Controversies on the Consequences of Iron Overload and Chelation in MDS

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Abstract
Many patients with MDS are prone to develop systemic and tissue iron overload in part as a consequence of disease-immanent ineffective erythropoiesis. However, chronic red blood cell transfusions, which are part of the supportive care regimen to correct anemia, are the major source of iron overload in MDS. Increased systemic iron levels eventually lead to the saturation of the physiological systemic iron carrier transferrin and the occurrence of non-transferrin-bound iron (NTBI) together with its reactive fraction, the labile plasma iron (LPI). NTBI/LPI-mediated toxicity and tissue iron overload may exert multiple detrimental effects that contribute to disease pathogenesis, complications and eventually evolution of MDS. Until recently, the evidence supporting the use of iron chelation in MDS was based on anecdotal reports, uncontrolled clinical trials or prospective registries. Despite not fully conclusive, these more recent studies, including the TELESTO trial, unravel an overall adverse action of iron overload and therapeutic benefit of chelation, ranging from improved hematological outcome, reduced transfusion dependence and superior survival of iron-loaded MDS patients. The still limited and somehow controversial experimental and clinical data available from preclinical studies and randomized trials highlight the need for further investigation to fully elucidate the mechanisms underlying the pathological impact of iron overload-mediated toxicity as well as the effect of classic and novel iron restriction approaches in MDS. This review aims at providing an overview of the current clinical and translational debated landscape about the consequences of iron overload and chelation in the setting of MDS.

Introduction
Myelodysplastic syndromes (MDS) are a heterogenous group of clonal myeloid neoplasms, characterized by dysplasia of at least one cell lineage and cytopenias in the bone marrow and peripheral blood. Approximately 80% to 90% of MDS patients present with anemia at diagnosis. In the past, complex pathophysiological interactions could be identified as main causative drivers of MDS, mostly associated with clonal events in hematopoietic stem cells. Recently, the bone marrow microenvironment has been described as an additional key player in disease initiation and progression.

Treatment of MDS has become more complex over time and asks for an individualized and risk-adapted approach. To allow risk stratification in MDS not only patients-related parameters such as age and comorbidities are taken into account but also disease specific aspects as blast counts, genetic abnormalities and number of cytopenias. The combination of these factors resulted in the prognostic scoring systems IPSS (International Prognostic Scoring System) and IPSS-R (International Prognostic Scoring System-Revised). IPSS and IPSS-R enable stratification of patients into risk categories (low, intermediate-1, intermediate-2, high for IPSS; very low, low, intermediate, high and very high for IPSS-R) and allow for a personalized therapeutic approach.

Independently from risk stratification into lower or higher risk subgroups, only limited therapeutic options can still be offered to MDS patients. In the case of LR-MDS (IPSS low/int-1, IPSS-R very low, low, intermediate up to 3.5 points) therapy is mainly aimed at improving cytopenia(s) (in order to prevent complications such as bleeding and severe infections), decreasing transfusion burden and improving quality of life. Higher-risk MDS patients may benefit from hypomethylating agents (HMA) or even induction chemotherapy (IC) followed by allogeneic hematopoietic stem cell transplantation (HSCT) in a small subset of patients.

Since the majority of MDS patients is of higher age, these patients often do not tolerate an intensive therapy, leaving symptomatic therapy centered on erythropoiesis-stimulating agents (ESA) or HMA as well as transfusion support as the only possible option. In fact, as anemia is a hallmark of MDS, red blood cell transfusions are mainstay of supportive care in most MDS patients, often leading to transfusion dependency.

As a result of chronic transfusions, MDS patients receive excessive amount of iron (250mg per RBC unit), which leads to
systemic and tissue iron overload (IO). Importantly, transfusion dependency has a negative impact on the clinical outcome of MDS patients and is predictive of a shortened overall as well as leukemia-free survival.15

Iron homeostasis and pathology of iron overload in MDS

Iron homeostatic mechanisms in health

Iron is an essential element for living organisms but becomes toxic when its systemic and tissue concentration overwhelms the physiological storage capacity. In light of its potential toxicity, iron homeostasis needs to be tightly regulated (Fig. 1). Iron is released into the circulation from duodenal enterocytes, which absorb daily 1 to 2 mg of dietary iron, and from macrophages, which recycle about 25 mg of iron from senescent red blood cells. Inorganic dietary iron is absorbed by duodenal enterocytes through divalent metal transporter 1 (DMT1),13 after iron reduction from ferric to ferrous form by the ferrireductase DcytB. Cytosolic iron is then exported into the circulation through the iron exporter ferroportin (FPN), assisted by the multicopper oxidase hephaestin, which facilitates iron loading onto transferrin by mediating iron oxidation.14 Since intestinal iron absorption accounts for less than 10% of the physiological iron needs, macrophages satisfy most of the daily iron requirement through erythropagocytosis and FPN-mediated hemoglobin-derived iron recycling. Iron circulates in plasma bound to its high affinity scavenger transferrin, which has two binding sites for iron and maintains it in a soluble, non-toxic form. Transferrin has the crucial role of delivering iron to target cells. The main consumer of iron is erythropoiesis, which utilizes around 20 to 25 mg of iron per day to sustain the daily production of 200 billion red blood cells (RBC).15,16 In humans, transferrin shows normally about 30% to 40% saturation with iron. Transferrin receptor 1 (TFR1) mediates the endocytic acquisition of dfferrc transferrin (TF-Fe₂) in erythroid progenitors, which express progressively reduced TFR1 levels along with erythroid maturation. A saturation >50% indicates iron overload and when it exceeds 60% to 70%, non-transferrin bound iron (NTBI), loosely bound to other plasma components, appears in the circulation.17,18

Within NTBI, the most reactive and readily chelatable fraction, the labile plasma iron (LPI), is considered the redox-active and toxic component of the NTBI. This form of iron is responsible for the generation of reactive oxygen species (ROS), interaction with biological molecules and accumulation into tissues, finally causing iron overload, organ dysfunction and cellular damage. Indeed, the key to iron homeostasis lies in the control of...
appropriate systemic iron levels and the maintenance of transferrin saturation within physiologic range. This is obtained through the critical regulation of duodenal iron absorption and macrophage iron recycling by the hepcidin/FPN regulatory system. Iron influx into the circulation can in fact be enhanced in condition of increased iron need (eg, hypoxia, stimulated erythropoiesis) and suppressed in condition of iron overload, through the modulation of hepcidin levels. The peptide hormone hepcidin is produced by hepatocytes based on ‘body iron status sensing’ and acts as key regulator of systemic iron homeostasis. Hepcidin-mediated control of systemic iron fluxes lies in its ability to bind the iron exporter FPN and trigger its internalization and lysosomal degradation, thus regulating cell iron efflux. Being FPN abundantly expressed on the surface of duodenal enterocytes and reticulo-endothelial macrophages, hepcidin levels determine circulating iron influx by increasing or decreasing cell iron export and retention, hence modulating iron absorption and recycling. Several, in part opposing signals are implicated in the regulation of hepcidin expression in hepatocytes, including iron stores, erythropoietic activity, hypoxia, and inflammation. When transferrin saturation and iron stores are increased, integrated signals lead to the induction of hepcidin synthesis, in order to reduce FPN-mediated systemic iron influx and alleviate the overall iron burden. Similarly, upon inflammatory and infection conditions, hepcidin levels are increased to restrict iron and reduce its availability for microbial pathogens, that rely on iron acquisition as a growth-limiting micronutrient. The inflammatory cytokines IL-6, IL-1β and activin B act both as inducers of hepcidin expression. A prolonged induction of hepcidin results in anemia of inflammation, an acquired disorder of iron homeostasis hallmarkd by macrophage iron retention usually associated with malignancies, infections and other causes of inflammation. Conversely, the inhibition of hepcidin expression by erythropoietic signals is critical to maintain proper de novo RBC production, which requires relevant amount of iron. Conditions including phlebotomy, severe blood loss, hemolysis and high altitude exposure lead to increased erythropoiesis as an attempt of the bone marrow to compensate for the resulting hypoxia. Hypoxia induces the renal production of erythropoietin (EPO) which is responsible for erythropoiesis stimulation. When erythropoiesis expands, the absorption of iron from the diet and its release from stores increase in parallel. Therefore, hepcidin levels are suppressed to enhance systemic iron availability for erythropoiesis. EPO controls hepcidin inhibition through the release of erythroferrone (ERFE), a negative erythroid regulator of hepcidin secreted by erythroblasts which communicates the increased erythron iron demands to enhance systemic iron influx. ERFE’s ability to suppress hepcidin production is mediated by its natural ligand trap function towards BMPs (bone morphogenetic proteins). By sequestering BMP ligands (BMP 5, 6, and 7), ERFE prevents the activation of the BMP/SMAD signaling pathway which controls hepcidin synthesis in hepatocytes. ERFE has been recently identified and the knowledge about its role in humans is still limited. Whether it is the only factor controlling hepcidin suppression during enhanced erythropoiesis is unclear and other molecules, including GDF15 (Growth Differentiation Factor 15), TWSG1 (Twisted gastrulation homolog protein 1) and PDGF-BB (Platelet-derived growth factor-BB), have been implicated, though not so definitely, in this mechanism. Recently GDF11, which is increased in MDS and β-thalassemia, has emerged as novel negative regulator of hepcidin. GDF11 suppresses hepcidin production by decreasing BMP-SMAD signaling, enhancing SMAD ubiquitin regulatory factor 1 (SMURF1) expression and inducing ERK1/2 (MAPK3/1) signaling. Besides regulating hepcidin through EPO/ERFE, hypoxia also increases duodenal iron absorption through HIF2α (hypoxia inducible factor 2α)-mediated induction of DMT1 and FPN in enterocytes, contributing to systemic iron elevation. Since the human body lacks any active system for iron excretion, iron overload develops when the mechanisms controlling iron absorption are altered (eg, genetic mutation, ineffective erythropoiesis) or bypassed (eg, transfusions, intravenous iron infusion). Common diseases of iron overload or deficiency as well as diseases with expanded erythropoiesis are hallmarkd by misregulation of the hepcidin/FPN axis, which affects iron release from enterocytes, macrophages and perportal hepatocytes. In these conditions transferrin saturation rises, leading to the formation of NTBI and LPI, which in turn deposit in tissue parenchyma. The liver, as main iron storage organ, is the first target of NTBI/LPI which enters hepatocytes through the importer ZIP14 and eventually accumulates in the iron storage protein, ferritin, which binds iron in a non-toxic form. When hepatic storage capacity is exceeded, other tissues, including the heart, pancreas, endocrine organs, kidney, start to accumulate iron, with potential detrimental effect on organ functionality.

Iron dyshomeostasis and overload in MDS

The main drivers of iron overload in MDS patients are (1) ineffective erythropoiesis and (2) transfusion therapy (Fig. 2).

Ineffective erythropoiesis

MDS patients might develop iron overload even before receiving transfusions, because of their underlying ineffective erythropoiesis. In this condition, when an insufficient number of mature RBCs is produced, the ensuing hypoxia induces EPO release and erythroid progenitor expansion in an attempt to recover the anemia.

This is typical of diseases such as b-thalassemia where anemia occurs as a result of decreased/absent b-globin synthesis, and MDS, where mutated clonal stem cells give rise to dysplastic ineffective hematopoiesis and peripheral cytopenia. These disorders are hallmarkd by stress but ineffective erythropoiesis, as the increase in the number of erythroid precursors unsuccessfully compensates for the underlying inherited or acquired genetic defect(s) which per se prevents adequate mature RBC production. Heparicin suppression has been now described as a key mediator of unrestrained intestinal iron absorption in some MDS subtypes as well as thalassemia. The absorbed iron is in part utilized for erythropoiesis in MDS patients with low hepcidin and the excess accumulates as NTBI in tissue parenchyma. In MDS iron accumulation is self-maintained in a vicious cycle, being iron, on the one hand, accumulated due to hepcidin inhibition induced by expanded bone marrow activity and, on the other hand, inadequately used after macrophage release, due to ineffective erythropoiesis and defective RBC maturation. Across the different MDS subtypes serum hepcidin absolute levels show a significant heterogeneity, with the lower values in patients with refractory anemia with ring sideroblasts (MDS-RS) and refractory cytopenia with multilineage dysplasia (MDS-MLD). This is consistent with the highest levels of NTBI and tissue iron overload that MDS patients with ring sideroblasts (RS) develop, even in absence of transfusion therapy, compared to
other MDS subtypes.\textsuperscript{40} MDS-RS is often associated with SF3B1 mutations (app. 90\% patients) and hallmarked by the presence of bone marrow erythroid precursors with mitochondrial iron accumulation (ring sideroblasts).\textsuperscript{41} Mitochondrial iron retention prevents iron incorporation into heme, which results in hypoxia and leads to increased but ineffective erythropoiesis with consequent hepcidin inhibition.\textsuperscript{37} The iron in ring sideroblasts plausibly deposits in mitochondrial ferritin, whose levels have been correlated with early apoptosis of RARS erythroblasts.\textsuperscript{42} Moreover, 20\% MDS-RS patients present with HFE gene polymorphism, which predisposes to higher iron loading than other MDS subtypes.\textsuperscript{37} Overall, due to existing pre-transfusional iron overload, MDS-RS patients may be further impacted by iron toxicities than solely transfused patients with other MDS subtypes.\textsuperscript{37} However, to existing pre-transfusional iron overload, MDS-RS patients may be further impacted by iron toxicities than solely transfused patients with other MDS subtypes. Importantly, the hepcidin/ferritin ratio is significantly decreased in untransfused MDS patients compared to healthy subjects, indicating that hepcidin levels are inappropriately low for the degree of iron loading (monitored as serum ferritin).\textsuperscript{38,39} Therefore, hepcidin is suppressed by the erythropoietic activity even when ample iron stores are available, suggesting that the effect of erythropoiesis on iron absorption is dominant over iron in regulating hepcidin. A lower hepcidin/ferritin ratio has been reported also in patients with 5q-syndrome, that together with MDS-RS patients, show the highest levels of serum ferritin, Tf saturation and NTBI.\textsuperscript{38} Our knowledge about the erythroid factors responsible for hepcidin is still limited in MDS and circulating ERFE concentration have not yet been extensively monitored in this patient population. First evidence indicates higher ERFE expression in bone marrow cells from MDS patients.\textsuperscript{43} Recently, an ERFE variant deriving from an alternative transcript and with preserved hepcidin suppressing activity has been described in MDS patients with SF3B1 mutation. Interestingly, plasma concentrations of ERFE are higher in these patients, suggesting that the ERFE variant accounts for the increased iron loading observed in these MDS subtype.\textsuperscript{40}

**Chronic transfusions**

Blood transfusions remain the dominant cause of iron overload in MDS patients.\textsuperscript{44,45} Especially for patients suffering from low-risk or intermediate-1-risk MDS, the improvement of anemia through RBC transfusions is a central point of supportive care.\textsuperscript{7} Iron overload can develop rapidly as every unit of transfused blood contains about 200 to 250 mg of iron, whereas natural iron losses reach only 1 to 2 mg daily. A requirement of 4 RBC units per month translates in the acquisition of 9.6 to 12 g iron/year.\textsuperscript{37} Considering that human annual iron intake is about 1.5 mg, 15 to 20 transfusions are enough to generate iron overload.\textsuperscript{10} However, a recent study demonstrated the negative impact of RBC transfusions even at very low density, this also applying to patients who would not be defined as transfusion dependent using standard (IWG) criteria. Paying special attention to this subgroup of patients this trials aimed to analyze the connection amongst transfusion rate and outcome, hypothesizing that patients in need of regularly RBC transfusion are generally more exposed to the assumed toxicity of transfusion. This study suggests that the effect of dose density continues to increase below 2 RBC units per month (3 RBC units per 16 weeks). Therefore, these low transfusion rate should also be considered as a possible indicator of decreased prognosis.
Differently from untransfused condition where duodenal iron absorption mainly contributes to iron loading via hepcidin suppression, transfusional iron overload initially occurs in hepatic and splenic macrophages which recycle transfused RBC once they become senescent. Moreover, the release of heme-derived iron from macrophages is likely accelerated due to inappropriately low hepcidin levels, resulting in accelerated NTBI formation and tissue iron deposition. Heparidin levels are significantly higher in transfusion-dependent MDS patients compared to healthy subjects and transfusion-independent MDS patients. In particular, hepcidin and serum ferritin positively correlate in MDS patients receiving fewer transfusions (<9) and negatively in those heavily transfusion-dependent (>24 transfusions). However, the decreased hepcidin/ferritin ratio in transfusion-dependent patients compared to healthy individuals suggests that hepcidin response to iron, although increased by transfusions, is still blunted and inadequate relatively to the degree of iron overload (indicated by serum ferritin elevation).

Finally, despite transfusion-triggered transient induction, hepcidin decreases towards the end of the transfusion cycle together with hemoglobin decrease. Therefore, hepcidin regulation is still sensitive to iron levels (at least in MDS patients with low transfusion requirements) but predominantly governed by erythropoiesis. Overall, in low-risk MDS patients, tissue iron accumulation results from a combination of iron-loading effects of (i) ineffective erythropoiesis and (ii) RBC transfusions. Ineffective erythropoiesis through hepcidin reduction is expected not only to contribute to body iron accumulation but also to promote transfusional iron overload redistribution to parenchymal cells in these patients.

Clinical consequences of iron overload in MDS

Despite some skepticism, evidence of iron overload in MDS is nowadays mounting. As patients present with MDS at an older age, have a shorter life expectancy and might show a number of comorbidities, the detrimental impact of iron overload and therapeutic benefit of iron chelation are a matter of debate. Controversies arise from the fact that elderly MDS patients are exposed to iron overload later in life, for a shorter period and to lower overall iron amount than those receiving life-long transfusion therapy, as thalassemia patients. Moreover, iron-related complications often overlap and might aggravate age-related clinical features and pathologies in MDS patients, which prevent from discriminating the specific additional contribution of iron overload to morbidities and mortality. Below we will review the emerging evidence of iron toxicity in MDS, with a focus on reduced survival, cardio-vascular and hepatic dysfunction, infectious complications, progression to leukemia and impact on the bone marrow microenvironment. Finally, we will consider whether certain tissues and cell types (eg, bone marrow, HSCs, MSCs, immune cells) might be more and differentially sensitive to NTBI and iron-mediated toxicities in MDS compared to thalassemia, even if the exposure to NTBI is overall lower/shorter in MDS patients than life-long transfused thalassemia patients.

Impact on overall survival

Retrospective studies showed that MDS patients with transfusion dependency had a significant shorter overall survival than those who did not require transfusion on a regular basis. The negative impact of iron on survival of low-risk MDS patients is dose-dependent, with a 30% increased risk of death every 500 μg/l additional serum ferritin above 1000 μg/l. Recently, it has been shown that the negative effect of transfusion treatment on progression-free survival already occurs at transfusion densities below 3 units/16 weeks – suggesting that transfusion dependency already at low dose densities, may be considered as an indicator of inferior progression-free survival. Importantly, serum ferritin and LPI have emerged as independent prognostic factors for patient survival. However, their elevation might result from higher transfusion requirements reflecting an underlying more severe course of the bone marrow failure as cause of shortened survival. Therefore transfusion dependency is likely associated with reduced overall survival because on the one hand, it clearly reflects the extent of bone marrow failure, but on the other one, it contributes to the establishment of an additional clinical issue which is iron overload and consequent toxicities. This might have differential impact based on the MDS subtype and related aspects, including life expectancy, predisposition to iron overload (eg, MDS-RS), progression of the disease. Importantly, several studies have now shown that iron chelation improves survival in transfusion-dependent MDS. (Table 1) Besides an ameliorated erythropoietic response, the therapeutic benefit of iron chelation in MDS might be mediated by substantial improvements in the functions of tissue which are significantly affected by iron overload, as described in the next sections.

Impact on cardio-vascular functionality

Cardiac events appear to be the most common non-hematological morbidity in transfusion-dependent MDS patients, leading to non-leukemic death, especially arrhythmias and heart failure. Nevertheless, the mechanisms leading to cardiac dysfunction and the specific contribution of cardiac iron overload remain still unclear in MDS. MDS-associated anemia and age-related cardiac comorbidities are in fact per se potential triggers of heart failure in this patient population. However, severe iron overload is a major and well-described cause of heart failure-related death in transfusion-dependent thalassemia. Myocardial iron overload develops in 17% to 27% of transfusion-dependent MDS patients monitored by cardiac T2 MRI in different studies and directly correlates with transfusion history. Since existing data regarding cardiac iron toxicity and its functional implications have been mainly generated in b-thalassemia patients, further investigations are needed in this direction in MDS patients. Interestingly, clonal hematopoiesis and related inflammation have been recently described as underlying mechanisms of MDS patients’ predisposition to atherosclerotic cardiovascular disease. Novel evidence has shown the implications of iron overload in atherosclerotic cardiovascular disease, through a multifactorial pro-atherogenic action of NTBI leading to vascular impairment, inflammation, ROS production and LDL oxidation. Importantly, the recently described NTBI deposition in vascular cells suggests that not only cardiac iron accumulation but also arterial iron loading plays a role in iron-driven complications of the cardiovascular system. Overall, these observations indicate that iron overload might further contribute to the propensity of MDS patients to develop cardiovascular disease, eventually accelerating its progression. However, experimental evidence in animal models and prospective human studies of how iron overload (and cardiac T2 MRI) contributes to cardiovascular events in low-risk transfusion-dependent MDS patients are
still needed. Retrospective analyses suggested that iron chelation therapy (ICT) delay cardiac events in transfusion-dependent MDS patients but requires confirmation from larger prospective studies. 

Impact on liver functionality

The liver is the first tissue which starts to accumulate iron when NTBI is generated. Being an iron-storage organ, the liver can tolerate significantly higher amount of stored iron compared to tissues such as the heart. However, a ten-fold increase in hepatic stored iron (>15–20 mg/g dry weight) compared to normal liver iron concentration (2 mg/g dry weight) leads to hepatic dysfunction and fibrosis and correlates with worse overall prognosis. 

Around 70% to 80% of regularly transfused MDS patients develop hepatic iron overload, correlating with RBC transfusion frequency. The German matched-pair analysis showed a reduced risk in hepatic-related deaths for chelated versus non-chelated MDS patients. Although some data suggest positive effects of iron chelation in reducing hepatocyte damage and possibly preventing dysfunction and cirrhosis, evidence for MDS patients is still scarce. 

Predisposition to infections

Despite still clinically and mechanistically understudied, an increased incidence of infections and infection-related mortality has been reported in MDS patients receiving transfusions. To what extent cytopenias and impairment of immune function due to MDS and iron overload due to transfusions contribute to infection susceptibility is unclear. 

Iron overload is thought to increase the risk of infections in patients with MDS through two major mechanisms: (1) a direct effect on pathogens, which acquire from the host free unbound iron (NTBI) essential for their growth; (2) a detrimental effect on the cellular and molecular mechanisms responsible for the natural resistance to infections, including the impairment of immune cell response (eg, macrophage, neutrophil, lymphocyte) as well as cytokine production and nitric oxide formation. 

A correlation between transfusional iron overload and infection risk has been established in transfusion-dependent MDS patients compared to transfusion-independent ones, scarce attention has been paid to the clinical benefit of ICT in reducing this risk. Bacterial, viral and fungal infections are more frequent in transfused MDS patients showing signs of iron overload and ICT seems to delay the appearance of the first infection from the initiation of transfusions. Non-chelated MDS patients also show an increased incidence of death due to infections compared to chelated patients. Whereas the use of deferoxamine in infections has to be avoided, because of its action as a siderophore promoting pathogen growth, patients treated with deferasirox does not increase the incidence of infections and has been suggested as potential adjunct therapy in patients with mucormycosis. 

Impact on erythropoiesis and leukemic progression

The detrimental and inhibitory effects of iron on erythropoiesis have been demonstrated over the last years through several in vitro and in vivo models of iron overload, leading to the concept that iron excess may aggravate bone marrow failure in MDS. Exposure of erythroid precursors to elevated iron induces dysplastic changes and significantly impairs erythroblast differentiation and RBC maturation, causing an overall reduction of burst-forming unit colonies formation and erythroblast apoptosis. These events are reversed by chelation and anti-oxidant agents. Consistently, hematopoietic stem cells (HSCs) from iron-treated MDS animal and from MDS patients with moderately elevated serum ferritin (>250 μg/l) show impaired proliferation – exclusively in the erythroid lineage. The enhanced sensitivity of erythroid precursors to iron toxicity might be due to the direct effect of NTBI exposure and/or mitochondrial iron retention, especially in MDS-RS. ROS formation and markers of oxidative DNA damage are elevated and further exacerbated by transfusional iron overload in bone marrow of MDS patients and corrected by ICT. 

Iron excess has also been implicated in the induction of epigenetic abnormalities and telomere erosion. Overall iron-induced oxidative stress, DNA damage and telomere shortening are thought to contribute to bone marrow mutagenesis, underscoring iron as a potential additional driver of genomic instability in MDS. Despite unable to trigger per se stem cell leukemic transformation, iron overload might accelerate leukemic progression by mediating genotoxic stress in highly proliferating stem cells. Moreover, given the critical regulation of hematopoietic cell stemness by ROS levels in the bone marrow niche, NTBI-driven ROS elevation likely induces HSC exit from quiescence, leading to the exhaustion of normal HSCs and eventually, the selective expansion of the MDS clone. 

Impact on the bone marrow microenvironment

An abnormal bone marrow microenvironment has been suggested to play a critical role in MDS pathogenesis and in the evolution of lower risk MDS to a more aggressive disease. Due to the key function of the bone marrow microenvironment in the maintenance, self-renewal and differentiation of HSCs, its alterations have been implicated in hematopoiesis impairment as well as progenitor cell apoptosis and dysplasia. 

Effect of iron on MSCs

Mesenchymal stromal cells (MSCs) from MDS patients shows to show cell dysfunction, inflammatory cytokine production and genetic abnormalities. Iron overload contributes and eventually exacerbates these effects as suggested by dysfunctional MSCs observed in a murine model of iron overload and delayed hematopoietic engraftment in iron-loaded animals receiving bone marrow transplant. In these contexts, iron might impair survival and triggers apoptosis of MSCs, events accompanied by mitochondrial fragmentation and autophagy, through ROS production. 

Effect of iron on immune cells

In contrast to stromal cells, the impact of iron on marrow resident innate and adaptive immune cells and its implications in MDS evolution are understudied. The immunomodulatory role of iron and transfusions through the induction of cytokine release and alteration of immune cell functions might in fact play a role in bone marrow niche dysfunction and deranged hematopoiesis in MDS patients who often present with a pro-inflammatory niche. Besides a potential role in dampened iron-driven inflammation, deferasirox has been reported to also have an iron-independent anti-inflammatory action. Whether the combined iron-dependent and -independent anti-inflammatory effect of deferasirox contributes to the hematopoietic improvement observed in deferasirox-treated patients needs still to be elucidated.
Effect of iron on bone cells

Finally, iron overload has been implicated in the development of osteoporosis, which MDS patients present more frequently. Interestingly, the osteogenic differentiation of bone-forming osteoblasts and their precursors, MSCs, is impaired by iron and rescued by iron chelation, thus explaining the association between iron overload and osteoporosis. Iron-induced alterations in osteoblast differentiation capacity might further interfere with the crosstalk between the osteo-hematopoietic niche and HSCs, thus promoting MDS evolution. As the interaction bone marrow microenvironment-HSCs is a relatively novel and still emerging area of investigation, detailed understanding of the mechanisms underlying iron-mediated niche disruption may help to define novel targets for diagnosis and possibly therapy of MDS.

Impact on allogeneic hematopoietic stem cell transplantation

For a subset of fit MDS patients allogeneic HSC transplantation (HSCT) might be a valuable therapeutic option and the only fully curative treatment. Patients selected for HSCT frequently present with elevated transferrin saturation and iron levels due to a history of recurrent blood transfusions. Ultimately, the conditioning prior to HSCT further exacerbates the presence of circulating NTBI and its reactive fraction LPI. Multiple studies have shown an inferior outcome after allogeneic HSCT in patients with iron overload, as it may add to overall comorbidities. A prospective multicenter observational German study, the Allogeneic Iron Investigators (ALLIVE) trial, recently investigated the influence of both stored iron and biologically active iron on post-transplantation outcomes in MDS and AML patients. Interestingly, patients with elevated NTBI at baseline show an increased incidence of non-relapse mortality in comparison to patients with pre-HSCT normal NTBI. Thus LPI positivity is predictive of an inferior overall survival (OS) in transplanted patients with MDS.

Although experimentally underexplored how NTBI excess is induced by conditioning, clinical evidence suggest that this event reflects a disruption of iron homeostasis consequent to myelosuppression and temporary reduction in iron utilization by the suppressed erythroid system. NTBI/LPI levels substantially drop after HSC engraftment when restored erythropoiesis allows iron reutilization. Other possible triggers of NTBI after conditioning include chemotherapy cytotoxicity on erythroid cells as well as other cell types, leading to intracellular iron release. Since NTBI increase might be induced by cytotoxic conditioning and in turn determines further cytotoxicity, with overlapping pattern, experimental proof of this additional mechanism is still limited.

Overall NTBI and LPI best reflect changes in iron homeostatic mechanisms in the HSCT setting, therefore being a potential target for chelation therapy pre- and peri-transplant. Recent evidence suggests that elevated NTBI in the bone marrow microenvironment may adversely affect the outcome of HSCT by (i) increasing the risk of infections in transplanted patients, who already present with a weak post-HSCT immune system; and (ii) impairing donor cell engraftment and/or differentiation due to detrimental effects of iron on HSCs and/or the receiving bone marrow niche. Whereas the ALLIVE study was able to demonstrate a causative link between the occurrence of NTBI and an increased incidence of infection-associated nonrelapse mortality in transplanted patients, evidence about the efficacy of iron restriction in this setting are still limited. The reduction of iron overload may be of benefit for MDS patients undergoing HSCT, who should be considered for ICT in the peri-transplant period, with the aim to avoid NTBI/LPI excess and its toxicities. Likewise, a recent work showed that fungal outgrowth in the sera of transplanted patients from the ALLIVE study was dependent on the presence of NTBI and rescued by deferasirox (but not the xenosiderophore deferoxamine), providing evidence of chelation benefit against invasive fungal infections in transplanted patients. Interestingly, a recent study showed that the reduction of iron burden early on after HSCT (started before 6 months) significantly improved relapse-free survival after transplantation (56% in the control group vs 90% in the treated group), whereas the administration of ICT prior to HSCT did not significantly influence HSCT outcome. Since studies are limited in the HSCT setting, new experimental evidence and prospective clinical trials are needed to elucidate the molecular mechanisms of NTBI-mediated toxicity during HSCT and demonstrate the actual feasibility and effectiveness of chelation therapy.

Overall, although several associations have been made between iron overload and altered erythropoiesis as well as dysfunctional bone marrow stem and niche cells, the underlying molecular mechanisms have been only partially explored. In particular, the current experimental evidence mostly relies on cell-based and murine models of ‘artificially-induced’ iron overload (eg, iron-dextrane injection) and little research has been conducted in mouse models of MDS which could provide more relevant insights with clinically translatable value. This highlights the need of further investigation on preclinical models of pathophysiological iron overload and MDS as well as prospective clinical trial to elucidate the mode of action of iron in erythropoiesis suppression and bone marrow niche impairment as well as ICT-mediated hematopoietic improvement in MDS.

Measuring and monitoring iron overload

Serum ferritin

In MDS serum ferritin levels are considered a clinically useful measure of body iron storage, which increase proportionally to tissue iron levels. While low serum ferritin levels indicate iron depletion, enhanced levels suggest iron overload. Circulating serum ferritin is mainly composed of L chain subunits and relatively poor in its iron content. Still its origin - from damaged cells or active cell secretion - and physiological functions are matter of debate in the field, despite its value as clinical parameter. In the interpretation of serum ferritin, it is critical to consider that inflammatory conditions associated to infections, cancer and other diseases, might affect its circulating levels, leading to a further elevation. Due to this relative accuracy as iron marker, the estimation of iron overload based on serum ferritin threshold might be approximative, especially in those MDS patients presenting with inflammation, and clinically relevant for highly elevated iron. However, in clinical practice serum ferritin levels >1000ng/ml are considered a marker of iron overload and commonly used as cut-off for the initiation of iron chelation. Currently, monitoring serum ferritin is the approach most widely applied for the clinical evaluation of iron overload as well as iron chelation therapy decision-making in MDS patients, and changes in this parameter are still useful to assess iron chelation efficacy.
Elevated serum ferritin levels significantly affect overall survival and when higher than 3000 ng/mL are associated with increased predisposition to cardiac events in MDS.49

Magnetic resonance imaging - MRI

The use of invasive biopsies has been nowadays progressively replaced by the magnetic resonance imaging (MRI) assessment of hepatic and cardiac iron, a non-invasive and reproducible method to detect iron overload via T2* measurement.37 MRI enables to estimate liver iron content (LIC) in a highly accurate and specific manner and therefore its utility in the management of transfusional iron overload is becoming increasingly important.123 Because of cost reasons, this technique is not yet fully integrated into clinical practice but expected to become soon a standard of care because of its reliability and possibility to offer a window for iron detection into pre-symptomatic organ dysfunction.

NTBI and LPI

Tissue iron overload is thought to arise from NTBI/LPI accumulation.17,18,19 Both these parameters are increased in transfused and subtypes of non-transfused MDS patients (e.g. RARS).124 NTBI and LPI elevation in MDS is of pathological concern, warranting iron chelation, with the aim to reach undetectable LPI levels. Despite the potential clinical relevance and the effort made in the last years to improve available techniques, numerous challenges remain with laboratory standardization and harmonization for the measurement and interpretation of NTBI and LPI.125 Nowadays still few but increasing laboratories have established reproducible and reliable methodology for NTBI and LPI quantification. We predict that with methodology improvement, the measurement of these parameters will be more widely adopted in clinical laboratory setting as marker and indicator of iron toxicity in iron-loading anemias, including MDS.126 Recent works show in fact that LPI appears a real-time indicator of iron overload closely reflecting body iron status (hepatic iron content).127

Interventional options

Iron chelation

An alternative to RBC transfusions is represented by the use of ESA (erythropoietic stimulating agent, eg recombinant EPO). However, these therapies are available only for a limited subset of MDS patients, depending on the severity and risk-score of their disease.128 Being other options for the correction of anemia still scarce in MDS, therapeutic approaches to prevent iron overload, such as iron chelation, are valuable strategy to limit iron overload and its toxicities.

Iron chelation is an established standard of care and well supported by high-quality evidence for patients suffering from other iron-loading anemias such as β-thalassemia.129-133 A relevant survival benefit has been proven for thalassemia patients under ICT.2,10,134,135 However, there is still a deficit of controlled randomized clinical trials on ICT in MDS patients.

Recommendations differ from when to transfuse and how to monitor iron overload to when to initiate iron chelation, which is why chelation in MDS has been ever since controversial.2,10,136 Despite differences from country to country, current guidelines indicate that patients to consider for ICT are low-risk MDS patients with RA, RARS and 5q-deletion who show a serum ferritin threshold of 1000–15000 ng/mL and received 20 to 25 transfusions.47,137

Currently, three different iron chelators are available on the market for the treatment of iron overload: deferoxamine, deferiprone and deferasirox. These agents differ in route and timing of administration as well as side-effect profiles, The chelator properties of a relatively novel compound, eltrombopag, used will be also discussed.

Deferoxamine

Deferoxamine has been the first iron chelator available on the market. It can be administered via intravenous, intramuscular and subcutaneous injection.138 However, its short half-life and parenteral administration are particularly inconvenient and result in poor patient compliance in.45 As side effects, injection site reactions and ocular/otic toxicity might occur. Randomized studies have been performed mostly in β-thalassemia, whereas evidence for MDS is scarce.

Deferiprone

Deferiprone is a valuable alternative oral chelator for patients showing signs of intolerance to either deferoxamine or deferasirox. However, due to (very rare) neutropenias as major adverse effect, tight monitoring of blood counts has to be performed.139 Clinical trials on the use of deferiprone in MDS are also limited.

Deferasirox

Deferasirox is the most recent iron chelator which enables iron excretion by intestinal passage. Being an oral drug, deferasirox has a higher treatment adherence.140,141

Compared to deferiprone, deferasirox shows superior efficacy and milder side effect profile in low-risk MDS patients with iron overload.142 More clinical data are available on the use of this chelator in MDS, as its use has been investigated in multiple trials. Typical side effects may include gastrointestinal adverse events and renal insufficiency, whereas hepatotoxicity is rare.143,144 The new and more convenient formula of deferasirox as a film-coated tablet has been proven even better tolerated by patients (ECLIPSE II trial), with great advantages for adherence,145 compared to the previous powder formulation.

Eltrombopag, a potential novel chelator

Eltrombopag is an orally bioavailable small molecule acting as thrombopoietin receptor (TPO-R) agonist, used in treatment of thrombocytopenia in MDS. Despite not approved as chelator, eltrombopag’s iron chelating properties are emerging over the last years. Recently, eltrombopag was shown to scavenge extracellular iron and mobilize cellular iron from hepatocyte, cardiomyocyte and pancreatic cells, with an overall ROS reduction. When combined with iron chelators, eltrombopag synergistically enhances cellular iron mobilization through iron donation to other chelators.145 Eltrombopag has antiproliferative effects on leukemia cells by directly reducing free intracellular labile iron in a TPO-R-independent manner.146 Moreover, eltrombopag stimulates multilineage hematopoiesis in patients with bone marrow failure syndromes through a stimulatory effect on stem cell numbers/functions which requires its iron-chelation activity.147 Eltrombopag-mediated iron chelation triggers a
bene
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cial metabolic and molecular reprogramming of HSCs, indicating that the labile iron pool plays a role in modulating HSC function and its restriction has potential therapeutic value.147 Due to its iron chelation action, iron de
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ciency might be a complication of eltrombopag therapy, especially in non-iron loaded patients, whereas its administration might exert multiple beneficial effects dependent on both its TPO-R- and chelator-related activities in iron-loaded individuals. Clinical trials are needed to assess the iron chelating properties of EPAG and related therapeutic benefit in MDS patients.

Alternative and novel iron restriction strategies and erythropoiesis modulators

Novel promising iron restriction strategies different from iron chelators have been recently proven successful in mouse models of b-thalassemia in their ability to reduce iron overload and improve hematologic outcome, anemia and ineffective erythropoiesis. These strategies include iron scavenging by apotransferrin and iron restriction by hepcidin agonists (e.g. hepcidin mimetics; small molecule FPN inhibitors; pharmacologic inhibition of the hepcidin negative regulator Tmprss6).148–151 Hepcidin agonists offer the advantage to reduce iron absorption and sequester iron in macrophages, thus limiting further iron accumulation and NTBI formation in iron-loaded patients, when the endogenous synthesis of hepcidin is not appropriate. However, high doses of hepcidin agonists might trigger anemia due to excessive iron restriction, underlying the importance of a careful titration of these compounds to obtain the desired therapeutic action. Based on the positive results from preclinical studies, hepcidin agonists are currently being tested in clinical trials for b-thalassemia. Their potential therapeutic value in MDS has not been explored yet.

Novel compounds have been recently reported to improve anemia by promoting terminal erythropoiesis in MDS, namely luspatercept152–154 and sotatercept,155–157 in both preclinical studies and clinical trials.158 Although luspatercept has no direct effect on iron metabolism, the correction of ineffective erythropoiesis results in increased hepcidin levels and reduction of iron burden, highlighting the close interconnection between erythropoiesis and iron homeostasis.159,160 The identification of a member of the TGF
b
superfamily, GDF11, as negative regulator of erythropoiesis and target of these compounds has been recently challenged,149 leaving open the question how

| Table 1 | Retrospective and Prospective Observational Studies Showing a Survival Benefit for Patients With Iron Chelation. |
|---------|---------------------------------------------------------------------------------------------------|
| Clinical studies | MDS risk IPSS | Study design | Number of Patients ICT | Type of ICT | Survival | Chelated patients | Non-chelated patients | p value | Ref. |
| Leitch et al 2006 | Low Intermediate-1 | Retrospective | 18 | 18 Deferoxamine | Median OS | 226 months | 40 months | p = 0.003 | 165 |
| Rose et al 2010 | Low Intermediate-1 | Prospective | 53 | 40 Deferoxamine | Median OS | 124 months | 53 months | p = 0.0003 | 166 |
| Komrokji et al 2011 | Low Intermediate-1 | Retrospective | 45 | 35 Deferasirox | Median OS | 59 months | 34 months | p = 0.013 | 172 |
| Neukirchen et al 2012 | Low Intermediate-1 | Matched pair analysis | 94 | 47 Deferasirox mono | Median OS | 75 months | 49 months | p = 0.002 | 170 |
| Delforge et al 2014 | Follow-up of a retrospective multicenter study | 80 | 14 Deferasirox followed by Deferasirox | Median OS | 10.2 years | 3.1 years | p < 0.001 | 173 |
| Lyons et al 2014 | Low Intermediate-1 | Prospective, 5-year, non-interventional, multicenter study | 263 | 28 Deferasirox | Median OS | 88 months | 47.8 months | p < 0.0001 | 174 |
| Raptis et al 2010 | ICT-eligible Low risk subgroup analysis | 54 | 20 Deferasirox | Median OS | 8.7 months | 4.7 months | p = 0.02 | 175 |
| Langemeijer et al 2016 | Low Intermediate-1 | Prospective | 195 | 140 Deferasirox | Median OS | ICT vs non-ICT: HR 1.5; 95% CI, 1.1-2.0 | p = 0.0005 | 164 |
| Parmar et al 2015 | Low Intermediate-1 | Prospective | 70 | 6 Deferasirox | Median OS | 8.62 years | 4.38 years | p = 0.0005 | 176 |
luspaterecot and sotarecept mechanistically improve anemia, what are the underlying molecular pathways and whether an interaction with iron homeostatic mechanisms exists. Interest-
ingly, GDF11 has been proposed as negative regulator of hepcidin synthesis in addition to ERFE.26

As an alternative iron depletion strategy phlebotomy may be considered, even if not always tolerated in elderly MDS patients. After HSCT phlebotomies demonstrated decent results, with the advantage of being inexpensive, accessible and highly efficient in removing iron.161,162 The number of phlebotomies required to decrease ferritin levels to normal range may differ depending on the initial values.

Interventional studies

Prospective interventional trials convincingly showing the benefit of iron chelation are still scarce or with limited statistical power in MDS. So far a clearly improved outcome for iron chelated patients suffering from other chronic anemias, particularly b-thalassemia, has been shown but the translation of these data to MDS is complicated by the different underlying disease.163 Therefore, concern remains whether or not to use ICT in lower risk MDS patients and accept possible side effects without having actual definitive proof of its benefit. However, some prospective as well as retrospective observational studies showed improved overall survival in MDS patients undergoing iron chelation.164

Table 1 shares an overview of the main studies for ICT in MDS

In 2006 in a retrospective study Leitch et al165 showed a significant improved survival for patients with ICT (median OS 226 months) compared to patients without ICT (median OS 40 months). A German matched-pair analysis of 188 patients showed similar results.71 Patients were matched for age at diagnosis, gender, MDS type according to WHO classification, and IPSS score. All patients showed signs of iron overload (serum ferritin ≥1000ng/ml, or a history of multiple transfusions and a serum ferritin≥300ng/ml). For ICT patient median OS was 75 month and for supportive care patients 49 month. However, a significant difference in median OS (ICT vs Non-ICT) could only be shown for lower-risk patients and not for high-risk MDS. Another prospective study enrolled 97 outpatients (low and int-1 IPSS) requiring RBC transfusions.166 Patients treated with iron chelation (n=53) showed a median OS of 124 month from diagnosis compared to non-chelated patients with a median OS of 53 months. The 3 year-Prospective US-03 trial assessed deferasirox safety and efficacy in MDS patients. (Table 2) 173 iron-chelated patients were screened for median serum ferritin levels (year 1: -23.2%, year 2: -36.36%, year 3: -35.53%), labile plasma iron (normalized mean LPI), and hematological responses (red blood cell response: 15% (26/173), neutrophil response: 15% (8/52), platelet response: 22% (17/77)). During this study a reduction of serum ferritin and LPI could be shown for patients with Deferasirox, a subset of patients even improved in hematologic and hepatic parameters.167

In the prospective GIMEMA trial, 152 transfusion-dependent patients received deferasirox therapy.168 Here, iron chelation was associated with reduction in median serum ferritin levels (−490 ng/mL, p<0.001), median alanine aminotransferase and aspartate aminotransferase (as marker of hepatic dysfunction) as well as transfusion independence (15.5%) of enrolled patients.

Recently the European Leukemia Net MDS Registry and the Canadian MDS Registry analyzed survival improvement by ICT, with adjustment for patient-related factors, including age, frailty, comorbidity, disability, performance status, transfusion dependence severity and time to ICT. In the adjusted analysis, the overall survival in both studies remained significantly better in chelated versus non-chelated MDS patients, adding evidence of the benefit of ICT, independently of several other patient-related aspects.164,169

The EPIC study, a prospective, open-label trial investigated the efficacy of deferasirox in 341 iron-loaded lower-risk MDS patients (Table 2).143 The study focused on individualized starting doses of deferasirox, which were adapted to RBC transfusion frequency of each patient. Of 341 patients 165 were chelation-naive at baseline. Patients receiving ≥2 to 4 RBC transfusion per month received 20mg/kg/day of deferasirox. The dose was adjusted to 10 or 30mg/kg/day in patients received less or more frequent RBC transfusions. Over the course of the study dose adjustments of 5–10mg/kg/day were applied, up to a potential dose escalation of 40mg/kg/day, which however is clinically not achievable in the daily routine practise. The study showed a significant decrease in serum ferritin (−253ng/mL from baseline to 1 year of therapy, p=0.002) and LPI (normalized from above normal at baseline to within normal range after 1 year), which consistently occurred in chelation-naive as well as previously chelated patients.143 A post-hoc analysis of EPIC evaluated hematologic response to deferasirox in this patient cohort and was able to demonstrate a significant improvement of hematologic parameters, with a 21.5% red blood cell response, 22.0% neutrophil response and 13.0% platelet response.170 Of major concern for this study was the high rate of chelation discontinuation due to side effects, which are nowadays improved with the availability of a new deferasirox formulation.

Although these studies (Table 2) showed significant improvement in decreasing serum ferritin and LPI levels along with hematological response, TELESTO has been the first randomized, placebo-controlled trial to evaluate event-free survival, safety and efficacy of deferasirox in iron-overloaded MDS patients (mostly with intermediate-1 risk).144 For this trial, 225 patients were randomized (2:1) double-blind into deferasirox group (n=149) or placebo group (n=76). The study enrolled patients who have received between 15 and 75 red packed red blood cell units (mean deferasirox 20.28, placebo 20.27), had serum ferritin of ≥1000ng/mL, and were free from cardiac, liver, and renal abnormalities prior to randomization. Patients who had received six months of cumulative ICT (with either deferasirox or deferiprone) and transfusions for more than three years prior to enrollment were excluded. TELESTO’s composite primary endpoint was the evaluation of time to first non-fatal event (due to cardiac and liver impairment and transformation to AML) or death in the deferasirox versus placebo group.172 The results show a prolonged median event-free survival of patients in the deferasirox (1440 days) compared to placebo group (1091 days) with a 36.4% risk reduction in event-free survival. In addition, the three-year estimated rate of event-free survival was greater in chelated patients (61.5% vs 47.3%). Relevant toxicities were not observed in the deferasirox versus placebo group. Overall, this study provided evidence of the clinical benefit of ICT by showing longer event-free survival, fewer cardiac- and liver-related events, and fewer transformations to AML in chelated MDS patients compared to the placebo group. However, the median overall survival was not significantly different. Patient recruitment unfortunately did not meet the expectation which might have limited the outcome and statistical power of this clinical trial.
Table 2
Prospective Interventional Studies of ICT in MDS Patients.

| Clinical studies | MDS n | MDS n | Study design | Number of patients with ICT | Type of ICT | Hematological response | Change in serum ferritin | Change in LPI | Change in transferrin saturation | Survival | Ref. |
|------------------|-------|-------|--------------|-----------------------------|------------|-----------------------|------------------------|-------------|----------------------------------|----------|------|
| Gattermann et al EPIC 2010 | 341 (MDS only, all patients: n=1744) | Low Intermediate-1 | Prospective, open-label multicenter | 341 | Deferasirox | RBC response: 21.5% (53/247) (p=0.002) | −253 ng/mL | Normalized (from above normal at baseline to within normal range) | Not reported | 1,7,14,43 |
| List et al US03 2012 | 176 | Low Intermediate-1 | Prospective, multicenter trial, 3 years | 173 | Deferasirox | RBC response: 15% (26/173) | −23.2% (year 1) | Normalized | 91% at baseline | 67% at EOS | 1,67 |
| Angelucci et al GIMEMA 2014 | 152 | Low Intermediate-1 | Prospective open-label, single arm multicenter | 152 | Deferasirox | 15.5% transfusion independent | −490 ng/mL (p=0.001) | Not reported | Not reported | 1,68 |
| Angelucci et al TELESTO 2018 | 225 | Low Intermediate-1 | Prospective, randomized, double-blind study | 149 | Deferasirox | Not reported | Not reported | Not reported | composite primary endpoint: death or non-fatal event | 1440 days Median Event-free survival (ICT) vs 1091 days Median Event-free survival (non-ICT) Estimated event-free survival at 3 years: 61.9% (ICT) vs 47.3% (non-ICT) Median overall survival 1907 days (ICT) vs 1509 days (non-ICT) | 1,44 |
**Table 3**

Major Controversies on Iron Overload and Iron Chelation in MDS.

| Major Controversies on IO and ICT in MDS | Impact on Overall survival vs cardio-vascular functionality | Impact on liver functionality vs NTBI and LIC levels | Impact on bone marrow micro-environment vs NTBI triggered | Impact on allo HCT vs Deferasirox | Impact on leukemic progression vs NTBI and LIC |
|------------------------------------------|---------------------------------------------------------------|-----------------------------------------------------|----------------------------------------------------------|-------------------------------|---------------------------------------------|
| How to define iron overload              | Serum ferritin levels vs MDA assessment vs NTBI and LIC levels | Serum ferritin and/or LIC levels                     | Serum ferritin and/or LIC levels                         | Serum ferritin and/or LIC levels | Serum ferritin and/or LIC levels            |
| When to initiate iron chelation          | Serum ferritin > 1000 μg/ml vs depending on RBC units         | NTBI triggered                                       | NTBI triggered                                           | NTBI triggered                                           | NTBI triggered                                           |
| Which ICT to use                         | Deferasirox vs Deferoxamine                                   | Deferasirox vs Deferoxamine                          | Deferiprone vs Deferoxamine                              | Deferiprone vs Deferoxamine                              | Deferiprone vs Deferoxamine                      |
| Response monitoring in ICT               | Serum ferritin vs Serum ferritin and LIC                      | NTBI triggered                                       | NTBI triggered                                           | NTBI triggered                                           | NTBI triggered                                           |

**Considerations on MDS patients who would benefit from iron chelation therapy**

Despite the large amount of evidence on the benefit of ICT in patients with MDS, a critical evaluation of patient-related parameters (such as age, comorbidities including gastrointestinal and renal function, patient’s adherence) has to be carried out before the initiation of ICT in iron-overloaded MDS patients. The major side effects associated with the administration of deferasirox, the most commonly used and recommended chelator in MDS, include gastrointestinal or renal impairment. Therefore patients need to be well selected and informed prior to therapy. Despite these considerations, it is sometimes challenging to decide when to initiate chelation therapy in an individual patient. International guidelines mostly recommend to use chelation therapy in low-risk MDS patients with a high serum ferritin level (usually at least > 1000 ng/mL) and transfusion history of about 25 units of packed RBCs. Selected patients with high-risk MDS undergoing hypomethylating agents (HMA)-based treatment and a life expectancy exceeding 1 year may also be candidates. In addition, all eligible MDS patients that are expected to undergo HSCT should be considered for ICT prior to conditioning.

**Conclusions**

Controversies regarding the consequences of IO and chelation in MDS (Table 3) mostly arise from the fact that still limited data are available from preclinical studies and randomized prospective trials and that observations on iron toxicity made in transfusion-dependent β-thalassemia patients are in most cases transposed, by extension, to those with lower-risk MDS without substantial evidence. Skepticism is driven by the differences in the underlying disorder, whereby MDS, even though sharing features of ineffective erythropoiesis, is a stem cell disease, developing in the elderly and presenting with comorbidities that might overlap to those induced by iron overload. Countless data support the concept that iron toxicity affects tissue function and erythropoiesis but most of them remain hypothetical and are mainly supported by evidence from in vitro system, in vivo models of ‘artificially’-induced iron overload and retrospective clinical studies. Similarly, the use of iron chelation in low-risk MDS patients is debated ever since. Novel data from the prospective randomized TELESTO trial are an important milestone and, despite a limited statistic power, confirm the beneficial effect of chelation in MDS, with a superior event-free survival in chelated patients. Still, at the moment, the final decision on management of iron overload is left to the treating physician. Further research in preclinical models of pathophysiologic iron overload is needed to explore novel mechanisms of iron-induced toxicity, paving the way for new randomized trials in MDS patients.

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