leukemia patients after one year of
324 chemotherapy.

325 Altogether, those limited data suggest that the iron status at diagnosis of acute leukemia is not
326 different from control subjects. However, larger cohorts and prospective studies are required to
327 definitively conclude of this point.

328

329 2.3. Body iron load increases in leukemias, mainly due to iron input from blood transfusions

330

331 Body iron status has mostly been evaluated while leukemia was being treated. Altogether, the
332 number of studies remains limited when referring to the direct evaluation of body iron stores using
333 appropriate techniques (MRI or histology). Halonen *et al.* reported that 30 children with acute
334 lymphoblastic leukemia (ALL) of whom 19 (63%) had moderate iron overload as assessed by the total
335 iron score (78, 79). Vag *et al.* (76) showed that the mean LIC of 15 children (nine ALL, six AML (acute
336 myeloblastic leukemia)) was significantly increased compared to non-leukemic children. The mean
337 LIC was then correlated with the number of transfusions. Armand *et al.* (80) found that 85% of 48
338 patients with AML (n=29), ALL (n=11) or MDS (n=8) had a LIC value above the upper limit of normal
339 and, in 42% of patients, significant iron excess corresponded to more than three times the upper
340 limit of normal. Again, iron overload was correlated with the number of transfusions. In Trottier *et*
341 *al.*'s cohort (4) consisting of 37 leukemia case, eight out of 10 ALL patients and all of the AML patients
342 had an iron overload. A poor relationship was found between LIC and transfusion history. Maximova
343 N *et al.* performed an MRI-based evaluation of multiorgan iron load in pediatric patients who
344 subsequently underwent hematopoietic stem cell transplantation (HSCT) (69). Among these patients,
345 21 had ALL and eight had AML. Thirteen ALL and all eight AML patients had a significantly elevated

346 LIC (>2-3 times the upper limit of normal) after HSCT. LIC was not found to be a reliable indicator of
347 total body iron stores as indicated by marked discrepancies between LIC data and the iron estimation
348 in the spleen or bone.

349 On the whole, for the previously mentioned studies, 49% (91/185) of the ALL or AML patients had
350 significant iron overload before HSCT. In these hematologic malignancies (2, 3), transfusions
351 appeared to be the main cause of the iron burden.

352

353 3. IS IRON A PREDISPOSING FACTOR TO LEUKEMIA?

354

355 3.1. The potential cellular toxicity of iron

356 Physiologically, most iron in the body is incorporated within molecular moieties that make it redox-
357 inactive. For instance, iron is bound to plasma transferrin that ensures its transport, and iron is stored
358 within cells in ferritin macromolecules that act as sponges preventing “unbound” or “free” cytosolic
359 iron from being toxic. At the cellular level, a large quantity of iron is incorporated into heme as part
360 of the hemoglobin molecule in erythroid cells, or myoglobin in muscle cells.

361 However, this protective process can be overridden in iron excess. This holds true when the plasma
362 transferrin saturation with iron is over 45%, with the appearance of non-transferrin-bound iron
363 (NTBI), and especially when the transferrin saturation becomes higher than 75% with the appearance
364 of labile plasma iron (LPI). NTBI is thought to be under the biochemical form of low-molecular weight
365 complexes (such as citrate and acetate), whereas LPI represents the potentially toxic form of
366 circulating iron, defined by its propensity to generate reactive oxygen species (ROS), capable of
367 damaging the membranes of the cells, intracellular organelles and nuclei (17, 81, 82).

368 In summary, while iron is physiologically redox-inactive, iron excess, characterized by a plasma
369 transferrin saturation with iron over 45%, is toxic for the cells.

370

371

372 3.2. Experimental data converge to indicate the promoting role of iron on leukemia development

373

374 The promoting role of iron on tumor development has been explored in numerous types of cancer
375 (83) and the proliferative effect of iron is well documented (84, 85). Iron is also known to favor
376 genetic instability (82). Focusing on leukemia models, the following data were reported. Inoculated
377 L1210 cells (a mouse lymphocytic leukemia cell line) proliferated more in mice injected with iron than
378 in non-iron-treated control animals (86). Further experimental data shed some light on the possible
379 mechanistic impact of iron on tumorigenesis. Iron has been shown to reduce tumor suppressor p53
380 activity (87); however, two types of data do not fit with a promoting role of iron on tumorigenesis
381 through decreasing p53: on the one hand, iron favors ferroptosis, whereas the mechanism by which
382 p53 could sometimes favor tumorigenesis has been reported to be its suppressing effect on
383 metabolic stress-induced ferroptosis (88); on the other hand, p53 has been shown to decrease the
384 NTBI transporter ZIP14 (also known as SLC39A14) (89), leading to decreased NTBI entry into tumor
385 cells and subsequently to decreased iron-related cell death. The precise role of iron on p53 regulation
386 and its effect needs further clarification. The Eltrombopag effect further illustrates the promoting
387 role of iron on leukemia cell growth. This compound is an oral small-molecule thrombopoietin
388 receptor (TPO-R also known as MLP) agonist used for treating chronic immune thrombocytopenic
389 purpura. However, independently of its TPO-R mediated effect, Eltrombopag is able to inhibit the
390 growth of human and murine leukemia cell lines by inducing differentiation and by slowing cell
391 division through blocking the cell cycle in the G1 phase. Interestingly, Eltrombopag decreased the
392 iron content within leukemic cells in a dose-dependent manner. Preloading cells with iron also
393 resulted in a rescue from the anti-proliferative and differentiation-inducing effects of Eltrombopag,
394 suggesting that the antileukemic effect of Eltrombopag is mediated through intracellular iron content
395 (90). Iron deprivation has been reported to induce monocyte differentiation of AML cells (91) and,
396 more generally, cellular iron-binding may be an interesting approach to counteract cancer through
397 the involvement of various signaling pathways (92). Finally, dihydroartemisinin has been reported to

398 induce acute myeloid leukemia cell death by inducing ferroptosis through the autophagy-dependent
399 degradation of ferritin (93).

400 In summary, experimental preclinical data converge to indicate the promoting role of iron on
401 leukemia development, and conversely, the role of iron chelation/deprivation to counteract cancer.

402
403 3.3. Is systemic iron overload in non-malignant hematological conditions a predisposition to
404 leukemia?

405
406 3.3.1. Lessons from hemochromatosis, the archetype of systemic iron overload

407 Hemochromatosis is a genetic disorder characterized by diffuse body iron overload caused by
408 increased intestinal iron absorption, which itself is related to hepcidin deficiency (10). *HFE* is by far
409 the most frequently mutated gene found in hemochromatosis, with *C282Y HFE* mostly present in
410 Caucasians. It is still being debated whether or not *HFE*-related genetic susceptibility to
411 hemochromatosis, and therefore iron excess, could favor the development of leukemia. A causative
412 link between the *C282Y HFE* mutation and ALL has not been clearly proven (94, 95) and the same
413 holds true for an association with the haplotype HLA-A3, known to be closely associated with the *HFE*
414 gene (95). However, in a US cohort of 161 childhood ALL cases, Kennedy *et al.* (96) reported that the
415 risk of ALL was not only associated with *C282Y* and *H63D HFE* variants and *S142G TFR1* (transferrin
416 receptor 1) variant, but also to some SNPs in other iron regulatory genes (*SLC11A2* and *TMPRSS6*
417 genes). Although no data were given on the iron status of this cohort, these results strengthen the
418 view that iron overload mediated by genetic variants could contribute to a risk of ALL. No *HFE*
419 association has been reported with AML (97). It should be noted that only exceptional cases
420 associating *HFE*-related hemochromatosis and ALL have been reported (98).

421 Altogether, a genetic correlation has been found between some variant genes involved in iron
422 metabolism and ALL. The impact of the level of the iron burden is not clearly demonstrated as a cause
423 of those ALL. Moreover, in our opinion, despite the absence of an established link between

424 hemochromatosis (iron overload) and leukemia, it is important in clinical practice to check *HFE*
425 mutations and, if negative, *non-HFE* mutations, in every leukemic patient with marked iron overload
426 exhibiting the characteristic profile of hepcidin deficiency (increased plasma transferrin saturation
427 associated with parenchymal, especially hepatocyte, iron overload (10, 52)) so as not to miss a
428 coincidentally associated hemochromatosis (10).

429
430 3.3.2. Lessons from sickle cell disease and thalassemia, archetypes of secondary iron overload
431 Sickle cell disease and thalassemia are inherited disorders treated with chronic blood transfusions
432 resulting in diffuse body iron overload. Here again, very little data are available on the promoting
433 role of iron overload in leukemia transformation in these diseases. In sickle cell disease, an over two-
434 fold increase in the risk of leukemia, mostly AML, has been reported in California (99) although it is
435 still unclear if iron overload was one of the risk factors in this study. In thalassemia, only exceptional
436 cases of leukemia have been described, suggesting a coincidental occurrence of these conditions
437 (100).

438
439 3.4. Is iron overload a factor favoring the transformation of myelodysplastic syndrome (MDS) into
440 leukemia?

441 MDS represents a particularly valuable experimental model and clinical situation for studying the role
442 of iron as a leukemia predisposing factor since MDS evolves towards AML transformation in
443 approximately one third of cases (101) (102). MDS encompasses a heterogeneous group of acquired
444 myeloid disorders leading to ineffective hematopoiesis with peripheral cytopenia(s) (103), among
445 which anemia is the most frequent. Patients with MDS can develop iron overload through repeated
446 blood transfusions and/or as a consequence of dyserythropoiesis.

447
448 3.4.1. Iron-related oxidative stress in MDS

449

450 It is now clear that MDS patients present increased oxidative stress that is further increased by iron
451 overload (104, 105), even in transfusion-independent cases (105). It has been proposed (106) that
452 iron deprivation by low-dose deferasirox can improve erythropoiesis in MDS. It should be noted,
453 however, that iron overload inhibited the proliferation of erythroid progenitor cells in MDS whereas
454 the myeloid compartment was not affected (107). All of the blood marrow cell types in 27 MDS
455 patients exhibited high ROS and low glutathion levels compared to 12 controls, with some correlation
456 with overall survival (108). Interestingly, Ghoti *et al.* found a correlation between ROS and serum
457 ferritin levels in the erythrocytes and platelets of low-grade MDS patients (109). Increased oxidative
458 DNA damage was found in MDS patients both on bone marrow cells (110) (however without
459 correlation with serum ferritin) and on peripheral blood mononuclear cells (111), with a protective
460 effect of oral iron chelation. Importantly, low-risk MDS patients have been reported to present the
461 highest ROS levels (108). Using NHD13 transgenic mice, a murine model of MDS, Chung *et al.* showed
462 that ROS may contribute to the progression of MDS to AML through ineffective DNA repair and
463 increased mutation frequency (112). Given the close relationship between iron and ROS, it is possible
464 that this progression was at least partly related to an iron effect (iron exposure, however, was not
465 used in this experiment).

466
467 3.4.2. The impact of dyserythropoiesis and erythroblastic mitochondrial iron overload in MDS to AML
468 transformation

469
470 Iron overload due to dyserythropoiesis is mainly found in MDS with ring sideroblasts (MDS-RS), a
471 subgroup characterized by erythroblastic mitochondrial iron overload and a high frequency of
472 somatic mutations in the spliceosome gene *SF3B1* (113). Mean hepcidin levels are heterogeneous
473 across the different MDS subtypes, with the lowest levels in refractory anemia with ring sideroblasts
474 (now classified as MDS-RS with single dysplasia), which present the highest plasma NTBI levels (114-
475 116). Thus, MDS-RS patients have inappropriately low hepcidin levels, a typical feature of iron-

476 loading anemia (117). Most patients with MDS-RS with single dysplasia are stratified into the lower-
477 risk groups by the revised IPSS (118), pointing out that abnormal mitochondrial iron accumulation
478 may not be a strong factor favoring leukemic transformation.

479

480 3.4.3. The impact of transfusional systemic iron overload in MDS to AML transformation

481

482 It has been reported that chronic red blood cell transfusions induce an iron overload that impacts
483 the survival of MDS patients (119). In a retrospective study of 467 MDS patients, the leukemia-free
484 survival of transfusion-dependent patients was significantly inferior to that of patients not requiring
485 transfusions, raising the possibility that transfusional iron overload may increase the risk of AML
486 transformation to leukemia (120). However, in a recent Brazilian study, hepatic iron overload,
487 measured by MRI and found in two-thirds of transfused and non-transfused MDS patients, was
488 associated with a lower overall survival, but was not correlated with an increased risk of AML
489 transformation (121). These results should nevertheless be considered in the light of the small cohort
490 size and the heterogeneous clinical status of the patients.

491 Although clinical data converge to indicate that blood transfusion dependency and an increasing
492 number of transfusions favor shorter overall survival and increase leukemic transformation in MDS
493 patients, it remains difficult to ascertain that these deleterious effects are related to iron overload
494 itself (5). Limiting and confounding factors may include: i) the fact that transfusion dependency could
495 reflect the overall disease severity; ii) the impact of comorbidities that are frequent in these elderly
496 patients; iii) the almost exclusive use of plasma iron parameters (especially ferritin levels) to estimate
497 body iron excess, which does not consistently reflect the iron store (as discussed in 2.1); and iv) the
498 risk of selection bias related to the possible proposal of chelation therapy to patients with a better
499 prognosis. Evaluating the impact of iron depletion by chelation therapy represents a “reverse”
500 approach to understanding the effect of iron excess on MDS to AML transformation. In this regard,
501 the repeatedly-found beneficial effect of iron chelation therapy (106, 122-126) represents an

502 important argument in favor of iron overload being responsible for promoting AML. In particular, the
503 TELESTO trial by Angelucci *et al.* (127), which is the sole prospective study randomizing deferasirox
504 vs. placebo in low-risk MDS patients, reports a favorable effect of iron chelation on event-free
505 survival, the rate of cardiac and hepatic events and the transformation to AML. However, it should
506 be kept in mind that part of the beneficial effect of the iron chelator deferasirox may be due not only
507 to iron chelation, but to well-documented associated hematopoietic effects (106, 125, 128-134).
508 Finally, regarding the specific effect of iron chelation on AML transformation, the Spanish Iron2 study
509 (126) identified a beneficial effect, although the German registry did not (123).

510
511 In summary, while systemic iron overload observed in hemochromatosis and sickle cell disease is not
512 clearly shown as a predisposing condition to leukemia, data are clearer for MDS. Some experimental
513 studies suggest that iron may be a factor facilitating MDS to AML transformation, and bioclinical data
514 show that there is an increase in ROS production in MDS likely causing oxidative stress, including
515 oxidative DNA damage. Clinical data are still lacking, however, to firmly establish the causal
516 relationship between iron overload and leukemia transformation in MDS.

517

518

519 4. THERAPEUTIC ASPECTS RELATED TO IRON OVERLOAD IN LEUKEMIA

520

521 4.1. Leukemia treatment and body iron burden

522

523 4.1.1. The impact of chemotherapy on iron metabolism: the transient appearance of NTBI

524 Chemotherapy impacts iron metabolism through two mechanisms. The first one is that
525 chemotherapy causes iron excess when red blood cell transfusions are needed to counteract
526 chemotherapy-related anemia. A high amount of iron bound to hemoglobin is brought together

527 with massive erythrocyte supplementation. This iron, released from the erythrocytes during
528 erythrophagocytosis, will not be extracted out of the body since no specific iron excretion exists,
529 and will be stored within the organism. This corresponds to a net addition of iron. Another
530 mechanism is that chemotherapy favors the appearance of plasma NTBI. Harrison P *et al.* were the
531 first to report the presence of NTBI during chemotherapy, often concomitant with neutropenia
532 (135). In 23 out of 25 patients, labile plasma iron (LPI) levels increased 48 hours after the start of
533 conditioning pre-autologous HSCT, with a peak before cell infusion and a return to the normal range
534 at engraftment (136, 137). Studying a cohort of 30 patients with acute leukemia (16 AML and 14
535 ALL), Belotti *et al.* (138) showed that this peak of NTBI was found for all subsequent high-dose
536 chemotherapy courses. The appearance of NTBI during chemotherapy may be essentially due to
537 the massive iron release from the degradation of hemoglobinized bone marrow cells leading to
538 elevated transferrin saturation (139). The presence of NTBI can also be explained on the one hand
539 by a decrease in NTBI uptake by the erythroid cells, given that such uptake has been demonstrated
540 in rat erythroid cells (140). Furthermore, it could be related to hypohepcidinemia due to the
541 absence of erythropoiesis. These different mechanisms can act simultaneously. Finally, high NTBI
542 levels were associated with a higher risk of sepsis (138). It should be noted that susceptibility to
543 infection may be, by itself, an important factor in terms of the survival of patients with leukemia
544 undergoing chemotherapy.

545 As a whole, chemotherapy causes iron excess when red blood cell transfusions are provided and
546 favors the appearance of plasma NTBI.

547
548 4.1.2. The prognostic impact of body iron burden on hematopoietic stem cell transplantation
549 (HSCT)

550 Numerous studies have concluded, overall, that high serum ferritin levels prior to HSCT are a
551 predictive factor of poor prognosis both in terms of morbidity and mortality. However, as previously

552 mentioned, hyperferritinemia clearly does not only reflect body iron overload, as this is notably an
553 inflammation marker (see reviews by Moukalled *et al.* (101), Isidori *et al.* (15); Leitch *et al.* (141),
554 Wang *et al.* (142), and Bertoli *et al.* (60)). This is why, in the present review, only studies using a
555 direct assessment, by MRI or histological studies, of the tissue iron concentration will be
556 considered.

557 A meta-analysis performed by Armand *et al.* (143) of four prospective studies (144) (4, 145, 146),
558 involving 144 AML, 90 ALL and approximately 50 MDS patients, concluded that iron overload was
559 not related to either overall survival or to non-relapse-mortality. However, one of the four studies
560 (145) was not in agreement with the other three, and meta-analysis bias due to a sample size issue
561 could not be totally excluded. Primarily, Wermke *et al.* (147) coordinated the first prospective,
562 German multicenter observational study (the ALLIVE study) assessing the relevance of
563 pretransplantation body iron overload measured by serum ferritin, transfusion burden, enhanced
564 LPI (defined as biologically active iron) and LIC (determined by MRI) in a cohort of hematopoietic
565 malignancies (92 AML and 20 MDS patients) undergoing HSCT. The results indicated that LIC values
566 more than three times the upper limit of normal were significantly associated with increased non-
567 relapse mortality. It is still a possibility that this impact could be restricted to patients having
568 undergone a reduced-intensity conditioning regimen (this case concerns 83% of the studied
569 patients). Moreover, the ALLIVE study showed a significantly increased incidence of non-relapse
570 mortality in patients exhibiting a raised baseline enhanced-NTBI concentration. Moafi *et al.* (77)
571 concluded that high levels of bone marrow iron were associated with a poor response to treatment
572 and to the risk of relapse.

573 To summarize, body iron overload assessed by direct methods has a predictive value for poor
574 survival after HSCT. However, the predictive value of increased serum ferritin levels, which reflect
575 not only body iron excess but also other mechanisms, of which the foremost is inflammation, may
576 be stronger.

577

578 4.2. The impact of iron removal during leukemia treatment

579 4.2.1. Iron chelation

580 We will focus on the results obtained using oral iron chelation, especially deferasirox (DFX). Sivgin
581 *et al.* (148) retrospectively investigated 80 patients including 45 AML and 18 ALL patients for whom
582 the pretransplant serum ferritin levels were ≥ 1000 ng/mL. Thirty-seven patients were given DFX
583 and compared to 26 patients who were phlebotomized due to DFX side effects or compliance
584 problems. Overall survival and disease-free survival were significantly better in the DFX group. The
585 first prospective multicenter clinical trial of DFX in adult allogeneic HSCT was carried out in Spain
586 (149). Thirty patients (including 17 AML patients and one ALL patient), with transfusional iron
587 overload (serum ferritin ≥ 1000 ng/mL or ≥ 20 units of packed red blood cells; the LIC assessment
588 was assessed by MRI, depending on equipment availability) received DFX at a starting dose of 10
589 mg/kg for 52 weeks or until the serum ferritin level was less than 400 ng/mL on two consecutive
590 occasions. There were no severe drug-related adverse events. A significant reduction in ferritinemia
591 was observed from baseline to 52 weeks (1444 to 755 ng/mL) in the intent-to-treat population. LIC
592 was also significantly reduced for the seven patients for whom basal and final LIC (at 52 weeks)
593 could be obtained. The multicenter German study (DE02) (150) assessed the safety and efficacy of
594 DFX in 76 recipients of allogeneic HSCT (comprising 52 AML) who started at a dose of 10 mg/kg with
595 an escalating design up to 20 mg/kg. The median exposure was 330 days. Seventy-one percent of
596 the patients experienced drug-related adverse events (increased blood creatinine, nausea and
597 abdominal discomfort) leading to occasional discontinuation. The median compliance rate was >
598 80%. A significant decline in serum ferritin was observed (from 2045 to 957 ng/mL) and a negative
599 iron balance was obtained in 84% of patients.

600 In summary, these studies indicate that DFX is effective for iron chelation therapy after HSCT, with
601 a manageable safety profile. Gastrointestinal side effects may be reduced with the new DFX film-
602 coated tablet formulation (151, 152).

603
604 4.2.2. Phlebotomy
605 Iron removal by phlebotomy is the alternative to iron chelation for therapeutic iron removal. As to
606 the impact of phlebotomies in leukemia patients, most studies have involved patients after
607 allogeneic HSCT. We will focus here on those studies that used a direct evaluation of tissue iron
608 load by liver biopsy (153, 154) or imaging techniques (MRI (155) and, in one study, SQUID
609 (superconducting quantum interference device) (156)). All of the studies converged to conclude
610 that phlebotomy was a safe and well-tolerated procedure. A significant reduction in the liver iron
611 concentration (and serum ferritin) was obtained. A single retrospective study reported that DFX
612 was more effective on iron excess than phlebotomy. However, only serum ferritin levels were
613 evaluated (148).

614 On the whole, further clinical studies should be carried out to ascertain if the long-term prognosis
615 after HSCT is favorably influenced by decreasing iron excess, through chelation or phlebotomy.
616 Irrespective of the therapeutic method, it should be emphasized that it is highly recommended to
617 avoid foods containing high amounts of iron since this is a natural and cheap way to counteract
618 intestinal iron absorption.

619
620 **CONCLUSION**

621 Close functional interactions exist physiologically between iron and bone marrow that concern the
622 leukocyte lineage as well as the erythroid cells. From a pathological point of view, iron acts as a co-
623 carcinogenic factor that experimentally promotes leukemia cell growth, whereas clinical data
624 promoting the role of iron in MDS transformation is still under debate. Body iron overload, best

625 assessed by direct MRI quantification, is usually not increased at the time of leukemia diagnosis; it
626 increases significantly during medical care, especially due to multiple transfusions. Iron overload,
627 notably through the damaging effects of NTBI, which is more likely to appear in the plasma with
628 chemotherapy, significantly contributes to a poor prognosis after HSCT. Hyperferritinemia only
629 partially reflects excess iron in the body but seems to be an interesting prognostic factor since it
630 also occurs in inflammation. Iron removal, mostly by oral chelation, must become the standard of
631 care whenever significant body iron overload has been firmly established.

632 **FUTURE CONSIDERATIONS**

633 In our opinion, increased awareness of the importance of diagnosing body iron excess in leukemia
634 patients remains a key objective. Further clinical trials evaluating the long-term prognostic effect
635 of iron removal by oral chelation are warranted, in principle, but may raise practical difficulties for
636 recruiting patients. Strategies to target iron removal from specific tissues should be considered.

637

638 **PRACTICE POINTS**

639 • Iron experimentally promotes leukemia cell growth; however, clinically, the promoting role of iron
640 overload in the transformation of MDS into leukemia is still being debated.

641 • The assessment of body iron status in leukemia patients requires the direct visualization and
642 quantification of iron excess, especially by magnetic resonance imaging (MRI).

643 • Iron overload, mainly due to transfusions, is likely to contribute to a poor prognosis after
644 hematopoietic stem cell transplantation.

645 • Hyperferritinemia is an interesting overall indicator of a poor prognosis; this parameter reflects
646 the inflammatory status and, albeit partially, the body iron burden.

647 • Iron removal by oral chelation or phlebotomies is well tolerated and acts efficient on the body
648 iron burden; its favorable effect on the long-term prognosis is uncertain.

649

650 **RESEARCH AGENDA**

651 • Further clinical trials investigating the impact of iron on MDS transformation, with appropriate
652 measurement and follow-up of iron burden.

653 • Exploration of the promoting role of iron in the development of
654 myelodysplastic/myeloproliferative syndromes from hematopoietic stem cells especially by using
655 appropriate transgenic mouse models.

656

657

658

659 **CONFLICT OF INTEREST**

660 PB has been an occasional consultant and member of advisory board for Novartis. No links of interest
661 for the other authors in the frame of the present article.

662

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1057

1058 **FIGURE LEGENDS**

1059

1060 **FIGURE 1.** Systemic iron homeostasis. Plasma iron comes from the duodenum (digestive iron absorption)
1061 and the spleen (iron originating from erythrophagocytosis). Once in the plasma, iron is bound to
1062 transferrin (TF) and mainly sent to the bone marrow for the production of new red blood cells. The “bone
1063 marrow-spleen-bone marrow” cycling process represents, quantitatively, every day, about 20 times the
1064 amount of iron coming from digestive absorption. If plasma iron increases, hepcidin production by the
1065 liver is increased, leading to decreased activity of ferroportin both at the duodenum and spleen levels,
1066 which in turn decreases plasma iron (the reverse regulation occurs in case of decreased plasma iron).

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1069 **FIGURE 2.** Bone marrow and iron metabolism. Due to iron toxicity, malignancy or chemotherapy, the
1070 functionality of bone marrow stem cells (BMSC) can be altered, leading, for the erythroid cell lineage, to
1071 dyserythropoiesis, which in turn causes an increased production of various factors including GDF15 and
1072 the hormone erythroferrone. These factors lead to a decrease in hepcidin production, then to an increase
1073 of plasma iron and, through non-transferrin bound iron, to iron deposition into various parenchymal cells
1074 (in first line the hepatocytes): it may lead to body iron overload (this iron excess can increase hepcidin but
1075 the increasing signal remains dominated by the reverse impact of dyserythropoiesis (and anemia itself)).

1076

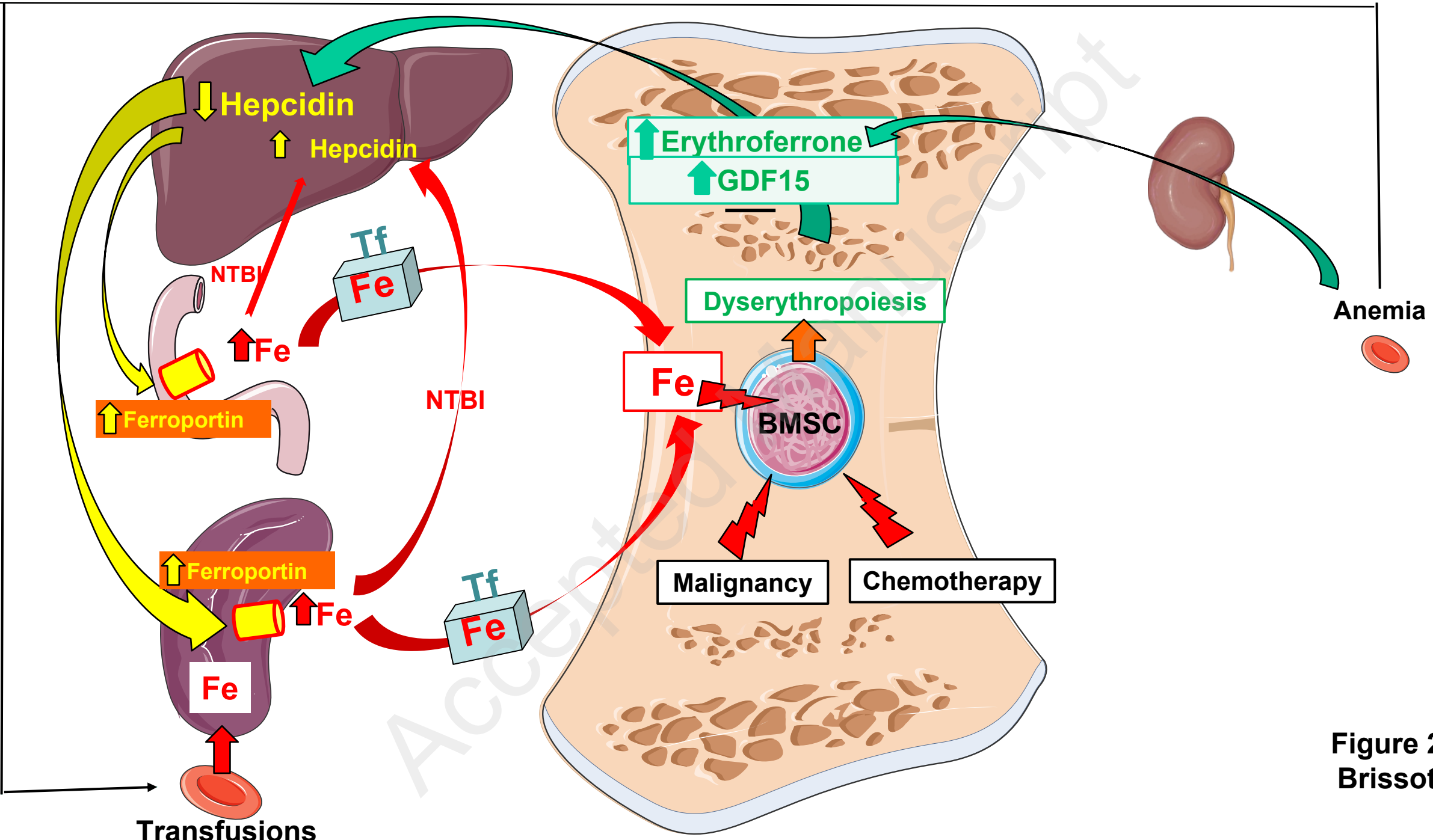


Figure 2
Brissot

Gene name and aliases	Gene symbol (Official, others)
aconitase 1, soluble	<i>ACO1</i> ; <u><i>IRP1</i></u> ; <i>IREB1</i> ; <i>IREBP</i>
<u>ceruloplasmin</u>	<i>CP</i>
<u>erythroferrone</u>	<u><i>ERFE</i></u> , <i>FAM132B</i>
<u>ferritin</u> , heavy polypeptide 1	<i>FTH1</i> ; <i>FTH</i>
<u>ferritin</u> , light polypeptide	<i>FTL</i> ; L-ferritin
<u>ferroportin</u> ; solute carrier family 40 (iron-regulated transporter), member 1	<i>SLC40A1</i> ; <i>IREG1</i> ; <i>MTP1</i> ; <i>SLC11A3</i> ; <i>FPN</i>
hemochromatosis	<u><i>HFE</i></u> ; <i>HH</i> ; <i>HFE1</i> ; <i>HLA-H</i>
<u>hepcidin</u> ; human anti-microbial peptide	<i>HAMP</i> ; <i>HEPC</i> ; <i>LEAP1</i>
<u>hephaestin</u>	<i>HEPH</i> ; <i>CPL</i>
iron-responsive element binding protein 2	<i>IREB2</i> ; <u><i>IRP2</i></u> ; <i>ACO3</i> ; <i>IRP2AD</i>
thrombopoietin Receptor	<i>MPL</i> , <u><i>TPO-R</i></u>
<u>transferrin</u>	<i>TF</i>
<u>transferrin receptor</u>	<i>TFRC</i> ; <u><i>TFR1</i></u> ; <i>TFR</i> ; <i>CD71</i>
transferrin receptor 2	<u><i>TFR2</i></u>

Table 1 : Principal iron regulatory genes presented in the review, with their multiple gene symbols. We have underlined the names and/or symbols used in the review.