

## Review Article

# Transfusion and Iron Overload: Where Are the Risks?

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### ABSTRACT:

Immunologic reactions were the first obstacle and risk, related to transfusions. They were resolved by the discovery of blood groups and the progress in immunohematology. Transmission of infectious agents became thereafter a 2nd problem with a lot of victims. Screening tests and viro-inactivation techniques have made transfusion safe (actual residual risk almost zero in Switzerland). Iron overload is still a problem in transfusion-dependent patients. What can we do to fight against iron overload? 1. Correct anemia (HSCT) or improve it (some forms of MDS with inefficient erythropoiesis, thalassemia intermedia) using anti-GDF11 drugs. 2. Try to correct iron hyperabsorption which is associated with low levels of hepcidin. 3. In case of sickle cell anemia correction of iron overload by erythrocytapheresis. 4. Sometimes (in case of low-risk MDS anemia), chelation removes the toxic effect of iron on erythropoiesis and transforms transfusion dependent anemia in transfusion-independent. 5. Chelation of iron by available drugs: the most effective way which applies to all cases. Increased concentrations of TGF $\beta$  ligand superfamily, including GDF11, are associated with inefficient erythropoiesis in MDS and other forms of anemia characterized by inefficient erythropoiesis. Luspatercept and Sotatercept, (hybrid molecules, fusion proteins, composed of the extracellular domain of the human activin IIA receptor and the Fc part of IgG1), are competitors of the activins and morphogenic proteins of the bone and thus block the signal transduction via the SMAD path. For anemic patients with inefficient erythropoiesis, the use of these inhibitors of the TGF $\beta$  pathway could improve Hb levels and decrease transfusion requirements. The results of Phase 2 studies go in this direction.

**KEY WORDS:** *Transfusion related reactions, Iron overload, Anti-GDF11 drugs, Erythrocytapheresis, Iron chelation, Anemia in MDS*

## INTRODUCTION

Chronic transfusion allows to avoid complications in patients suffering from sickle cell anemia, to survive in patients with beta thalassemia major, or with Diamond-Blakfan anemia or with a form of CDA (Congenital Dyserythropoietic Anemia), or even suffering from anemia secondary to MDS, myelofibrosis or aplastic anemia. However chronic transfusion presents risks of serious, sometimes mortal complications. Because human beings do not possess effective mechanisms to get rid of iron, chronic transfusion

leads to iron overload responsible of lesions in parenchymal organs and or in dysfunctions of endocrine glands.

Pathophysiology of iron overload in chronically transfused patients may be summarized as follows: In a first step transfused erythrocytes are phagocytized by RES (reticulo-endothelial-system). The iron is then liberated from heme and cumulates in the cytoplasm. Macrophages release iron excess in the plasma where iron is bound to transferrin. This progressively leads to increased saturation of serum transferrin. Hepatocytes are another site of iron storage. Chronic transfusion exceeds loading capacity of iron in macrophages and hepatocytes. A huge amount of iron is released in the plasma which exceeds transferrin's capacity to transport iron and thus we have production of NTBI (non-transferrin bound iron), which is the toxic form of iron to cells via the formation of ROS (reactive oxygen species).<sup>1</sup>

Figure 1 schematically represents organ lesions sec-

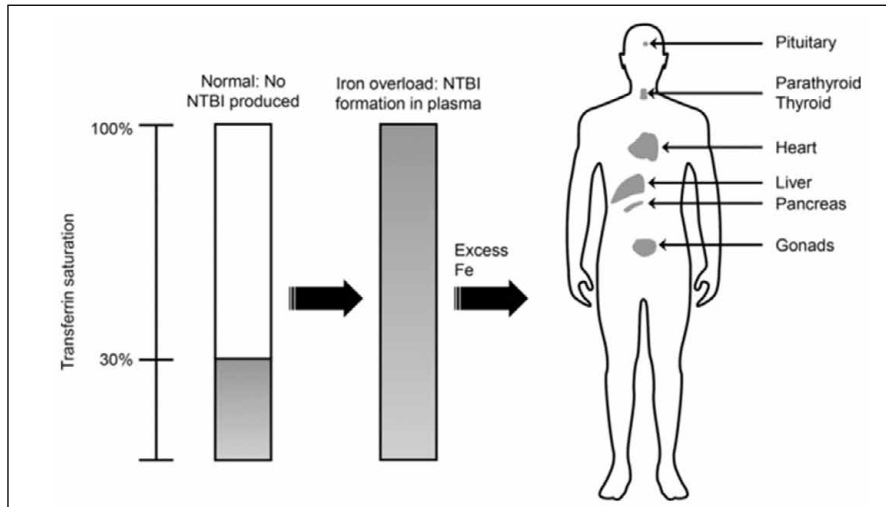
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**Figure 1.** Schematically representation of organ lesions secondary to iron overload in chronically transfused patients (From reference 1).

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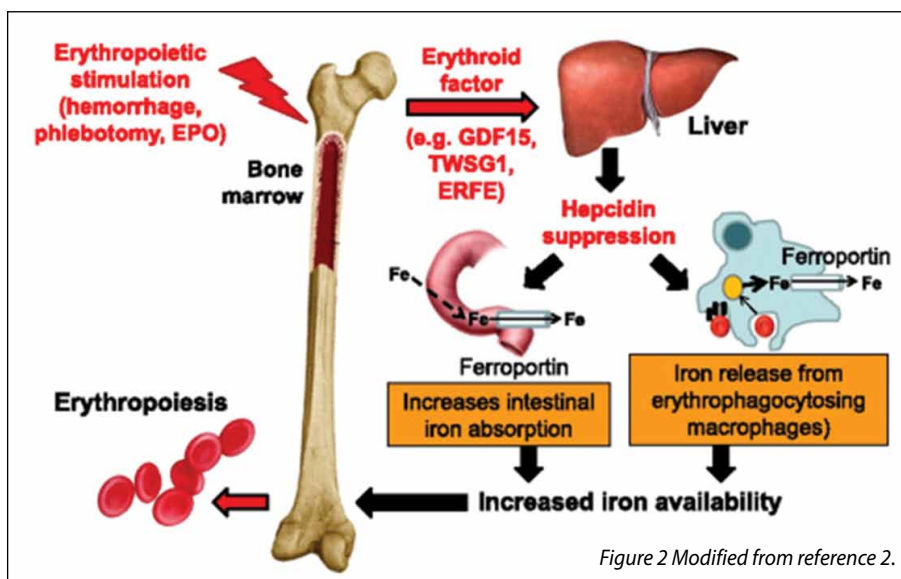
However, transfusion is not the only source of excessive iron input. We must remind here the role of hepcidin and particularly the role of erythroferrone in certain forms of anemia characterized by accelerated and ineffective erythropoiesis.

Figures 2 and 3 illustrate the role of these two proteins in iron absorption.

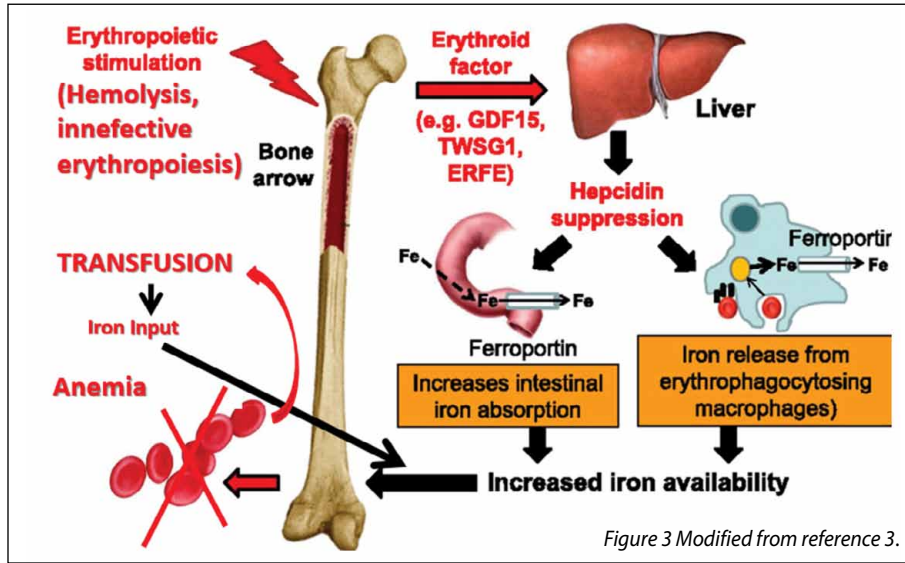
In brief erythropoietic stimulation leads to production of erythroid factors among which is erythroferrone (ERFE). These factors suppress hepcidin production leading to increased iron absorption from the intestine

and to iron release from macrophages. The increased iron availability covers iron needs for accelerated erythropoiesis (Figure 2). In case of ineffective erythropoiesis, the increased iron absorption and the iron brought by transfusions lead to iron overload (Figure 3, Reference 2).

In our laboratory, we have studied hepcidin levels in autoimmune hemolytic anemia induced in mouse. In Figure 4 it is clearly seen that induction of autoimmune anemia in mice leads to significantly decrease of hepcidin levels in day 4 (Figure 5) after administration of anti-erythrocytic antibody.<sup>3</sup> We believe that any form of accelerated erythropoiesis leads at a collapse of hepcidin and this because of the hyper-production of erythroferrone.



**Figure 2.** Role of erythroferrone and hepcidin in iron absorption in normal situation.



**Figure 3.** Role of erythropoietin and hepcidin in iron absorption in hemolysis and ineffective erythropoiesis.

Let us see now the risks of chronic transfusion in different clinical settings:

**1. CHRONIC TRANSFUSION IN CASE OF MDS: WHERE ARE THE RISKS**

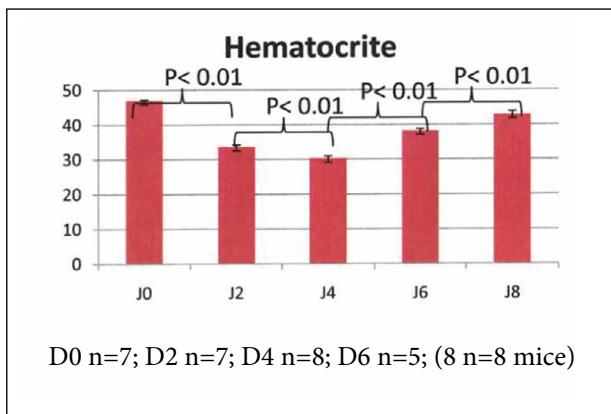
Iron overload in case of MDS starts before the patients become transfusion-dependent because, as we saw before, ineffective erythropoiesis inhibits production of hepcidin.<sup>4</sup> However chronic transfusion is the main and most important source of iron overload in anemic MDS patients: For example, if a patient receives 4 units/month this corresponds in 100 units per two years, which means 20 gr of iron.<sup>5</sup> Clinical studies have shown that iron overload in MDS decreases survival in patients with low risk MDS.<sup>6</sup> Furthermore, iron overload leads at endothelial

cell dysfunction which aggravates arteriosclerosis.<sup>7</sup> Iron overload because of oxidative stress of ROS favors the evolution to acute leukemia.<sup>8</sup> It was recently shown that iron overload deteriorates erythropoiesis which means aggravates anemia.<sup>9</sup>

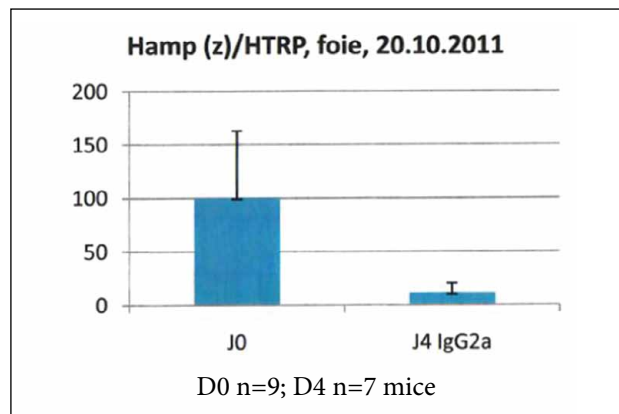
Figures 6 and 7 illustrate the possible role for iron overload in the pathophysiology of MDS (Figure 6 from Reference 5; Figure 7 from Reference 10)

**2. THE ROLE OF IRON OVERLOAD IN HSCT IN CASE OF THALASSEMIA, MDS BUT ALSO IN AL**

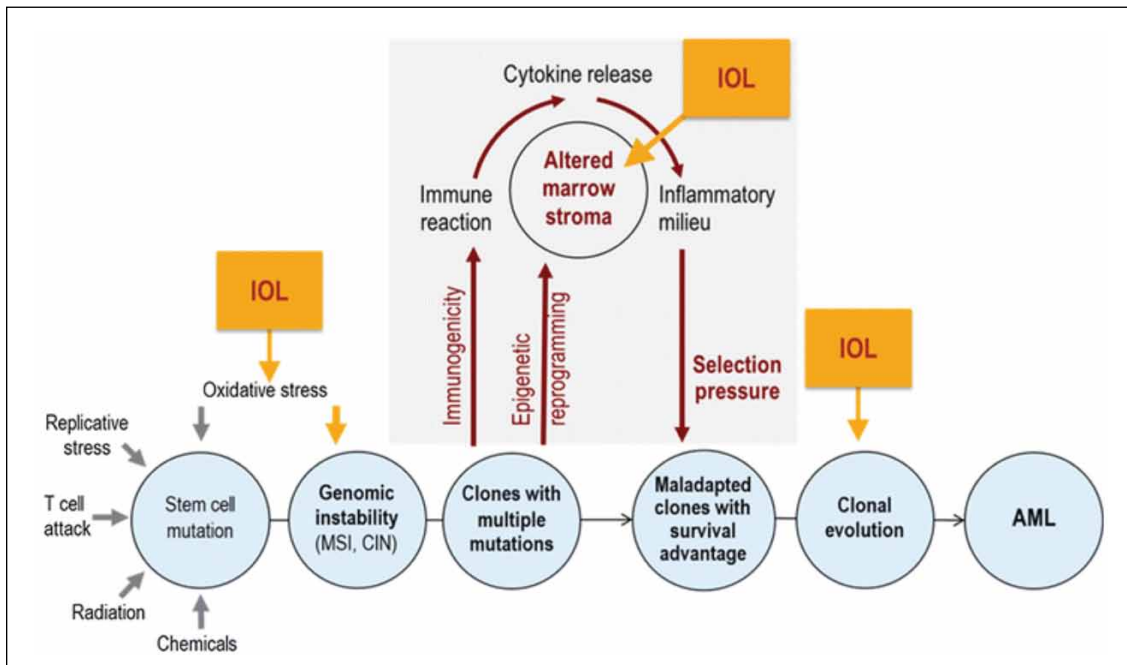
In case of iron overload in patients submitted to HSCT (Haematopoietic Stem Cell Transplantation), elevated liver iron concentration (LIC) is associated



**Figure 4.** Induction of immune hemolytic anemia by using anti-erythrocytic antibody in mice.



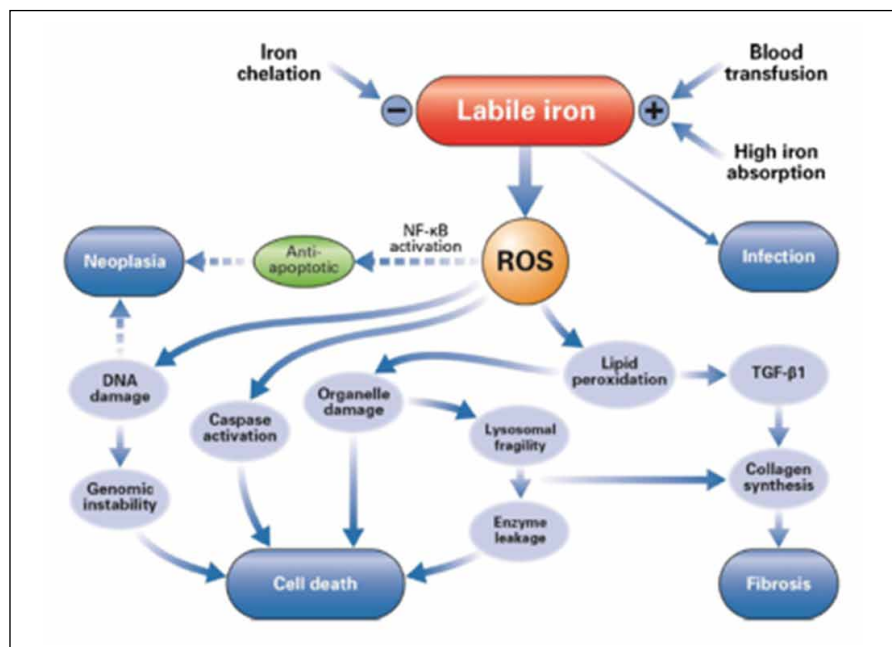
**Figure 5.** Induction of autoimmune anemia in mice leads to significantly decrease of hepcidin levels in day 4.



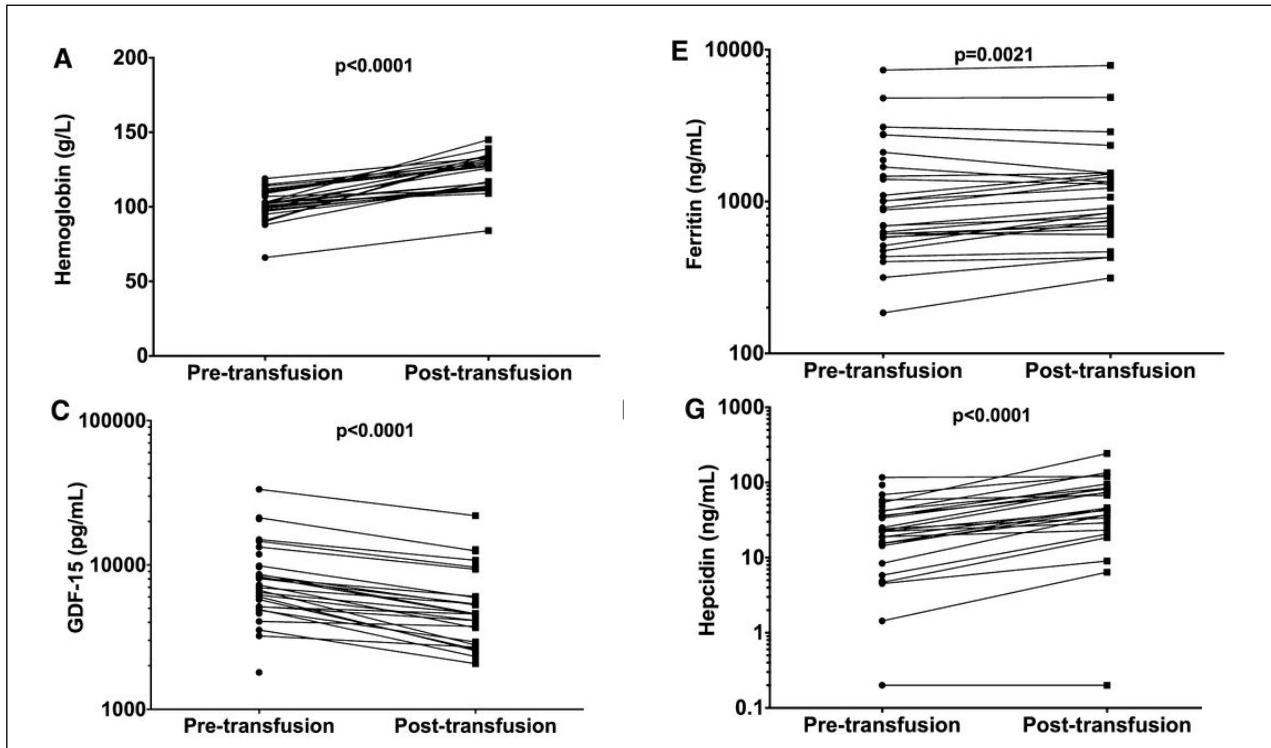
**Figure 6.** Iron overload because of oxidative stress of ROS favors the evolution to acute leukemia (From reference 5).

and, this significantly, with reduced overall survival post HSCT.<sup>11</sup> Furthermore, there is an increased risk of infectious complications particularly by fungi. If LPI (labile plasma iron), is detectable before transplantation, then patients are at high risk for mortality non-linked to relapse of the malignant hematologic disease.<sup>12</sup> After

transplantation, treatment by phlebotomy or by chelators improves hepatic function and decreases GvHD.<sup>13</sup> It is highly suspected that chelation before transplantation may be extremely important but this should be proved by prospective randomized studies before routine application in the clinic.<sup>10</sup>



**Figure 7.** Possible role for iron overload in the pathophysiology of MDS (from reference 10).



**Figure 8.** Transfusion suppresses erythropoiesis and increases hepcidin in adult patients with  $\beta$ -thalassemia major. This leads to decreased iron absorption which explains why pre- and post-transfusion ferritin levels remain almost the same (From reference 17).

### What can we do to fight against iron overload in such settings?

1. If possible correct anemia by HSCT or improve it in certain forms of MDS and ineffective erythropoiesis or in thalassemia intermedia, by using the new category of drugs with anti GDF11 properties (see later in this text).
2. Try to decrease iron hyperabsorption linked with low hepcidin levels (see also Figure 8).
3. In case of sickle cell anemia, anemia and iron overload can be corrected by erythrocytapheresis (see also Figure 9).
4. It was recently shown, in cases of low risk MDS, that iron chelation reduces toxic effect of iron to erythropoiesis transforming thus the patients in transfusion indepen-

dent ones or reducing transfusion needs (Table 1).

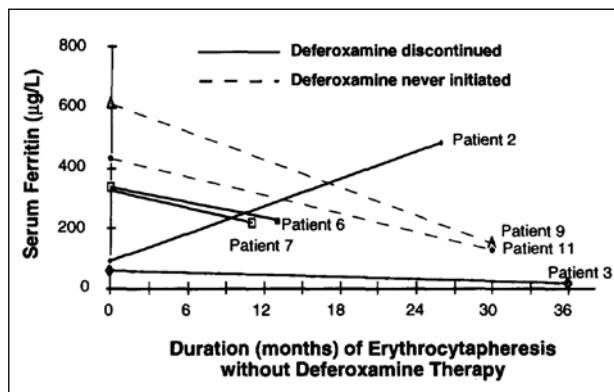
#### 5. The most important: Iron chelation by drugs

It is possible that suppression of oxidative stress improves hemopoiesis by slowing down malignant clonal genetic evolution and by offering a better support by the bone marrow stroma.<sup>5</sup>

In  $\beta$ -thalassemia major, hepcidin levels are simultaneously associated with erythropoiesis and iron loading pre- and posttransfusion. Transfusion improves anemia, suppressing erythropoiesis and in turn increasing hepcidin in patients with  $\beta$ -thalassemia major. This was shown by Pasricha et al in a longitudinal study published in Blood in 2013.<sup>17</sup> It is clearly seen in Figure 8 that transfusion leads to decreased production of erythroid factors like GDF15

**Table 1.** Mentions Clinical trials showing erythropoietic improvement during iron chelation therapy in patients with lower risk MDS (5)

Study	Risk IPSS	RBC response	Neutrophil response	Plateletesponse
List et al (13)	Low/Int-1	15% (n=173)	15% (n=52)	22% (n=77)
Gattermann et al (14)	Low/Int-1	21.5% (n=247)	22% (n=50)	13% (n=100)
Nolte et al (15)	Low/Int-1	11% (n=50)	NR	NR
Angelucci et al (16)	Low/Int-1	Transfusion independence in 15.5% (n=152)	NR	NR



**Figure 9.** Serum ferritin levels in 6 patients with HbSS disease, transfusion-dependent and treated by erythrocytapheresis.<sup>18</sup>

(Fig 8C), with as a consequence an increase of hepcidin in the post transfusion period (Fig 8G) and this explains the stability of ferritin levels (Fig 8E).

Erythrocytapheresis in case of iron overload in Sickle cell anemia combines phlebotomy with transfusion. In fact, reduction of HbS concentration to 25-30% allows to « pump » iron from the iron stores of the patient, by producing new Hb S and this up to 50-55%, value when a new erythrocytapheresis will be done (in general every 4 to 8 weeks).

The main indications in sickle cell anemia are CVA (cerebrovascular accidents), acute chest syndrome, pulmonary hypertension. This therapeutic strategy is very effective against iron overload in this setting (Figure 9). In our hands it leads to iron deficiency anemia! However, the principal obstacle for a routine use is the vein access to perform the procedure.<sup>18-21</sup>

### Treatment of iron overload in patients with MDS: indications and therapeutic scheme

Treatment by chelators should started in MDS patients with low or intermediate risk according to IPSS-R (RA, RARS or isolated 5q-), in patients transfusion-dependent, with ferritin >1000 µg/l (= after 25 units). However sig-

nificant association between survival and ferritin was found when a ferritin level of  $\geq 500 \mu\text{g/l}$  was considered. In these patients it is possible that chelation treatment by improving ineffective erythropoiesis, improves survival.<sup>22</sup> Chelation should also start in patients with MDS candidates for allogeneic HSCT.

Concerning choice of chelator, the most frequently used is deferasirox in its new formulation FCT (film-coated tablet)<sup>23</sup> and in the following dosages:

→15mg/kg (FCT) if transfusion needs are <2 Units/month;

→20mg/kg (FCT) if transfusion needs are intermediates

→25-30mg/kg (FCT) if transfusion needs are 2 or more units /month.<sup>5</sup>

### Treatment of iron overload in patients with Thalassemia

The following Table 2 gives thresholds values to evaluate iron overload in patients with thalassemia.<sup>1,10</sup>

In thalassemia chelation should start at mild iron overload state. It is considered effective if the patients stays at this state and does not progress to moderate or severe iron overloaded state (Table 2). However, if this is the case then intensive iron chelation is recommended (see below).

### Intensive iron chelation

Is indicated in thalassemic children (patients) with moderate or severe iron overload, leading to heart failure. In the literature we find many such cases successfully treated with intensive iron chelation.<sup>24</sup> This is also valid for similar situations in patients with MDS.<sup>25</sup>

The applied regimen is deferoxamine (DFO) 30-50mg/kg/d 5x/week associated to deferasirox 15-20mg/kg/d or to deferiprone 50-75mg/kg/d divided in 3 doses.

The evaluation of treatment is done by measuring T2\*, ferritin levels and pro-BNT levels and by performing cardiac ultrasounds.<sup>25,26</sup>

**TABLE 2.** Iron overload state

Parameter	Normal	Mild	Moderate	Severe
Serum ferritin (µg/l)	<300	>1000 -	<2500	>2500
Transferrin Saturation (%)	20-50		>50	
LPI (µM)	0 - 0.4		>0.4	
LIC (mg Fe/g dw)	<1.8	3 - 7	>7	>15
Cardiac MRI T2* (msec)	>20	14 - 20	8 - 14	<8

Dw, dry weight; LIC, liver iron concentration; LPI, labile plasma iron; MRI magnetic resonance imaging

**TABLE 3.** Iron parameters at baseline and after 1 year of treatment with deferasirox, by underlying anemia (from reference 27)

Parameter	Base line	End of study
Mean ± SD LIC, mg Fe/g dw		
TM	22.1 ± 12.6 (n= 311)	17.1 ± 12.5 (n= 310)
MDS	14.8 ± 10.5 (n= 15)	11.9 ± 8.3 (n= 16)
SCD	14.4 ± 8.2 (n= 13)	12.4 ± 10.1 (n= 18)
Mean ± SD LPI, μ mol /l		
TM	1.25 ± 2.33 (n= 472)	0.59 ± 1.54 (n= 818)
MDS	0.53 ± 0.62 (n= 221)	0.14 ± 0.32 (n= 147)
SCD	0.11 ± 0.54 (n= 55)	0.10 ± 0.21 (n= 46)

### Measurement of NTBI and LPI (=labile plasma iron)

LPI represents the most toxic component of NTBI and is an active redox factor. It penetrates cellular membranes and destroys cells and tissues by the production of ROS. It was demonstrated that LPI has a significant relation with LIC and it provides a supplementary indication of treatment response by iron chelators as this is shown in the next Table 3.

For the time being there is no standardized method for the quantification of serum non-transferrin bound iron and labile plasma iron, in iron overload patients. We quote here the comments of the 2<sup>nd</sup> international round robin for the quantification of NTBI & LPI published in *Haematologica*:<sup>29</sup> “Absolute levels differed considerably between assays and were lower for LPI than for NTBI. Four assays also reported negative values. Assays were reproducible with high between-sample and low within-sample variation. Assays correlated and correlations were

highest within the group of NTBI assays and within that of LPI assays. Increased transferrin saturation but not ferritin, was a good indicator of the presence of forms of circulating NTBI. The possibility of using NTBI & LPI measures as clinical indicators of overt iron overload and/or of treatment efficacy would largely depend on the rigorous validation and standardization of assays”.

### Chelators used in the clinical practice

In the following Table 4 we give some characteristics of the three approved chelators actually used in clinical practice.<sup>10,28</sup>

### Improvement of anemia in certain forms of MDS with ineffective erythropoiesis and in patients with thalassemia intermedia by using drugs with anti GDF11 effect: inhibition of the TGFβ pathway

Increased concentrations of the ligands of the super-family TGFβ including GDF11 are linked with ineffective erythropoiesis in some cases of MDS.

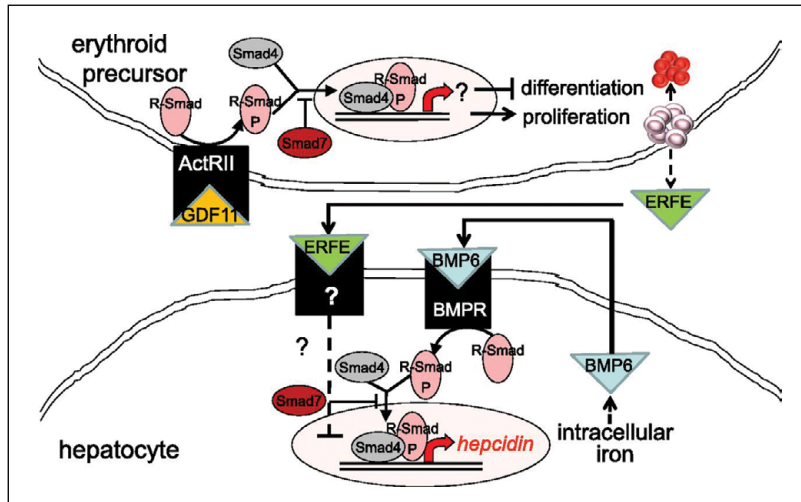
Luspatercept and Sotatercept, are hybrid molecules, representing fusion proteins, composed of the extracellular domain of the receptor IIA of human activin and the Fc part of IgG1. They are competitors of activins and of bone morphogenetic proteins blocking thus the transduction of signal via the SMAD pathway.<sup>30,31</sup>

Their stimulatory activity of erythropoiesis was found in women treated by Sotatercept for osteoporosis.<sup>32,33</sup> The following Figure 10 illustrates how inhibition of the TGF-β family member GDF11 stimulates the last phase of erythropoiesis. It offers also a Model of crosstalk between erythropoiesis and iron metabolism.<sup>2</sup>

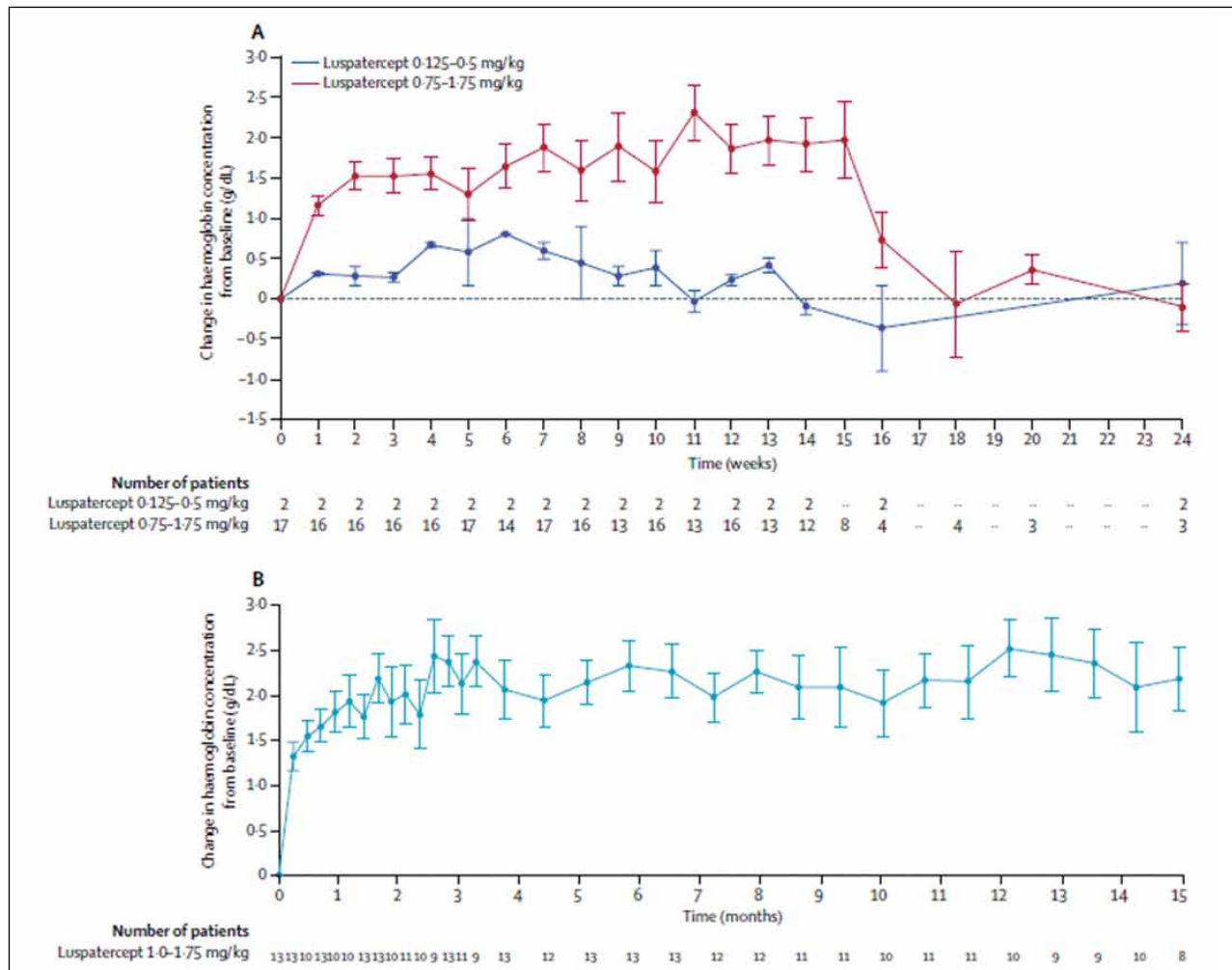
Phase 2 studies in patients with lower-risk myelodysplastic syndromes as well as in thalassemia intermedia patients have already shown the effectiveness of these

**TABLE 4.** Iron chelators available for the management of iron overload secondary to chronic transfusions

Agent	Approval	Administration & daily dose	Adverse events
Deferoxamine (Desferal®)	Chronic iron overload due to transfusion-dependent anemia	Continuous iv or sc administration, up to 50 mg /kg/d over 8-24h, 4-7d/week	Allergic reactions, hepatotoxicity, pain at the site of injection
Deferasirox (Jadenu®)	Chronic iron overload due to transfusion or non-transfusion dependent anemia	Oral administration, 14 to 28 mg/kg/d, once daily	Gastrointestinal events, increased creatinine, allergic reactions
Deferiprone (Defferiprox®)	In association with deferoxamine or deferasirox when intensive iron chelation is needed.	Oral administration, 75 to 100 mg/kg/d, 3 doses daily	Gastrointestinal side effects, agranulocytosis, arthritis, hepatotoxicity



**Figure 10.** Model of crosstalk between erythropoiesis and iron metabolism involving TGF- $\beta$  family member GDF11. It is also shown that inhibition of the TGF- $\beta$  family member GDF11 stimulates the last phase of erythropoiesis (From reference 2).



**Figure 11.** Change in Hb concentration from baseline in patients with low transfusion burden in the base study (A) and patients with low transfusion burden who were treated across both base and extension studies.<sup>31</sup>



drugs in anemia and consequently in decreasing iron overload. Hb concentration may improve up to 25 g/l as is shown in the following Figure 11.<sup>31</sup>

In fact 32 of 51 patients (=63%) by receiving a dose of Luspatercept of 0.75-1.75 mg/kg/ weeks achieved an HI-E (Hematological improvement-erythroid).

Severe side effects were: muscle pain in 2% of patients and deterioration of physical condition in 2% of the treated patients. Basal EPO levels were predictive of response. Patients with acquired sideroblastic anemia and/or SF3B1 mutation respond better (77%). A phase 3 study is going on in case of lower risk MDS (NCT02631070).

### The future: hepcidin agonists as therapeutic tools<sup>34</sup>

We saw earlier that hepcidin is the key factor in iron metabolism. Decreased hepcidin levels characterized all iron overload disorders. It is thus reasonable to prevent iron overload and improve erythropoiesis by creating drugs that either act as hepcidin agonists or stimulators of hepcidin production or even acting as ferroportin inhibitors.<sup>34</sup> A recent paper in *Blood* provides the list of these drugs as well as the advance of clinical studies.<sup>33</sup> These drugs will certainly change treatment of hereditary hemochromatosis. However, they are expected to be also beneficial, for the management of iron loading anemias characterized by low hepcidin levels because of hyper production of erythroferrone. These drugs may also complement other novel treatments mentioned before, that target ligands of the transforming growth factor- $\beta$  family.<sup>35</sup>

## CONCLUSIONS

- Immunologic reactions were the first obstacle and mortal risk linked with transfusion. The discovery of blood groups and progress in immunohematology allowed to completely resolving them.
- Transmission of infectious agents became the next major problem creating many victims. Serologic screening tests, diagnostic tests based in recombinant DNA technology as well as virus-inactivation techniques, allowed transfusion to become safe with a residual transmission risk close to zero in Europe, USA and some other countries.
- Iron overload however continues to be a problem in chronically transfused patients.
- If iron chelation by the new chelators, given orally, brings an acceptable solution to the problem, understanding iron metabolism in all its detail, will allow to arrive to an equilibrium in iron metabolism more easily, and in certain patients will even improve anemia with possibility to transform them transfusion-independent.

- Such an example is given by findings, indicating increased concentrations of the ligands of the superfamily of TGF $\beta$ , including GDF11 in some anemic iron overloaded patients. These cases are characterized by ineffective erythropoiesis and belong mainly to low/intermediate-1 risk MDS patients as well as to thalassemia patients.
- Luspatercept and Sotatercept are competitors of activin and of bone morphogenetic proteins inhibiting thus transduction of signal via the SMAD pathway.
- For patients with anemia secondary to ineffective erythropoiesis, the use of these inhibitors of the TGF $\beta$  pathway, could improve Hb levels decreasing thus transfusional needs. Preliminary results of phase 2 studies, go in that direction.

**Conflict of Interest:** None.

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