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Hematologic improvement with iron chelation therapy in myelodysplastic syndromes: Clinical data, potential mechanisms, and outstanding questions

Heather A. Leitch[a,*], Norbert Gattermann[b]

[a] Hematology, St. Paul's Hospital and the University of British Columbia, Vancouver, BC, Canada
[b] Dept. of Hematology, Oncology and Clinical Immunology, Heinrich Heine University, Düsseldorf, Germany

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ABSTRACT

Myelodysplastic syndromes (MDS) are clonal hematopoietic stem cell disorders characterized by cytopenias and progression to acute myeloid leukemia (AML). Although several treatments for MDS are available, the mainstay of therapy for most patients remains supportive care. This includes red blood cell (RBC) transfusion to correct anemia, which leads to iron overload. RBC transfusion dependence and iron overload portend inferior overall survival. Some studies indicate that iron chelation therapy (ICT) may have beneficial effects on clinical endpoints in MDS; however, these data are from non-randomized trials and the validity of the results is vigorously debated. A consistent observation in clinical studies of ICT in MDS has been hematologic improvement (HI) in some patients, including a reduction in RBC transfusion requirements and even transfusion independence. Here, we review data on HI with ICT in lower risk MDS, preclinical data examining mechanisms by which HI may occur, and identify areas for future investigation.

Abbreviations: αKG, α-ketoglutarate; AML, acute myeloid leukemia; AMPK, adenosine monophosphate kinase; ARNT, aryl hydrocarbon receptor nuclear translocator, also known as hypoxia inducible factor 1β; BCL-2, B-cell lymphoma 2; BFU-E, burst forming unit erythroid; β-TM, β-thalassemia major; c-FOS, cellular FBJ osteosarcoma virus; CFU-E, colony-forming unit erythroid; CFU-GM, colony-forming unit granulocyte macrophage; CFU-Mix, colony-forming unit mix; CXCL12, C-X-C motif chemokine 12; Cys, cysteine; DFO, deferoxamine; DFP, deferiprone; DFX, deferasirox; DMT1, divalent metal transporter 1; DNA, deoxynucleic acid; dROM, derivatives of reactive oxygen metabolites; DSB, double strand breaks; E2F1, E2F transcription factor 1; eLPI, enhanced labile plasma iron; ETC, electron transport chain; EPO, erythropoietin; ESA, erythropoiesis-stimulating agents; FAB, French-American-British; Fe^{2+}, ferrous; Fe^{3+}, ferric; Fe–S, iron–sulfur cluster; GCS, γ-glutamyl cysteine synthetase heavy subunit; GDF, growth differentiation factor; GI, gastrointestinal; GLUD, glutamate dehydrogenase; GLUL, glutamate-ammonia ligase; GM-CSF, granulocyte macrophage colony-stimulating factor; GPX, glutathione peroxidase; GRIM, genes associated with the retinoid-interferon-induced mortality; GSH, glutathione; H-, heavy; H_2O_2, hydrogen peroxide; HI, hematologic improvement; HIF-1α, hypoxia inducible factor 1α; HMA, hypomethylating agent; HOX, homeobox; HPC, hematopoietic progenitor cell; HSC, hematopoietic stem cell; HSPC, hematopoietic stem/progenitor cell; IC_{50}, 50% inhibitory concentration; ICAM, intracellular cell adhesion molecule; ICT, iron chelation therapy; IOL, iron overload; IκBα, nuclear factor of κ light polypeptide gene enhancer in B-cells inhibitor, α; iNOS, inducible nitric oxide synthase; IPSS, International Prognostic Scoring System; IRE, iron response element; IRP, iron responsive protein; IWG, International Working Group; JNK, c-Jun *N*-terminal kinase; L-, light; LCI, labile cellular iron; LDH, lactate dehydrogenase; LIP, labile iron pool; LPI, labile plasma iron; MAPK, mitogen activated protein kinase; MCV, mean cellular volume; MDA, malonyldialdehyde; MDS, myelodysplastic syndrome; MDS-CI, MDS comorbidity index; MnSOD, manganese superoxide dismutase; mRNA, messenger ribonucleic acid; MSC, mesenchymal stem/stromal cells; mTOR, mammalian target of rapamycin; NAC, *N*-acetyl cysteine; NADP/H, nicotinamide adenine dinucleotide phosphate (oxidized, H = reduced); NFκB, nuclear factor κB; NK, natural killer; NOX, NADP/H oxidase; NRAS, neuroblastoma retrovirus-associated DNA sequences; NS, not significant; NTBI, non-transferrin bound iron; NUP, nuclear pore complex protein; OCT-4, octamer-binding transcription factor, also known as POU5F1; OGG1, 8-oxoguanine glycosylase; OS, overall survival; PKC, protein kinase C; POU5F1, OCT-4, octamer-binding transcription factor; PYGL, glycogen phosphorylase, liver form; RANTES, regulated on activation, normal T cell expressed and secreted; RBC, red blood cell; RCMD, refractory cytopenia with multilineage dysplasia; REDD1, regulated in development and DNA damage response 1; RES, reticuloendothelial system; Rev-1, DNA repair protein; RIP, receptor interacting protein; ROS, reactive oxygen species; SCF, stem cell factor; SCT, stem cell transplantation; SF, serum ferritin; SOX, sex determining region Y-box; STAT, signal transducer and activator of transcription; Tc1, cytotoxic T cell type 1; Tc2, cytotoxic T cell type 2; TCA, tricarboxylic acid; TfR, transferrin receptor; Th1, T helper cell type 1; Th2, T helper cell type 2; TLR, toll like receptor; TNF, tumor necrosis factor; Treg, regulatory T cell; TSC, tuberous sclerosis; VEGF, vascular endothelial growth factor; WHO, World Health Organization; 2-HG, 2-hydroxyglutarate; 8-OG, 7,8-dihydro-8-oxoguanine; 8-OH-dG, 8-hydroxy-2′-deoxyguanosine

* Corresponding author at: 440–1144 Burrard Street, Vancouver, BC, V6Z 2A5, Canada.
E-mail address: hleitch@providenchematology.com (H.A. Leitch).

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1. Introduction

The myelodysplastic syndromes (MDS) are a group of acquired clonal hematopoietic stem cell disorders characterized by cytopenias and progression to acute myeloid leukemia (AML). Overall survival (OS) and AML progression risk are predicted by the International Prognostic Scoring System (IPSS) and newer scores (Greenberg et al., 1997, 2012; Kantarjian et al., 2008; Malcovati et al., 2007; Schanz et al., 2012). Lower risk MDS is characterized by ineffective hematopoiesis and cytopenias, with many patients having a relatively long survival and low risk of AML progression. Higher risk MDS, in contrast, is characterized by genomic instability, short expected survival and substantial risk of progressing to AML. Several treatments are available for patients with MDS, including erythropoiesis-stimulating agents (ESA), lenalidomide, and hypomethylating agents (HMA), which improve cytopenias and may improve OS. Luspatercept, a ligand-trapping substance binding the transforming growth factor-β family member growth differentiation factor (GDF)-11, has also shown great promise in clinical trials and may become another erythropoiesis-supporting drug for treating MDS in the foreseeable future (Platzbecker et al., 2017a). However, the mainstay of therapy for the majority of patients with MDS remains supportive care (Fenaux et al., 2010; List et al., 2006; Platzbecker et al., 2017a, b). Without specific MDS treatment, cytopenias inevitably progress, and most patients with MDS at some point in the course of their disease require transfusion of red blood cells (RBC) because of anemia (Balducci, 2006). RBC transfusion dependence portends inferior OS with or without MDS progression, and negatively impacts on patients' quality of life (Buckstein et al., 2009; de Swart et al., 2015; Malcovati et al., 2005; Sanz et al., 2008). Because RBCs contain significant amounts of iron and the body has no mechanism to excrete excess iron, iron overload (IOL) inevitably develops. Excess iron can deposit in the tissues and organs, including the liver, heart, endocrine organs, and bone marrow, leading to clinical sequelae, and some data indicate that IOL is associated with inferior OS in patients with MDS (De Swart et al., 2011; Malcovati et al., 2005; Pileggi et al., 2017; Sanz et al., 2008). Clinical endpoints may be impacted at least in part by the presence of labile iron and oxygen free radicals, formed through Fenton chemistry because of the ability of iron to transfer electrons (Fig. 1).

Iron chelation therapy (ICT) refers to medications that bind iron, rendering it non-toxic and in a form that is excretable by the body. The benefits of ICT in congenital anemias such as β-thalassemia major (β-TM) are well established, including improvement in organ function and OS (Brittenham et al., 1994; Davis and Porter, 2000; Olivieri and Brittenham, 1997). Patients with β-TM are transfused from a young age, and an association between organ iron reduction and clinical outcome is well documented (Borgna-Pignatti et al., 2004; Davis et al., 2004; Gabutti and Piga, 1996; Telfer et al., 2000). In contrast, the median age of onset of MDS is 65–70 years. Since it takes months to years to develop clinically relevant IOL, and months to years to offload significant amounts of organ and total body iron, the relevance of IOL and ICT in patients with a shorter life expectancy has been called into question. However, the organs of patients with MDS are also older, possibly making them more susceptible to a deleterious effect of iron. A number of studies indicate that ICT in patients with MDS may have beneficial effects on organ function and OS; however, these data are from non-randomized studies and the results are therefore debated, pending full results from the prospective, randomized controlled trial of ICT with deferasirox (DFX) in MDS (NCT00940602) (Abraham et al., 2017; Angelucci et al., 2018; Chee et al., 2008; ClinicalTrials.gov, 2016; Gattermann et al., 2010b; Guariglia et al., 2011; Hoeks et al., 2017; Leitch, 2007; Leitch et al., 2008, 2017b; Lyons et al., 2017; Mainous et al., 2014; Neukirchen et al., 2012; Remacha et al., 2015; Rose et al., 2010; Steensma, 2009; Tefferi and Stone, 2009; Wong and Leitch, 2018; Zeidan et al., 2015).

A consistent, and initially surprising observation in clinical studies of ICT in MDS is hematologic improvement (HI) in a significant minority of patients, including a reduction in RBC transfusion requirements and even transfusion independence (Angelucci et al., 2014; Gattermann et al., 2012; Nolte et al., 2013). This is an effect that does not occur in β-TM and may be relevant to clinical outcomes such as survival in MDS. Other factors that may contribute to a possible survival benefit in patients receiving ICT are an improvement in organ function (Gattermann et al., 2010a; List et al., 2012); reduced infection (Wong et al., 2018); decreased progression to AML (Lyons et al., 2017); and improved outcomes around hematopoietic stem cell transplantation (SCT; reviewed in (Leitch et al., 2017a)). In this review, we summarize data on HI in patients with MDS receiving ICT, focusing on erythroid improvement in lower IPSS risk patients in the usual (non-SCT) clinical setting. We also review preclinical studies examining the mechanisms by which IOL may suppress hematopoiesis and by which HI with ICT may occur and identify areas for future investigation of this phenomenon. Although there are multiple reports of HI in other bone marrow failure syndromes, including aplastic anemia and primary myelofibrosis, this review focuses on MDS (Cheong et al., 2014; Di Tucci et al., 2007; Park and Han, 2008).

1.1. Iron physiology

Iron is essential for living organisms, but it must be regulated as excess iron is toxic and the body has no mechanism to excrete an excess. Iron is necessary for heme and hemoglobin production, while heme and iron–sulfur clusters are incorporated into many enzymes. Iron is involved in oxygen transport and exchange, cellular respiration, metabolic reactions, DNA synthesis and repair, ribosome function and translation to polypeptides, proliferation, and inflammation. Because iron is able to transfer electrons, it switches between ferrous (Fe^{2+}) and ferric (Fe^{3+}) states. Iron is stored in macrophages and hepatocytes as ferritin or hemosiderin. The serum ferritin (SF) level is a reasonable reflection of body iron stores, and despite its drawbacks, such as fluctuations influenced by factors including inflammation, it remains the most convenient measure of iron load in clinical practice. Most body iron is found in hemoglobin, with additional iron in myoglobin, cytochromes, and other enzymes (Kohgo et al., 2008; Leitch and Thachil, 2016; Muckenthaler et al., 2017).

Under normal conditions, about 1–2 mg of iron is absorbed daily via the gastrointestinal (GI) tract, and the same amount is lost by turnover of GI and skin epithelial cells. Dietary iron is largely in the Fe^{3+} form, but the intestinal epithelial iron importer, divalent metal transporter 1 (DMT1), transports only Fe^{2+}. Iron is reduced by duodenal cytochrome *B* and transported into enterocytes, where it can be stored; for this, Fe^{2+} is oxidized to Fe^{3+} by the H-subunit of ferritin. Alternatively, Fe^{2+} may be transported into the portal circulation via ferroportin. Once transported across the basolateral membrane, iron is oxidized to

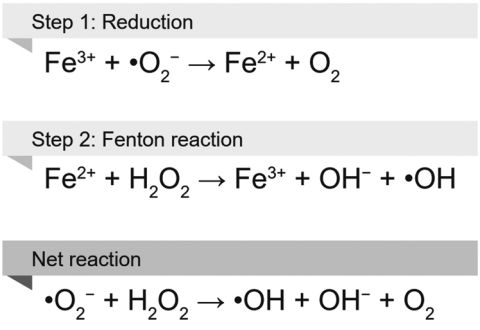

Fig. 1. The Haber–Weiss and Fenton reactions, which result in the generation of reactive oxygen species in the presence of iron.
Fe^{2+}, ferrous iron; Fe^{3+}, ferric iron; H_2O_2, hydrogen peroxide; OH^-, hydroxide; $^{\cdot}OH$, hydroxyl radical; $^{\cdot}O_2^-$, superoxide; O_2, oxygen. Modified from Koppenol WH. Redox Rep 2001;6(4):229–34, with permission.

Fe^{3+} by ceruloplasmin. Intestinal iron absorption is negatively regulated by hepcidin, which binds to ferroportin, inducing its internalization and proteasomal degradation. Hepcidin levels are in turn regulated by iron signals, erythropoiesis, and inflammation (Muckenthaler et al., 2017). The erythropoietic demand for iron seems to be signaled, at least in part, by erythroferrone, produced by erythroblasts, although other as yet undefined mediators may be involved (reviewed in (Kohgo et al., 2008; Leitch and Thachil, 2016)).

Under usual conditions, iron is transported by transferrin in the circulation and taken up into cells via the transferrin receptor; normal transferrin saturation is 15–50%. Each transferrin molecule can bind two molecules of Fe^{3+}. Diferric transferrin (holotransferrin) binds to the transferrin receptor (TfR1), is taken up by receptor-mediated endocytosis, then released by acidification and transported into the cytoplasm by DMT1. Regulation of the synthesis of TfR1, DMT1, ferroportin, and ferritin are controlled at a post-transcriptional level via iron response elements (IREs) on their RNA that bind iron regulatory proteins (IRPs)-1 and -2. When iron is low, the conformation of IRP-1 allows it to bind to the 3′ IRE of TfR mRNA, stabilizing it and allowing transcription of more TfR protein, and to the 5′ IRE of ferritin mRNA, decreasing ferritin translation. Cellular iron storage is mainly in ferritin, a complex of subunits that renders iron redox inactive and insoluble. The function of circulating ferritin is incompletely understood (Kohgo et al., 2008; Leitch and Thachil, 2016; Muckenthaler et al., 2017).

Cells maintain a pool of labile iron (labile cellular iron; LCI) by controlling iron uptake via expression of receptors and storage via ferritin. Most cells have no mechanism for iron efflux; exceptions are GI epithelial cells (on the basolateral surface), macrophages of the reticuloendothelial system (RES) and hepatocytes. Causes of IOL include repeated blood transfusions, ineffective erythropoiesis, and mutations in genes leading to decreased production of or resistance to hepcidin (Kohgo et al., 2008).

2. Clinical data on hematologic improvement with iron chelation in MDS

Initial data describing HI with ICT in MDS was a case series reported by Jensen et al (Jensen et al., 1996, 1992). In this study, patients received deferoxamine (DFO), a parenteral agent which, because of a short half-life, is given as a continuous subcutaneous infusion over at least 8–12 h/day. A significant reduction in transfusion requirements occurred in three patients (Jensen et al., 1992). In an extension study, 11 patients received ICT for 6–60 months, and the hemoglobin requirement decreased in seven patients. Five patients became transfusion independent at a median duration of 20 months of ICT, and remained transfusion independent for 3 months to 3 years (Jensen et al., 1996).

Several case reports of erythroid improvement in patients with MDS or other acquired anemias followed. Although most patients with erythroid improvement were receiving the oral chelator DFX, there was one report of HI using deferiprone (Badawi et al., 2010; Del Río Garma et al., 1997; Di Tucci et al., 2007; Guariglia et al., 2011; Latagliata et al., 2016; Messa et al., 2008b; Sanford and Hsia, 2015; Smeets et al., 1996). In some patients, transfusion independence was maintained for several months after stopping ICT (Kochhar et al., 2015). An improvement in mean cellular volume (MCV) was also seen in some patients with erythroid response, suggesting a contribution to erythropoiesis from normal erythroid precursors. However, in other patients, no change in MCV was seen, suggesting that erythroid improvement may have originated with MDS progenitors (Jensen et al., 1992). Case reports of patients with MDS achieving transfusion independence with ICT are summarized in Table 1. Other patients with MDS and erythroid improvement who did not become transfusion independent have been reported (Jensen et al., 1992; Messa et al., 2008a). These results were initially surprising, as with ICT the goal was to control SF levels and organ iron, rather than to improve hematopoiesis. However, several reports in sizeable cohorts followed, as discussed below.

Investigators for the Evaluation of Patients' Iron Chelation with Exjade (DFX dispersible formulation; EPIC) trial in patients with transfusion-dependent anemias performed a *post hoc* analysis examining the HI rate in 341 patients with MDS, using International Working Group (IWG) 2006 criteria (Cheson et al., 2006; Gattermann et al., 2012). To be evaluable for erythroid response, patients had a hemoglobin < 110 g/l and were not receiving ESA. The number of patients eligible for analysis of erythroid, neutrophil and platelet responses was 247, 50 and 100, respectively. The median DFX dose (dispersible formulation) was 19 mg/kg/day and median duration of chelation 12 months. Rates of improvement in the erythroid, neutrophil and platelet lineages were 21.5%, 22% and 13%, respectively, and ongoing HI rates at 1 year were 60%, 0% and 80%, respectively. There were no significant differences between patients with and without HI in the reduction of SF levels, or in suppression of labile plasma iron (LPI). However, LPI was probably suppressed very rapidly in all treated patients, and could thus not be expected to be a good discriminator, as discussed further in Section 5 (Gattermann et al., 2012). This study gives a denominator to the HI previously reported in only a handful of cases; a substantial minority of patients with MDS experienced HI during ICT.

Several other analyses have described HI rates in patients with MDS, with erythroid improvement rates ranging from 7% to 45%. In two studies, the rates of RBC transfusion independence were 11% and 9% overall (Angelucci et al., 2014; Molteni et al., 2013). One study examined the cumulative incidence of transfusion independence over time and is discussed in more detail below (Angelucci et al., 2014).

In the US03 trial of DFX in 176 patients with lower IPSS risk MDS, 173, 52 and 77 patients were eligible for analysis of erythroid, neutrophil and platelet responses, respectively. The mean DFX dose was approximately 20 mg/kg/day and median duration of ICT 12 months. Fifty-one patients (28%) had HI by IWG 2006 response criteria while 26 (15%), 8 (15%) and 17 (22%) patients had erythroid, neutrophil and platelet responses, respectively. The median time to response was 5.6 (range 2.8–12.7) months. LPI reduction did not differ between hematologic responders and non-responders (List et al., 2012).

In one study of 40 patients with lower IPSS risk MDS receiving a median dose of DFX of 1125 mg/day for a median duration of 12 months, four patients (10%) achieved an erythroid response. Among responders, the median RBC transfusion requirement decreased from 5.5 to 1 unit/month (Breccia et al., 2012). In another study of 50 patients with lower IPSS risk MDS receiving DFX at a mean daily dose of 19 mg/kg/day, 33, 6 and 10 patients were evaluable for analysis of erythroid, neutrophil and platelet responses, with 2 (6%), 1 (17%) and 3 (30%) responses, respectively (Nolte et al., 2013).

In a study of 51 patients with MDS and two patients with myelofibrosis, 36 received DFX and 17 DFO. Fifty-three, 11 and 13 were eligible for erythroid, neutrophil, and platelet response analysis, respectively, with 18 (34%), 5 (45%) and 7 (54%) responses, respectively. The rate of RBC transfusion independence was 9%. The median time to response was 3 months, and median response duration 6, 8 and 9 months for erythroid, neutrophil and platelet responses, respectively. None of the following factors were predictive for erythroid or any hematologic response in multivariate analysis: age; RBC transfusion burden; neutrophil and platelet counts; ICT agent; serum iron; serum transferrin; SF; lactate dehydrogenase (LDH); erythropoietin (EPO) level; SF reduction; specific French-American-British (FAB) or World Health Organization (WHO) MDS subtype; number of cytopenias; cytogenetic risk; presence of fibrosis; and cellularity (Bennett et al., 1982; Molteni et al., 2013; Swerdlow et al., 2008). The authors commented that the absence of predictors of response may have been due to small sample size.

In 159 patients with lower IPSS risk MDS receiving DFX, 68 (43%) continued on ICT at 12 months. Patients receiving other medications

Table 1
Characteristics of 14 patients with MDS reported in the literature who achieved transfusion independence after receiving iron chelation therapy.

Pt	Age at diagnosis (years)	Gender	MDS type	IPSS score	Initial transfusion requirement (RBC units/mo)	Initial Hb requirement (g/mo)	ICT agent and dose	Approximate time to TI (mo)	Duration of TI (mo)	Hb before ICT (g/l)	Hb during ICT (g/l)	Ref
1	47	F	RA	Low	NR	350	DFO*	24	3+	87	NR	(Jensen et al., 1996)
2	46	F	RA	Int-1	NR	251	DFO*†	20	24+	88	NR	(Jensen et al., 1996)
3	69	M	RARS	Int-1	NR	339	DFO*	20	3+	96	NR	(Jensen et al., 1996)
4	41	F	RA	Int-1	NR	174	DFO*†	36‡	20+	86	NR	(Jensen et al., 1996)
5	18	F	RAEB	Int§	NR	186	DFO*	20	6+	89	NR	(Jensen et al., 1996)
6	71	F	RA	NR	NR	141	DFO¶	12	NR	78	101	(Del Río Garma et al., 1997)
7	25	M	RARS	NR	NR	244	DFO¶	15	NR	65	143	(Del Río Garma et al., 1997)
8	71	M	RCMD	Int-1	4	NR	DFX**	12	NR	83	100††	(Messa et al., 2008b)
9	74	M	RAEB	High	4	NR	DFX**	3	NR	75	100‡‡	(Messa et al., 2008b)
10	69	M	RA	Int-1	3	NR	DFX**	2	28+	60	122	(Badawi et al., 2010)
11	74	M	MDS-U	Int-1	TD-NOS	NR	DFX**	1.5	12+	80	105	(Guariglia et al., 2011)
12	63	F	RARS-T	Low	2.5	NR	DFX**	1.5	22	76	133	(Kochhar et al., 2015)
13	74	M	RCUD-A	Low	3	NR	DFX**	12	60+	< 80	130	(Sanford and Hsia, 2015)
14	48	M	NR	Low	2	NR	DFX**	2	29+	NR	95	(Oliva et al., 2010)

* 2 g/day, 5 days/week; †5 days/week; ‡ICT was stopped at 20 months; §Blast count not be reported so IPSS score could not be determined more exactly; ¶30 mg/kg, 3 days/week; **20 mg/kg/day, dispersible formulation; ††At 12 months of ICT; ‡‡At 3 months of ICT; DFO, deferoxamine; DFX, deferasirox; F, female; Hb, hemoglobin; ICT, iron chelation therapy; Int-1, intermediate-1; IPSS, International Prognostic Scoring System; M, male; MDS, myelodysplastic syndrome; MDS-U, MDS unclassifiable; mo, months; NR, not reported; Pt, patient; RA, refractory anemia; RAEB, refractory anemia with excess blasts; RARS, refractory anemia with ringed sideroblasts; RARS-T, RARS with thrombocytosis; RCMD, refractory cytopenia with multilineage dysplasia; RCUD-A, refractory cytopenia with unilineage dysplasia, anemia; Ref, reference; TD-NOS, transfusion dependent not otherwise specified; TI, transfusion independence.

that could influence hematologic response were excluded. The median dose of DFX was 10–15 mg/kg/day. The number of patients eligible for erythroid, neutrophil and platelet response analysis was 145, 125 and 125, respectively, with responses in 16 (11%), 4 (3%) and 19 (15%) patients, respectively. None of the following factors were associated with achieving transfusion independence: age; IPSS risk; MDS comorbidity index (MDS-CI); interval from MDS diagnosis to enrollment; number of RBC units transfused; duration of transfusion dependence; baseline SF; and cytogenetics; however, this analysis was also limited by small numbers. The cumulative incidence of transfusion independence, adjusted for death and disease progression, at 6, 9 and 12 months was 2.6%, 12.3% and 15.5%, respectively (Angelucci et al., 2014; Della Porta et al., 2008).

One study examined 118 patients with all IPSS risk groups of MDS receiving DFX; 13.5% had intermediate-2 or high IPSS risk. The median dose and duration of DFX was not reported, nor were numbers eligible for response analysis. Erythroid, neutrophil and platelet responses were seen in 17.6% (7.1% transfusion independent), 7.1% and 5.9%, respectively, in patients who were not receiving other agents (azacitidine, lenalidomide, or ESA). In patients receiving azacitidine or lenalidomide, erythroid improvement rate was 18.2% (n = 6) and one (3%) patient became transfusion independent. The SF level decreased from a median of 1790 to 1304 ng/ml at 12 months of chelation ($p < 0.0001$) and transaminases normalized in 38.1% of patients. Higher starting doses of DFX were associated with transfusion independence at 24 months ($p = 0.02$) (Maurillo, *et al* 2015). Studies examining HI in cohorts of ≥ 40 patients with MDS are summarized in Table 2.

Differences in response rates between studies may be attributable to differences in inclusion/exclusion criteria, pre-ICT hemoglobin levels, RBC transfusion requirements, degree of IOL, and the dose and duration of ICT. Important questions that remain unanswered include factors predictive of HI in patients with MDS receiving ICT, and whether the rate of HI varies between MDS subtypes. Regarding the first point, a preliminary study suggested that HI with DFX may be related to starting the medication at a low dose (5–10 mg/kg/day of the dispersible formulation), which appears to be associated with alterations in expression of the nuclear transcription factor nuclear factor κB (NFκB) and to be independent of iron reduction; this observation is discussed further in the section on potential mechanisms of HI (Messa et al., 2010; Meunier et al., 2017). In contrast, another study suggested that higher starting doses of DFX were associated with transfusion independence (Maurillo et al., 2015). Regarding the second point, there are differences in iron physiology between MDS subtypes that are incompletely understood. For example, hepcidin levels vary widely between subtypes, suggesting that the contribution of GI iron absorption and release from the RES to IOL will vary by subtype, possibly impacting on relative levels of non-transferrin bound iron (NTBI) and the redox-active LPI, as discussed further in Section 4.3. In addition, GDF-15 and EPO levels differ between MDS subtypes (Cui et al., 2014; Santini et al., 2011; Zipperer et al., 2013). For example, in patients with refractory anemia with ringed sideroblasts, SF levels were higher, hepcidin levels lower, and erythroblasts, transferrin saturation and GDF-15 levels were higher, all indicative of increased (ineffective) erythropoiesis (all $p < 0.05$; (Zhu et al., 2016)). Similarly, gene expression of the more recently described iron regulator erythroferrone varies between MDS subtypes, though protein levels have not yet been documented (Mossner et al., 2014). In one analysis, the endogenous EPO level and diagnosis of refractory cytopenia with multilineage dysplasia (RCMD) were significantly associated with achieving transfusion independence in univariate analyses, but neither factor retained significance in multivariate analysis (Molteni et al., 2013). Similarly, three of 15 (20%) patients with lower IPSS risk RCMD receiving ICT had clear erythroid improvement, including one who achieved long-term RBC transfusion independence (Wong and Leitch, 2018). Clarification of which patients are more or less likely to experience HI with ICT would be helpful in clinical practice. More information on these points may become available from the TELESTO trial; however, given that the sample size was reduced by two-thirds due to slow enrollment, it may no longer be powered to illuminate these points (Angelucci et al., 2018). One point that is clear is that patients who achieve a significant reduction in transfusion requirements with ICT may experience a significant improvement in quality of life (Badawi et al., 2010; Buckstein et al., 2009; Efficace et al., 2016).

Three studies examined HI with ICT in higher IPSS risk MDS. In the first, 51 patients received ICT, 36 concomitantly with azacitidine. Findings included a decrease in SF levels over the period of chelation. One patient receiving ICT without other therapy achieved RBC transfusion independence lasting 12 months until interruption of DFX because of a normal SF level, and regained transfusion independence for another 11 months upon re-introduction of DFX (Musto et al., 2014). A second study examined 29 patients receiving azacitidine, 17 of whom also received DFX at 10 mg/kg/day (dispersible formulation). Of 18 patients with any hematologic response by IWG 2006 criteria, 15 had received DFX (Improta et al., 2013). In a third study of 98 patients, 13 with higher risk MDS, the median time to HI was 15 and 3 months for DFO and DFX, respectively. The response to DFO appeared to parallel a reduction in SF levels, but the same was not true for DFX (Messa et al., 2017).

3. Mechanisms of hematologic suppression from iron overload

In this section, we examine mechanisms by which IOL may have a suppressive effect on hematopoiesis. Proposed mechanisms include: direct suppressive effects of ferritin; and oxidative stress-induced damage to macromolecules leading to cell death or malignant progression. Progress has been made in elucidating some of the molecular pathways involved in these effects, which are described below.

3.1. Hematopoietic stem cell physiology and pathophysiology

The regulation of hypoxia, metabolism, cell proliferation and the microenvironment is an area of active investigation. Hematopoietic stem cells (HSC) reside in the stem cell niche, adjacent to the sinusoids in the bone marrow (Parmar et al., 2007). Oxygen tension in the marrow is lowest in this area, with a negative gradient from the endosteal arteries, and cellular energy production in HSCs is mainly via glycolysis. Stressors disrupting the oxygen gradient lead to cellular consequences (Eliasson and Jonsson, 2010; Zhang and Sadek, 2014). HSCs are relatively quiescent, with a low metabolic rate, have fewer mitochondria than hematopoietic progenitor cells (HPC), and their location in a relatively hypoxic area of the bone marrow suggests a lower generation of reactive oxygen species (ROS, a measure of oxidative stress, produced largely from oxidative phosphorylation, see Fig. 1) than in more mature hematopoietic cells. HSCs interact with mesenchymal stromal cells (MSC), which helps to regulate their level of metabolic activity. Stromal cells secrete cytokines which stimulate endothelial cell proliferation, and in turn, MDS cells induce stromal cell reprogramming. Communication between hematopoietic cells and stromal cells may occur in part via exchange of vesicles between cells (reviewed in (Korn and Mendez-Ferrer, 2017)). A transfer of mitochondria from stromal to AML cells via tunneling nanotubules driven by ROS was recently demonstrated; however, it remains to be seen whether this mechanism applies to MDS (Marlein et al., 2017). HPCs have a greater capacity for cell division and differentiation than HSCs and are associated with a greater energy requirement; therefore metabolism in HPCs is primarily aerobic via oxidative phosphorylation. In moderate levels, ROS produced by oxidative phosphorylation and/or derived from nicotinamide adenine dinucleotide phosphate (NADPH) oxidase 2 (NOX) are thought to be important in cellular signaling. In IOL, cellular ROS increase, which could have adverse consequences for cells; these points are discussed in Section 4.3. Increased ROS results in HSCs exiting their quiescent state with a loss of self-renewal capacity

Table 2
Rates of hematologic improvement in studies of 40 or more patients with lower IPSS risk MDS receiving iron chelation therapy with deferasirox.

n	IPSS risk	ICT	ICT duration	Erythroid response (%)	Median response duration	Neutrophil response (%)	Median response duration	Platelet response (%)	Median response duration	Factors not associated with HI	Ref
40	NR	DFX	Median: 12 mo	13*	NR	NR	NR	NR	NR	NR	(Breccia et al., 2012)
173† 52‡ 77§	Low/Int-1	DFX	Median: 12 mo	15¶	NR	15¶	NR	22¶	NR	NR	(List et al., 2012)
247† 50‡ 100§	Low/Int-1	DFX	Median: 12 mo	21.5†,	83.5 (range 29–204) days	22¶	NR**	13¶	NR††	NR	(Gattermann et al., 2012)
33† 6‡ 10§	Low/Int-1	DFX	≥1 dose; median duration: NR	6¶	NR	17	NR	30	NR	NR	(Nolte et al., 2013)
53† 17‡ 13§	NR	DFX 36 DFO 17	≥6 mo	35.1¶,‡‡	6 mo	76.4	8 mo	61¶	9 mo	(a)	(Molteni et al., 2013)
152	Low/Int-1	DFX	43% continued at 1 yr	11¶,‡‡	NR	3	NR	15	NR	(b)	(Angelucci et al., 2014)
55 34 14 2	Low Int-1 Int-2 High	DFX	NR	17.6 (7.1§§)	NR	7.1	NR	5.9	NR	(c)	(Maurillo et al., 2015)
98 (84 with MDS/CMML)	Low/Int-1	DFX 68 DFO 30	≥3 mo	41.8¶	NR	NR	NR	NR	NR	HI not related to effective reduction in SF	(Messa et al., 2017)
57	Low/Int-1	DFX	≤3 mo at study entry	19% at 12 mo	123 days	NR	NR	NR	NR	(d)	(Rose et al., 2016)

(a) Factors associated with achieving an erythroid response (in univariate analysis): transfusion burden, serum erythropoietin level, and FAB/WHO MDS diagnosis (RARS vs RA or RARS vs RCUD, respectively); serum LDH showed a trend towards being associated with erythroid response. The following factors were not significantly associated with erythroid response (in univariate analysis): age, neutrophil count, platelet count, ICT medication, serum iron, transferrin, SF, response to ICT (reduction in SF of > 35% at 12 mo), number of cytopenias, cytogenetic risk (intermediate vs low), presence of medullary fibrosis, and marrow cellularity. Transfusion burden, serum erythropoietin level, RARS, and serum LDH were not associated with erythroid response in multivariate analysis.
(b) The following factors were not significantly associated with achieving transfusion independence in univariate analysis: age, IPSS risk, MDS-specific comorbidity index, MDS diagnosis to enrolment interval, number of RBC transfusions, duration of transfusion dependence, baseline SF level, and karyotype (IPSS).
(c) Higher initial doses of DFX (20 mg/kg) were associated with transfusion independence (type of analysis not specified). Other variables evaluated were not reported.
(d) Factors not significantly associated with erythroid response (type of analysis not specified): cytologic classification (WHO 2008), time since MDS diagnosis, serum ferritin at inclusion, and number of RBC transfusions.

* Four out of 30 patients treated with DFX only; †eligible for erythroid response analysis; ‡eligible for neutrophil response analysis; §eligible for platelet response analysis; ¶according to International Working Group 2006 criteria; **response duration in two patients was 56 and 252 days; ††response duration was 168 days in one patient; ‡‡9% of patients were transfusion independent; §§rate of RBC transfusion independence; CMML, chronic myelomonocytic leukemia; DFO, deferoxamine; DFX, deferasirox; FAB, French-American-British; HI, hematologic improvement; ICT, iron chelation therapy; Int, intermediate; IPSS, International Prognostic Scoring System; LDH, lactate dehydrogenase; MDS, myelodysplastic syndrome; mo, months; NR, not reported; RA, refractory anemia; RBC, red blood cell; RCUD, refractory cytopenia with unilineage dysplasia; RS, ringed sideroblasts; SF, serum ferritin; vs, versus; WHO, World Health Organization; yr, year.

and an increase in mutations, possibly leading to stem cell exhaustion (Walter et al., 2015; Zhang and Sadek, 2014). The increased cycling of HSCs in the presence of increased ROS increases the risk of genetic hits and cell transformation (Chen et al., 2008; Takizawa et al., 2012).

A recent study showed a role for the mitochondrial heat shock protein mortalin in regulating HSC ROS levels; when mortalin is downregulated, ROS increase, and HSC numbers decrease (Tai-Nagara et al., 2014). In mortalin-negative zebrafish, ineffective hematopoiesis similar to that of MDS occurs (Craven et al., 2005). In addition, improvement in growth of MDS colonies occurs in hypoxic conditions approximating conditions in the bone marrow, compared to normoxic conditions; in 18.6% O_2, normal cells produced myeloid and erythroid colonies at a 2.3- and 1.3-fold reduction, respectively, compared to cultures in 3% O_2. However, MDS colonies in 18.6% O_2 were 7- and 4-fold less than in 3% O_2 (p-value not reported). These results suggest that MDS progenitor cells may be particularly sensitive to oxidative stress and may compete with normal HSC in hypoxic regions of the bone marrow (Thompson et al., 2007). The regulation of hypoxia, metabolism, cell proliferation and angiogenesis involves hypoxia inducible factor (HIF)-1α (discussed in Section 4.3) (Parmar et al., 2007; Zhang and Sadek, 2014).

3.2. Iron overload in the stem/progenitor cell niche

In the 1980s, it was demonstrated that ferritin has a suppressive effect on normal early erythroid progenitors (burst-forming units erythroid [BFU-E]; the mean number of colonies was 35 versus 26.3 per 5×10^4 to 1×10^5 peripheral blood mononuclear cells, $p < 0.05$). This activity was independent of glycosylation status and iron (Broxmeyer, 1986, Broxmeyer, et al 1991, Broxmeyer, et al 1986, Broxmeyer, et al 1989). Ferritin activated a pro-inflammatory state in hepatic stellate cells by inducing protein kinase C (PKC) and NFκB signaling, and increased expression of pro-inflammatory mediators such as interleukin-1β, inducible nitric oxide synthase (iNOS), regulated on activation normal T cell expressed and secreted (RANTES), nuclear factor of κ light polypeptide gene enhancer in B-cells inhibitor-α (IκBα), and intracellular cell adhesion molecule (ICAM)-1 (Ruddell, et al 2009). Inflammatory signals appear to be involved in the direction of hematopoiesis; interferon-γ induces myeloid differentiation in some HSCs, and interleukin-1β induces myeloid differentiation via NFκB activation.

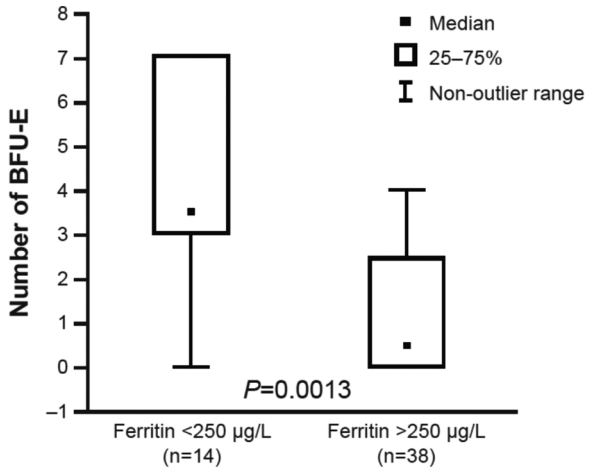

Fig. 2. Inhibition of BFU-E in patients with MDS according to serum ferritin level (< 250 or > 250ng/ml).
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Thus, inflammation-induced imbalance in hematopoiesis may lead or contribute to the development of hematologic malignancies such as MDS. The early experiments with ferritin may provide a link between IOL, associated with increased SF levels, and abnormal hematopoiesis (Matatall et al., 2016, 2014; Pietras, 2017). More recently, BFU-E production was reported to be impaired in patients with MDS with higher SF levels (SF ≥ 250 ng/ml; median BFU-E 0.5 versus 3.5, p = 0.001; Fig. 2 (Hartmann et al., 2013)). This also held true in transfusion-independent patients with MDS (Cui et al., 2014; Santini et al., 2011; Zipperer et al., 2013).

A series of experiments in mice with IOL, with an increased labile intracellular pool (LIP) in bone marrow mononuclear cells following injections of iron dextran, demonstrated decreases in HSCs, HPCs, BFU-E, colony-forming unit [CFU]-erythroid [E], CFU-granulocyte macrophages [GM], CFU-Mix, and MSCs (all p ≤ 0.05). The induction of ROS production inhibited osteogenic differentiation gene expression, induced adipogenic genes, and inhibited the production of several chemokines and adhesion molecules (stem cell factor, C-X-C motif chemokine [CXCL] 12, and vascular endothelial growth factor [VEGF]) by bone marrow MSCs (all p ≤ 0.05). These effects were partially reversed by the addition of either DFX or the antioxidant *N*-acetyl cysteine (NAC) to the culture (both p ≤ 0.01; (Zhang et al., 2015)). These data indicate that IOL not only impacts on hematopoietic cells, but also on the bone marrow microenvironment (Raaijmakers, 2014; Raaijmakers et al., 2010).

3.3. Oxidative stress in the stem/progenitor cell niche

Once transferrin saturation reaches 70–80%, NTBI is detectable in the serum. A redox active subset of NTBI is LPI. NTBI and LPI enter cells independently of the Tfr; in particular, NTBI is taken up into erythroid cells (in the presence of NTBI, the increase in LIP in RBC and reticulocytes was 15% and 21%, respectively, p < 0.01), where it contributes to ROS generation (the increase in ROS in RBCs and reticulocytes was 35% and 35%, respectively, p < 0.01) but cannot support hemoglobin production (Leimberg et al., 2003; Prus and Fibach, 2011). By virtue of its ability to transfer electrons, iron can catalyze the production of ROS, or oxygen free radicals (Fig. 1). ROS, when present in moderate amounts, participate in cell signaling, but excess ROS may have significant consequences for organelles, cells, tissues and organs, including the bone marrow (Nathan, 2003; Nathan and Cunningham-Bussel, 2013; Nathan and Ding, 2010). ROS involved in cell signaling include hydrogen peroxide (H_2O_2) and superoxide (Table 3). ROS at least in part confers signal specificity by covalent modification of amino acids as opposed to the phosphorylation and macromolecular interactions that occur with classically defined mechanisms of cell signaling; H_2O_2 oxidizes cysteine residues at the active site of phosphatases (Denu and Tanner, 1998). H_2O_2 also participates in signaling by growth factors; for example, it increases tyrosine phosphorylation of the granulocyte macrophage colony-stimulating factor (GM-CSF) receptor β chain and signal transducer and activator of transcription (STAT) 4, inducing the expression of cellular FBJ osteosarcoma virus (c-FOS) and other genes involved in G1/S cell cycle transition, an effect that was partially inhibited by NAC (Sattler et al., 1999). H_2O_2, though weakly oxidizing, is long-lived and membrane permeable, so its effects could manifest at a distance. Moreover, ROS may suppress hepcidin, increasing GI iron import and RES iron export (Choi et al., 2007). Reactive nitrogen species may also be important in cell signaling and toxicity (Nathan, 2003; Nathan and Cunningham-Bussel, 2013; Nathan and Ding, 2010). More damaging than H_2O_2 are the highly reactive hydroxyl radicals. Cancer cells commonly overproduce ROS, which can not only cause damage to macromolecules, organelles and cells, leading to programmed cell death or malignant progression, but also increase genomic instability, leading to DNA double strand breaks (DSBs) and altered repair, creating further errors through upregulation of alternative DNA repair pathways (Rassool et al., 2007; Sallmyr et al., 2008).

Lower risk MDS is characterized by an increase in apoptosis in the bone marrow. In higher risk MDS, an accumulation of mutations results in cellular outgrowth and malignant progression. Multiple preclinical studies have described adverse effects of increased ROS on the structure and function of proteins, lipids, and nucleic acids (reviewed in (Gattermann and Rachmilewitz, 2011)). Damage to proteins may lead to altered signaling, cell proliferation and fibrosis. Damage to lipids may lead to membrane damage and lysis of cells or organelles, causing changes in membrane fluidity and permeability, and resulting in altered ion transport and metabolism. Affected organelles may include mitochondria, leading to ineffective energy metabolism, apoptosis and cell senescence; the endoplasmic reticulum, compromising protein processing; and lysosomes, causing toxic compounds to be released into the cytoplasm. DNA is unreactive with oxidants in the absence of a transition metal such as iron. DNA damage from oxidative stress in IOL includes strand breaks, base modification, DNA–protein cross-links, covalent reactions with lipid peroxidation products and bulky DNA adducts. A common oxidative DNA lesion is incorporation of 7,8-dihydro-8-oxoguanine (8-OG), which mispairs with adenine resulting in GC to TA transversion; these changes may lead to apoptosis or mutagenesis. Cellular damage due to elevated NTBI and LPI may lead to increased LCI and ROS, and contribute to clinical endpoints in MDS with IOL.

In a study of 40 patients with MDS, the serum reactive oxygen metabolite levels were higher in patients than in controls, and there was a correlation between ROS levels and SF levels (p = 0.01 (Saigo et al., 2011)). In 20 patients with MDS with IOL (SF ≥ 1000 ng/ml), an increase in malonaldehyde (MDA), a lipid peroxidation product, and nitrites was seen compared with 56 patients with MDS without IOL; the MDA level was 14.3 µM in MDS with IOL versus 9.8 µM in MDS without IOL and 3.9 µM in normal controls, while nitrite levels were 6.7, 3.8, and 0.4 nM, respectively (all p < 0.0001 (de Souza et al., 2013)). These clinical findings underscore the point that toxic metabolites appear under conditions of oxidative stress. Mechanisms of cell death and mutagenesis with IOL are discussed below.

In mice, IOL measured by increased LIP and ROS inhibited the clonogenic capacity of HSCs and HPCs, as well as long-term culture-initiating cells, and inhibited engraftment following bone marrow transplantation (all p < 0.0001); DFX or NAC partially reversed these effects (all p < 0.0001) by inhibiting NOX4 and P38 mitogen-activated protein (MAP) kinase, improving long-term and multilineage engraftment following bone marrow transplantation (Chai et al., 2015). In a

Table 3
Types and biological characteristics of reactive oxygen species formed by the Haber–Weiss and Fenton reactions.

Name	Formula	Oxidizing capacity	Participate in cell signaling	Half-life	Membrane permeable
Hydrogen peroxide	H_2O_2	Weak	Yes	Long	Yes
Superoxide	$^{\cdot}O_2^-$	Strong	Yes	Short	No
Hydroxyl radical	$^{\cdot}OH$	Strong	Unclear	Short	No

transgenic mouse model, overexpressing both human mutant neuroblastoma retrovirus-associated DNA sequences (NRAS) and the anti-apoptotic protein B-cell lymphoma (BCL)-2 resulted in an increase in error-prone DNA repair by non-homologous end-joining, with a 26% increase in misrepair frequency for double mutants, $p < 0.0001$; and for increased ROS, $p < 0.05$, compared with single transgenic and normal control mice. These changes were partially reversed by NAC, which was associated with a 35% decrease ($p < 0.05$) in DNA damage (Rassool et al., 2007).

Several distinct mechanisms of cell death have been described; including apoptosis, necrosis, necroptosis, and iron-dependent ferroptosis (Dixon and Stockwell, 2014). IOL can disrupt enzymes, including those containing iron–sulfur clusters, resulting in the release, through superoxide and H_2O_2, of iron into the cellular LIP. This has two consequences. First, the enzyme is inactivated, and if required for essential cellular processes, cellular function and integrity may be compromised. Second, the released iron can participate in Fenton chemistry, further damaging cells and cellular components. The presence of cellular ROS leads to expression of pro-apoptotic genes, and mitochondrial ROS to apoptotic signaling. Mutations in mitochondrial DNA lead to increased superoxide production, possibly due to alterations in the electron transport chain (ETC), and superoxide activates adenosine monophosphate-activated protein (AMP) kinase, which upregulates nuclear E2F transcription factor-1 (E2F1), leading to transcriptional upregulation of pro-apoptotic genes. Alternatively, the hemoprotein cytochrome C catalyzes peroxidation of cardiolipins, leading to further cytochrome C release and activation of caspase-3 and caspase-7. These enzymes cleave the p75 subunit of complex 1 of the mitochondrial ETC, disrupting it, and resulting in further generation of ROS, which amplify apoptotic signaling. Necroptosis is initiated by activation of the tumor necrosis factor (TNF) receptor, initiating a cascade including activation of receptor interacting protein (RIP)-1 and -3, resulting in increased ROS, possibly via phosphorylation of STAT-3, which interacts with genes associated with the retinoid-interferon-induced mortality (GRIM)-19 subunit of mitochondrial ETC complex 1, again leading to increased ROS production. RIP-3 may interact with the cytoplasmic enzymes glutamate-ammonia ligase (GLUL), glutamate dehydrogenase (GLUD), and glycogen phosphorylase, liver form (PYGL), also increasing ROS production. Finally, NOX may interact with the TNF receptor, increasing ROS production and leading to cell death. Ferroptosis is initiated by inhibition of the function of the cell surface cysteine/glutamate antiporter system, leading to a decrease in intracellular cysteine and lower levels of the antioxidant glutathione, which results in an increase in lipid ROS, leading to cell death. From these models it is clear that not all IOL-sensitive mechanisms of mammalian cell death involve identical lethal processes. It is possible that different iron species (for example, labile versus enzyme bound [iron–sulfur clusters; Fe–S] and heme) or iron pools (for example, lysosomal versus mitochondrial versus cytosolic) may be involved in mediating unique cell death phenotypes in response to different stimuli. In addition, though many of the molecular mechanisms of these death pathways have been identified, the sources, types and targets of ROS relevant to cell death and cell signaling remain incompletely defined. IOL may also trigger cell death under anaerobic conditions, through enzyme mis-metalation, abrogation of essential functions, and production of sphingolipids, which activate a kinase signaling cascade leading to cell death (reviewed in (Dixon and Stockwell, 2014)). In MDS, therefore, exposing cells to IOL and ROS leads to replication stress of HSC by loss of quiescence; dysplasia through loss of HSC quality control; aggravated marrow failure through increased HSC mutation load, resulting in cell death; and further mutations promoting MDS disease progression. However, do these findings regarding cell death pathways in IOL translate to clinical endpoints?

At a cellular level, Shao et al described a moderate increase in ROS stimulating HSC cycling, leading to HSC exhaustion, with a further increase leading to the induction of HSC senescence. This occurred via oxidative DNA damage, leading to: DSBs and induction of P53 and P16 expression, resulting in decreased progression through the cell cycle; replicative senescence, with telomere shortening; and premature senescence, which may involve stabilization of P16 mRNA via induction of P38, inhibiting cell cycle progression (reviewed in (Shao et al., 2011)). A second group described an increase in erythroid apoptosis in MDS with IOL via regulation of HIF-1α/ROS. Upregulation of HIF-1α attenuates cell injury from IOL and ROS, as indicated by improved colony formation and decreased rates of apoptosis (both $p < 0.01$) (Zheng et al., 2017). Bowen et al described increased expression of antioxidant enzymes including glutathione peroxidase (GPX)-1, manganese-dependent superoxide dismutase (MnSOD), and γ-glutamyl cysteine synthetase (GCS) heavy subunit in bone marrow cells from patients with MDS and AML, including $CD34^+$ HSCs (all $p < 0.05$ (Bowen et al., 2003)). The authors suggest that these increases in antioxidants are insufficient to protect MDS cells from cell death. Gu et al showed that retroviral expression of the aryl hydrocarbon receptor nuclear translocator (ARNT; HIF-1β) in human myeloid leukemia HL60 cells increased the expression of ROS defense genes and protected against troglitazone, which otherwise induces apoptosis (Gu et al., 2013). Thus, HIF-1α and HIF-1β are expressed in myeloid cells, activate ROS defense, and appear to be involved in the adaptation of myeloid cells to oxidative stress.

A ROS-induced pathway also predisposing to AML progression involves alterations of natural killer cells and CD34+ cells in MDS. In a study of 34 patients with MDS, 15 with IOL (received > 20 units of RBCs [200 ml/unit] or SF > 1000 ng/ml) and 19 without IOL, IOL increased ROS, which led to decreased P38, decreased c-Jun N-terminal kinase (JNK, also known as MAP kinase 8), decreased apoptosis of $CD34^+$ cells, and increased blast counts in the marrow (all $p < 0.05$; (Hua et al., 2017)). ROS may also alter T cell and macrophage subsets, altering immune surveillance (discussed further in Section 5) (Chen et al., 2017; Vinchi et al., 2017).

What about nucleic acids? Oxidative DNA lesions distort the DNA helix and could potentially lead to apoptosis or malignant progression. Protection requires repair by global genome nucleotide excision repair and Rev-1 (a core factor) dependent trans-lesion synthesis. In a nuclear pore complex protein (NUP)-98–homeobox protein (HOX)-D13 transgenic mouse model of MDS, ROS levels were increased in HPCs, with an increase in DSBs leading to cell cycle arrest and increased mutation frequency (all $p < 0.05$ (Chung et al., 2014)). In humans, comet assays revealed an increase in oxidative DNA damage in $CD34^+$ cells from patients with lower risk MDS compared to cells from healthy controls (Novotna et al., 2009). More specific oxidative DNA lesions seen in patients with MDS with IOL, such as 8-hydroxy-2′-deoxyguanosine (8−OH-dG), are discussed further in the section on mechanisms of HI with ICT (Kikuchi et al., 2012).

What about epigenetic changes? Using whole RNA sequencing of

Table 4
Studies indicating hematologic suppression by iron overload and potential mechanisms.

Observation HSC/HPC/MSC	Cell/model type	Comment	Ref
Ferritin suppressed BFU-E and CFU-GM ($p < 0.05$)	Murine and human cells *in vitro*	Activity in ferritin H-chain, requires ferroxidase activity, independent of glycosylation and iron	(Broxmeyer et al., 1986)
BFU-E impaired in patients with MDS with ferritin > 250 ng/ml ($p = 0.001$)	MDS patient cells *ex vivo*	CFU-GM not suppressed in this model	(Hartmann et al., 2013)
Reduced BFU-E, increased immature population, induced apoptosis in IOL ($p < 0.05$ for all comparisons)	Mice and humans, $CD34^+$ cells *in vitro*	Via increased intracellular ROS and suppression of BCL-2 in erythroblasts Partially reversed by DFO or NAC	(Taoka et al., 2012)
Increased expression of antioxidant enzymes in bone marrow cells from MDS/AML patients, including $CD34^+$ cells ($p < 0.05$ for all comparisons)	Bone marrow cells from MDS/AML patients, including $CD34^+$ cells	GPX1, MnSOD, GCS Suggest oxidative stress involved in ineffective hematopoiesis of MDS, insufficient increase in antioxidants to protect from cell death	(Bowen et al., 2003)
Decreased HPC, HSC, CFU-E, BFU-E, CFU-GM and CFU-Mix, osteogenic gene expression, adipogenic genes induced, inhibition of SCF, CXCL12, VEGF by MSC in IOL ($p < 0.05$ for all comparisons)	Murine model	HSC, HPC and the microenvironment are influenced by IOL	(Zhang et al., 2015)
Alterations of NK and $CD34^+$ cells occurred in MDS via a ROS-induced pathway in IOL, decreased apoptosis of $CD34^+$ cells, increased blasts ($p < 0.05$ for all comparisons)	MDS cells *in vitro*	ROS causes conditions favoring AML progression Effects via decreased P38 and JNK (MAPK8)	(Hua et al., 2017)
Nucleic acids			
ROS levels increased in proliferative and early hematopoietic progenitors Increased DSB leading to cell cycle arrest Increased mutation frequency ($p < 0.05$ for all comparisons)	Transgenic mouse model of MDS	Suggests oxidative stress leads to cellular consequences in HSC and HPC	(Chung et al., 2014)
Increased glucose metabolism enzymes: aconitase and isocitrate DH, and the TCA cycle Increased αKG, 2-HG and DNA methylation in IOL ($p < 0.05$ for all comparisons)	Murine model	Increased DNA methylation is associated with adverse prognosis in MDS	(Yamamoto et al., 2016)
Increased 8-OH-dG* levels in PBMC and $CD34^+$ cells of MDS with IOL (SF > 500 ng/ml) ($p = 0.001$)	MDS cells *ex vivo*	Partially reversed by DFX	(Kobune et al., 2013)
Immune surveillance			
Altered ratio of T cell subtypes via inhibition of apoptosis in MDS with IOL ($p = 0.01$)	MDS cells, murine cells	Implications for immune surveillance, partially reversed by DFX	(Chen et al., 2017)
Altered ratio of inflammatory to regulatory mitogen-stimulated T cells in normal donors (p-value not reported)	Normal human cells	Implications for immune surveillance, partially reversed by DFX	(Banerjee et al., 2015)

* An indicator of oxidative DNA damage that results in abasic sites and DSBs. 2-HG, 2 hydroxyglutarate; 8-OH-dG, 8-hydroxy-2′-deoxyguanosine; αKG, α-ketoglutarate; AML, acute myeloid leukemia; BCL-2, B cell lymphoma 2; BFU-E, burst-forming unit erythroid; CFU-E, colony-forming unit erythroid; CFU-GM, colony forming unit granulocyte-macrophage; CFU-Mix, colony-forming unit mix; CXCL12, C-X-C motif chemokine ligand 12; DFO, deferoxamine; DFX, deferasirox; DH, dehydrogenase; DSB, double strand breaks; GCS, γ-glutamyl cysteine synthetase heavy subunit; GPX, glutathione peroxidase; HPC, hematopoietic progenitor cell; HSC, hematopoietic stem cell; IOL, iron overload; JNK, c-Jun N-terminal kinase; MAPK8, mitogen-activated protein kinase 8; MDS, myelodysplastic syndrome; MnSOD, manganese-dependent superoxide dismutase; MSC, mesenchymal stem cells; NAC, *N*-acetyl cysteine; NK, natural killer; PBMC, peripheral blood mononuclear cells; ROS, reactive oxygen species; SCF, stem cell factor; SF, serum ferritin; TCA, tricarboxylic acid; VEGF, vascular endothelial growth factor.

bone marrow cells from mice with IOL (injected with 10 mg iron dextran daily for 5 days), Yamamoto et al demonstrated an increase in enzymes involved in glucose metabolism, including aconitase and isocitrate dehydrogenase, and the tricarboxylic acid (TCA, Krebs, citric acid) cycle, resulting in increases in α-ketoglutarate and 2-hydroxyglutyrate, as well as an increase in DNA methylation, which in turn was associated with an adverse prognosis in MDS (all $p < 0.05$ (Yamamoto et al., 2016)).

In summary, ROS are involved in cell signaling, are increased in IOL, increase HSC cycling leading to senescence, inhibit myelopoiesis, induce cell death, alter lymphocyte and macrophage subsets, increase mutation rates in mitochondrial and nuclear DNA, alter DNA methylation, and may promote MDS/AML progression. Mechanisms of hematologic suppression with IOL are summarized in Table 4.

4. Mechanisms of hematologic improvement with iron chelation

Identifying the mechanisms by which HI with ICT occurs in MDS is an area of active investigation. Proposed mechanisms include: a direct effect on the MDS clone or bone marrow microenvironment; an increase in iron release from iron stores, allowing use by hematopoietic tissue; an alteration in NFκB expression, leading to altered transcription of anti-apoptotic factors, cytokines or adhesion molecules that may affect erythroid inefficacy; inhibition of m-TOR signaling; and a reduction in oxidative stress. In this section, evidence for these and other mechanisms of HI with ICT are reviewed.

4.1. Serum ferritin levels

Multiple clinical trials and analyses have demonstrated a reduction in SF levels during ICT (Angelucci et al., 2014; Breccia et al., 2012; Gattermann et al., 2012; List et al., 2012; Maurillo et al., 2015; Messa et al., 2017; Molteni et al., 2013; Nolte et al., 2013). Given the suppressive effects of purified ferritin on BFU-E, the reduction in SF itself could have positive effects on erythropoiesis (Broxmeyer, 1986; Broxmeyer et al., 1991, 1986; Broxmeyer et al., 1989). However, in clinical studies, HI often occurs without SF reduction (see Section 2), so the importance of SF reduction in HI with ICT is likely minor.

4.2. Deferoxamine; increased erythropoietin levels, decreased oxidative stress

There are only a few patients reported who achieved HI following treatment with DFO, and few preclinical studies have investigated the possible mechanisms of HI with this agent (Del Río Garma et al., 1997; Gu et al., 2017; Jensen et al., 1996, 1992). One study of 16 healthy subjects examined their responses to 8 h of continuous intravenous infusions of DFO at doses of 1–4 g/70 kg body weight. The investigators observed increases in EPO levels at 8 and 12 h ($p < 0.05$) that were possibly mediated via HIF-1α, or by depletion in the iron pool affecting

redox signaling (Ren et al., 2000). In seven patients with MDS who achieved RBC transfusion independence with DFO, the median time to transfusion independence from initiation of chelation therapy was 20 (range 15–36) months (Jensen et al., 1996). In that study, achieving transfusion independence did not appear to be associated with a significant reduction in SF levels. In 22 patients with MDS receiving DFO, there was a significant decrease in LPI over 26 weeks of chelation ($p < 0.001$). There was a correlation between LPI and LIC ($r = 0.739$, $p = 0.001$), suggesting that LPI may be a practical means to monitor the efficacy of ICT (Gu, et al 2017). Taoka et al found that IOL negatively affected the differentiation of erythroblasts from murine and human $CD34^+$ cells, and that this effect was reversed by incubation with 100–150 μM DFO (Taoka et al., 2012). They also showed that iron accumulation in human erythroblasts resulted in increases in ROS levels and apoptosis, which was accompanied by reduced BCL-2 expression. However, the concentrations of iron used in these experiments were 10–100 times higher than the levels of NTBI found in the serum of patients with β-TM, making the clinical relevance of these findings uncertain.

4.3. Deferasirox; effects on oxidative stress and cellular signaling

The majority of studies addressing HI with ICT have focused on DFX. In reports of seven patients who achieved RBC transfusion independence with DFX, the median time to transfusion independence was 2 (range 1–12) months (Table 2). In the US03 study (List et al., 2012), the median time to HI was 5.5 (2.8–12.5) months, while in the EPIC study (Gattermann et al., 2012), the median time to onset of erythroid response was 3.6 (0.03–9.4) months. The median time to platelet response was 5.6 (0.9–10.5) months and to neutrophil response 7.4 (1.9–11.1) months. Nearly 1000 lower IPSS risk patients with MDS have been examined in major studies (Table 2), and a significant reduction in SF level was not found to be associated with HI in any of these studies. Two studies examined LPI, the US03 and the EPIC studies, and both found that LPI was decreased to within the normal range very quickly, within 3 months of starting DFX. Neither study showed an association between suppression of LPI and HI, but this is probably due to the fact that LPI levels are easily suppressed in all patients and therefore cannot discriminate between hematologic responders and non-responders (Gattermann et al., 2012; List et al., 2012). A smaller study of 16 patients with MDS receiving DFX for a mean of 3 (1.2–3.9) months also showed a significant decrease in mean LPI from 0.39 to 0.11 ($p = 0.02$) (Ghoti et al., 2010). In addition, a significant reduction in RBC ROS and RBC lipid peroxidation was seen ($p < 0.05$ and < 0.01, respectively) while glutathione levels rose significantly in RBCs, platelets, and neutrophils (all $p < 0.01$).

Several preclinical studies have suggested that improvements in oxidative stress may be linked to HI. In normal hematopoietic stem/progenitor cells (HSPCs), exposure to DFX (100 μM, equivalent to 20–30 mg/kg/day of the dispersible formulation in patients) increased ROS production, which led to decreased CD34 expression, increased mitochondrial mass, and TfR upregulation (all $p \leq 0.05$), suggesting an upregulation of erythropoiesis via alterations in redox signaling; these effects were not seen with DFO. These changes were accompanied by changes in the expression of transcription factors and regulatory proteins in $CD34^+$ cells, including POU5F1 (octamer-binding transcription factor [OCT]-4), and sex determining region Y-box (SOX) 2 and 17, all of which mediate embryonic stem cell pluripotency. The authors proposed that flow cytometric analysis of ROS levels in patient samples might help to identify patients likely to experience an erythroid response to DFX (Tataranni et al., 2015). Pullarkat et al observed an increase in ROS in $CD34^+$ cells from patients with MDS following incubation with 20 μM DFX (equivalent to median trough levels in patient plasma), along with reductions in cell viability and colony formation (all $p \leq 0.01$); these effects were not observed in normal cells (Pullarkat et al., 2012). The results indicate that early MDS progenitors are inhibited by increased ROS, while differentiation appears to be induced in normal progenitors. This suggests a differential sensitivity of MDS cells to intracellular events precipitated by DFX, possibly including cellular programs that protect against oxidative stress. The finding that suppression of MDS progenitors occurs at trough doses of DFX suggests that lower doses introduced earlier to patients with MDS with anemia might have favorable effects on hematologic status, as discussed in the following paragraphs (Pullarkat et al., 2012).

One study examined neutrophil function before and after treatment with DFX in nine transfusion-dependent patients. There were significant reductions in derivatives of reactive oxygen metabolites (hydroperoxide levels related to lipid, peptide and amino acid oxidation; 409 versus 309 units, $p = 0.03$) over a median of 5 (3–15) months of DFX treatment. Neutrophil ROS production was significantly decreased following incubation with 50 μM of DFX ($p < 0.0001$) but not with 100 μM of DFO, possibly protecting them from oxidative stress-induced damage (Saigo et al., 2013).

4.3.1. Oxidative stress effects on DNA damage and repair

Oxidative DNA damage, indicated by levels of 8-OH-dG, was increased in PBMCs from patients with MDS with IOL (SF > 500 ng/ml) compared with those from patients with MDS without IOL or normal controls. An increase in 8-OH-dG was seen in MDS with IOL, and also in higher versus lower IPSS risk patients. The 8-OH-dG levels were greater in patients with karyotypic abnormalities than in patients without abnormalities and were increased further with increasing cytogenetic risk group (all $p \leq 0.03$), suggesting that oxidative DNA damage may induce chromosomal abnormalities and that ICT may confer protection against this damage. The 8-OH-dG levels decreased significantly ($p \leq 0.002$) after 3 months of ICT with DFX (Fig. 3) (Kikuchi et al., 2012).

Although IOL could directly result in an increase in intracellular ROS and molecular products of oxidative damage, some evidence indicates that a defect in DNA repair mechanisms might also contribute to oxidative DNA damage. Eight-oxoguanine glycosylase (OGG1) is the primary enzyme responsible for excision of 8-OG, a mutagenic base byproduct that occurs as a result of exposure to ROS; it both cleaves the

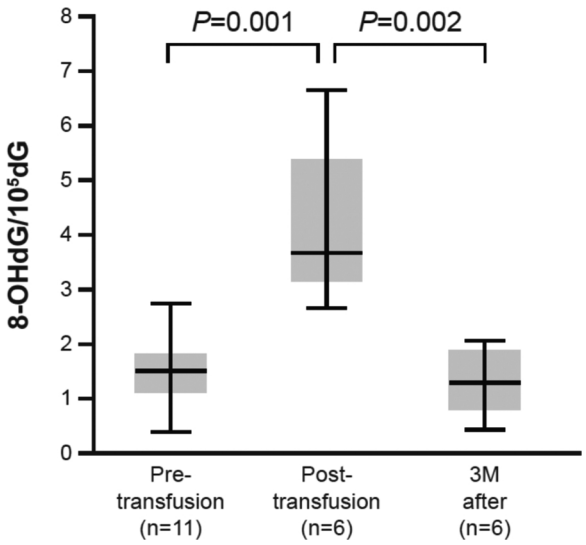

Fig. 3. Serial changes in levels of 8-OH-dG, a marker of oxidative DNA damage, in peripheral blood mononuclear cells from patients with MDS (serum ferritin level < 500 ng/ml prior to RBC transfusion dependence; ferritin > 500 ng/ml following RBC transfusion dependence; following 3 months of chelation with deferasirox).
MDS, myelodysplastic syndrome; RBC, red blood cells; 8-OH-dG, 8-hydroxy-2′-deoxyguanosine. Modified from Kikuchi S, et al. *Free Radic Biol Med.* 2012;53(4):643–8.

glycosidic bond of the mutagenic lesion and causes a strand break in the DNA backbone. Elevated levels of 8-OG were observed in $CD34^+$ cells from patients with MDS. A subgroup of patients had increased expression of OGG1, and the increase in oxidative DNA damage was correlated with the presence of the OGG1-Cys326 allele, which has reduced ability to repair abasic DNA sites secondary to oxidative DNA damage ($p \leq 0.05$ (Jankowska et al., 2008)). Therefore, extrinsic factors (transfusional IOL) and cellular intrinsic factors (defects in DNA repair) may have an additive or synergistic effect on nucleic acid damage.

4.3.2. Oxidative stress effects on lymphoid cells, macrophages and hepcidin levels

The effects of oxidative stress are not limited to myeloid cells. T helper type 1 (Th1) cells ($CD4^+$) generate cellular immunity, while Th2 cells generate humoral immunity, and similarly, CD8+ cytotoxic T-lymphocyte type 1 (Tc1) and Tc2 cells also generate cellular and humoral immunity, respectively. The effect of DFX on mitogen-stimulated T cells from normal volunteers was investigated. Findings included a dose-dependent increase in interferon-positive T cells (Th1, Tc1) and a decrease in regulatory T cells, suggesting that changes in the ratio of inflammatory to regulatory T cells may improve immune surveillance, resulting in suppression of abnormal myeloid precursors and allowing recovery of normal myelopoiesis (p-value not reported (Banerjee et al., 2015)). In another study, the percentage of $CD3^+$ T lymphocytes was lower, and the ratio of CD4/CD8 T cells and percentage of regulatory T cells (Tregs) was higher in patients with MDS with IOL ($n = 20$) than in patients with MDS without IOL ($n = 10$) (Chen et al., 2017). The investigators also found decreases in the Th1/Th2 and Tc1/Tc2 T lymphocytes subset ratios in patients with IOL (all $p < 0.01$). The decrease in the percentage of $CD3^+$ cells was reproduced in a murine model of IOL ($p < 0.0001$), and this effect was partially abrogated by treatment with DFX or NAC (both $p < 0.0001$), though the B-cell frequency was unaffected (p = NS). IOL-induced apoptosis of $CD4^+$ and $CD8^+$ cells was abrogated by DFX or NAC (all $p < 0.0001$), suggesting that cellular immune responses are disrupted in IOL, and may be mediated via T cell intracellular ROS (Chen et al., 2017). An alteration in macrophage subsets in transfusional IOL with an enhancement of anti-inflammatory phenotype was recently described (Vinchi et al., 2017). These findings have potential implications for MDS progression via disrupted immune surveillance as well as infectious complications of MDS. Further, hepcidin was suppressed by ROS (Choi et al., 2007). In 19 patients with lower IPSS risk MDS and transfusional IOL, treatment with DFX for 3 months significantly increased serum hepcidin levels compared to baseline ($p = 0.005$). The increase in hepcidin levels would inhibit iron absorption via the GI tract and iron mobilization from the RES, reducing parenchymal iron loading (Ghoti et al., 2011). Thus, lymphocyte and macrophage ratios are disrupted by oxidative stress, and hepcidin levels are suppressed, which would be predicted to increase iron loading. Conversely, lymphocyte ratios and hepcidin levels are restored by ICT and antioxidants.

4.3.3. Oxidative stress effects on NFκB and myeloid blasts

An intriguing finding is an alteration in expression of NFκB following exposure to DFX in human myeloid leukemia cell lines (K562) and in MDS patients (Messa et al., 2010). NFκB is activated by a broad range of signals including ROS. NFκB was inhibited by DFX at 50 μM, and not by either DFO or deferiprone. All three chelators effectively depleted iron and suppressed LPI and ROS, suggesting that the inhibition of NFκB by DFX was independent of changes in these parameters. Addition of iron to the cultures did not affect NFκB inhibition by DFX, suggesting this effect is independent of iron status (Messa et al., 2010). It was previously demonstrated that the H- and L- subunits of ferritin activate NFκB signaling in an iron-independent manner in hepatic stellate cells; whether this is also the case in HPCs remains to be determined (Ruddell et al., 2009). The downstream target of NFκB relevant to DFX is currently unclear; however, Messa et al speculated that DFX may specifically target blasts since the NFκB pathway is activated in this cell population. In lower risk MDS, this would lead to inhibition of the MDS clone and improvement in ineffective erythropoiesis (Messa et al., 2010). These results suggest that NFκB could be a therapeutic target in MDS. In myeloblasts, DFX synergized with the differentiating agent vitamin D to kill AML blasts in preclinical models ($p < 0.0001$) and improved the OS of elderly patients with AML following failure of HMA at starting doses of 20–30 mg/kg/day ($p = 0.002$ (Braun et al., 2006; Callens et al., 2010; Paubelle et al., 2013)). In contrast, Meunier et al reported that NFκB expression was activated at a low concentration of DFX (3 μM; equivalent to 5 mg/kg/day of the dispersible formulation), and associated with increased cell cycling, decreased apoptosis, and increased CFU-E in MDS progenitors; effects mediated by intracellular ROS and independent of iron reduction. Of six EPO-refractory patients receiving 5 mg/kg/day of DFX, all became transfusion independent (Meunier et al., 2017). While these two studies appear to show contradictory effects of DFX on NFκB expression, both studies indicate that this pathway is important in HI with DFX and further investigation may resolve the mechanism of action. In summary, expression of NFκB in myeloid cells is activated by ROS, which is expected to result in outgrowth of blasts, and conversely, NFκB expression is inhibited by DFX, resulting in suppression of myeloid blasts.

4.4. Deferasirox; effects on mTOR and cell survival

Other molecular pathways that may be relevant to HI with DFX include mammalian target of rapamycin (mTOR), which is important in the initiation of protein translation and regulates cell proliferation and survival. In myeloid leukemia cell lines (K562, U927, and HL60) and a xenograft model, mTOR was inhibited by DFX at a 50% inhibitory concentration of up to 50 μM in cell lines and up to 172 μM in primary AML samples. The mRNA expression of REDD1 (regulated in development and DNA damage response 1), a stress response gene induced by hypoxia, was upregulated in this system (from < 0.5 to > 2.0 arbitrary units in all three cell lines and from < 0.2 to > 0.6 arbitrary units in two of four fresh leukemia samples). REDD1 activated the tuberous sclerosis (TSC) 1–2 complex, which inhibited mTOR activation (from 1.0 to approximately 0.6 arbitrary units in K562 cells). The net effect of oral administration of DFX at 50 mg/kg/day was a significant reduction in tumor volume from > 20,000 to 0 mm^3 at day 50 ($p < 0.0001$) in the xenograft model (Ohyashiki et al., 2009).

4.5. Eltrombopag, stimulation of hematopoietic stem cell growth and differentiation

In 2012, it was demonstrated that eltombopag (ELT) inhibits AML cells via iron chelating activity (Roth et al., 2012). Pre-clinical data indicate that ELT offloads organ iron (Koumoutsea et al., 2016, 2015). In 2018, using LTC-IC and serial colony assays, HSC self-renewal and differentiation commitment of cells isolated from human or mouse bone marrow was assessed in vitro. These investigations demonstrated that the growth and differentiation of long-term culture initiating cells (LTC-IC) was stimulated via the iron chelating activity of ELT, and independent of the thrombopoietin receptor (Kao et al., 2018). Using highly enriched human HSC, the addition of ELT resulted in a nearly two-fold increase in LTC-IC ($p < 0.0001$). When HSC were preloaded with iron (ferrous ammonium citrate), however, then treated with ELT, LTC-IC remained at baseline levels. Gene expression profiling indicated molecular changes consistent with intracellular iron reduction. Therefore, ELT triggers a molecular response that is distinct from TPO-R signaling in HSC and stimulates mouse HSCs independently of the TPO-R, and this effect is critically dependent on the iron chelating property of ELT. Fewer data indicated similar effects on LTC-IC by DFX ($p < 0.05$). Whether ELT can be employed as an iron chelator in clinical practice requires further study.

Table 5
Studies indicating hematologic improvement with iron chelation and potential mechanisms of this effect.

Mechanism	Study type and source of samples	DFX	DFO, DFP	Comments	Ref
Normal cells					
Production of ROS in normal HSPCs	*In vitro* Normal cells	Decreased CD34, increased mitochondrial mass, increased CD71 (TfR)	Effects not seen with DFO	$p < 0.05$ for all Differentiation induced in normal progenitors	(Tataranni et al., 2015)
Increased serum EPO levels in healthy subjects	Clinical, healthy subjects	–	DFO, $p < 0.058$	8-hour continuous intravenous DFO at 1–4 g/70 kg Possibly mediated via HIF-1α, or by affecting redox signaling?	(Ren et al., 2000)
Inhibited NFκB-dependent transcription of TNF in T cells Improved ratio of inflammatory to regulatory mitogen-stimulated T cells from healthy subjects	*Ex vivo*, healthy subjects	p-value NR	NR	Improved immune surveillance Possibly involves suppression of abnormal myeloid precursors, allowing recovery of normal myelopoiesis?	(Banerjee et al., 2015)
MDS					
Ferritin reduction	Clinical, patients with MDS	Achieved; does not appear to be related to HI $p < 0.01$ in US03	Achieved, possibly related to HI	$p < 0.01$ in US03	(Angelucci et al., 2014; Gattermann et al., 2012; Jensen et al., 1996; List et al., 2012; Molteni et al., 2013)
LPI reduction	Clinical, patients with MDS	Achieved; unclear if related to HI	Achieved, unclear if related to HI	Median ± 0.07 U at baseline	(Gattermann et al., 2012; Ghoti et al., 2010; Gu et al., 2017; List et al., 2012)
Reduction of RBC ROS in MDS	*Ex vivo*, patients with MDS	Reduced lipid peroxidation, increased GSH $p \leq 0.05$	–	ICT appeared to have antioxidant effect by decreasing intra- and extracellular toxic iron species	(Ghoti et al., 2010)
Production of ROS in $CD34^+$ cells from MDS	*In vitro*, MDS progenitor cells	Reduction in cell viability and colony formation $p \leq 0.01$ for all	–	Inhibition of early MDS progenitors, not seen in normal cells	(Pullarkat et al., 2012)
Restored ability of erythroblasts to differentiate	*In vitro*, MDS, murine cells	–	DFO, $p < 0.05$ for all	Reversed IOL effects: increased ROS in $CD34^+$, induced apoptosis, and suppressed BCL-2 in erythroblasts	(Taoka et al., 2012)
Reduced dROM, ROS production in neutrophils of patients with MDS	*Ex vivo*, patients with MDS receiving DFX	DFX, $p \leq 0.03$	Effects not seen with DFO	Effect of DFX was associated with a reduction in iron stores and inhibition of ROS production	(Saigo et al., 2013)
Decreased 8-OH-dG* levels in PBMC and $CD34^+$ cells of MDS with IOL (SF > 500 ng/ml)	Clinical, patients with MDS	3 months of ICT with DFX $p \leq 0.002$	NR	Not seen in MDS without IOL or in normal controls	(Kikuchi et al., 2012)
Restored T cell subtypes ratio altered by IOL via inhibition of apoptosis in IOL	*Ex vivo*, patients with MDS, mice	$p < 0.0001$ for all Also partially restored by the antioxidant NAC	NR	Implications for infections and MDS progression via disrupted immune surveillance	(Chen et al., 2017)
Serum hepcidin levels increased	Clinical, patients with MDS	3 months of chelation with DFX $p = 0.005$	NR	Expected to inhibit GI iron absorption and mobilization from RES, reducing parenchymal iron loading	(Ghoti et al., 2011)
Inhibition of NFκB by DFX	*In vitro*, *ex vivo*, MDS cells, cell lines	Effect seen at 50 μM $p \leq 0.03$	Effect not seen with DFO or DFP (p = NS)	All MDS risk types and sAML Independent of iron reduction and LPI/ROS suppression*	(Messa et al., 2010)
Activation of NFκB by DFX	*In vitro*, MDS cells, patients	Effect seen at 3 μM $p \leq 0.04$	Effect not seen with DFO or DFP	Independent of iron reduction Supported cycling and CFU-E formation in MDS cells ROS-mediated	(Meunier et al., 2017)
MDS/IOL models					
Inhibition of mTOR via upregulation of REDD1	*In vitro*, xenograft model	IC_{50} 50–170 μM Oral administration at 50 mg/kg/day $p < 0.0001$	NR	mTOR is a cellular checkpoint protein kinase important in translation initiation Significant reduction in tumor volume	(Ohyashiki et al., 2009)

(*continued on next page*)

Table 5 (*continued*)

Mechanism	Study type and source of samples	DFX	DFO, DFP	Comments	Ref
Decreased HPC, HSC, CFU-E, BFU-E, CFU-GM CFU-Mix, osteogenic gene expression, increased adipogenic genes, inhibited SCF, CXCL12 and VEGF with IOL	*In vitro*, murine model	Partially reversed by DFX	NR	$p \leq 0.01$ Partly reversed by NAC	(Zhang et al., 2015)

*Iron-free ferritin induces NFκB by a cytokine-like mechanism. In lower risk MDS, NFκB inhibition would inhibit MDS cells, allowing restoration of normal myelopoiesis. In higher risk MDS, NFκB inhibition would inhibit blasts. 8-OH-dG, 8-hydroxy-2'-deoxyguanosine; BCL-2, B-cell lymphoma 2; BFU-E, burst-forming unit erythroid; CFU-E, colony-forming unit erythroid; CFU-GM, colony-forming unit granulocyte-macrophage; CFU-Mix, colony forming unit mixed; CXCL12, C-X-C motif chemokine ligand 12; DFO, deferoxamine; DFP, deferiprone; DFX, deferasirox; dROM, derivatives of reactive oxygen metabolites; EOS, end of study; EPO, erythropoietin; GSH, glutathione; HI, hematologic improvement; HPC, hematopoietic progenitor cell; HSC, hematopoietic stem cell; HSPC, hematopoietic stem/progenitor cell; IC50, 50% inhibitory concentration; ICT, iron chelation therapy; IOL, iron overload; LPI, labile plasma iron; MDS, myelodysplastic syndrome; mTOR, mammalian target of rapamycin; NAC, N-acetyl cysteine; NFκB, nuclear (transcription) factor kappa B; NR, not reported; NS, not significant; p, probability; PBMC, peripheral blood mononuclear cells; RBC, red blood cells; REDD1, Regulated in DNA Damage and Development response 1; RES, reticuloendothelial system; ROS, reactive oxygen species; sAML, secondary acute myeloid leukemia; SCF, stem cell factor; SF, serum ferritin; TfR, transferrin receptor (CD71); TNF, tumor necrosis factor; U, units; VEGF, vascular endothelial growth factor.

4.6. Summary, mechanisms of hematologic improvement with iron chelation,

Thus, HI with ICT appears to occur at an earlier time point with DFX than with DFO. All three commercially available chelators suppress LPI; DFO appears to increase EPO levels; and DFX may, by suppressing ROS, improve erythropoiesis; reduce oxidative mitochondrial and nuclear DNA damage; improve immune surveillance via NFκB and mTOR suppression; and have an MDS/AML suppressive effect, resulting in reduced blast burden and improved survival in mice and humans. From these investigations, a model emerges in which inflammatory, angiogenic, immunomodulatory, matrix remodeling effects, increased SF levels, and IOL may lead to MDS initiation and progression (Banerjee et al., 2015; Raaijmakers, 2014; Raaijmakers et al., 2010). Therefore, IOL reduction may attenuate MDS progression. Studies investigating the mechanisms by which ICT with DFX, DFO and DFP may result in HI are summarized in Table 5, and the mechanisms of suppression of hematopoiesis, support of MDS initiation/progression, and points at which IOL may exacerbate these effects are shown in Fig. 4.

5. Hematologic improvement with iron chelation; unanswered questions and future directions

Multiple clinical and preclinical studies have established that ICT leads to HI in MDS. Going forward, it will be important to determine which clinical or laboratory parameters predict which patients will experience HI with ICT, and whether these parameters differ between chelation agents or MDS subtypes. Suggested parameters predicting HI include a negative iron balance or longer duration of LPI suppression, but this remains to be demonstrated in clinical studies. Enhanced LPI (eLPI) is measured using an agent that displaces LPI from serum carriers such as albumin and citrate. In the SCT setting, the presence of elevated eLPI prior to conditioning was strongly associated with increased early non-relapse mortality in a multivariate analysis ($p = 0.002$) and OS ($p < 0.0001$); whether eLPI is associated with clinical outcomes in the non-SCT setting is particularly worthy of investigation (Wermke et al., 2018). Alterations in factors such as hepcidin or EPO, and alterations in ROS levels leading to alterations in redox signaling and/or cytotoxicity may be important. Balance between subsets of T cells, natural killer cells, and macrophages are altered in IOL and by ICT, and may be important in influencing cell growth and differentiation. A number of molecular mechanisms have been suggested by which HI may occur, but it remains to be seen whether these are applicable to MDS patients in clinical practice. It will be of interest to determine whether HI occurs by restoration of effective myelopoiesis in normal hematopoietic precursors, within the MDS clone, or both. Ultimately, the mechanism underlying HI may depend on the cellular context, including MDS subtype, cytokine milieu, stromal function, degree of remaining normal myelopoiesis, cytogenetic risk group, blast count, immune tumor surveillance, and degree of IOL. Given the induction of mutations in IOL, it will be interesting to determine whether somatic mutations associated with MDS initiation and progression are accelerated by IOL (Bejar, 2017; Kennedy and Ebert, 2017).

Clinical guidelines for MDS recommend starting ICT for lower risk patients after receiving 20–50 RBC units with SF levels of 1000–2500 ng/ml (Bennett, 2008; Gattermann, 2008). Myeloid maturation of normal cells and decreased colony formation of MDS cells occur at the equivalent of a low therapeutic dose of DFX, raising the question whether ICT should be started earlier than recommended by guidelines, at a lower dose (Pullarkat et al., 2012; Tataranni et al., 2015), which would also reduce side effects (Meunier et al., 2017). This approach would ideally be taken in the context of a clinical trial with correlative studies performed. Studies suggesting that DFX inhibits myeloblasts are intriguing (Callens et al., 2019; Messa et al., 2010; Paubelle et al., 2013), raising the question whether combinations of ICT with standard MDS treatments such as HMA or lenalidomide might

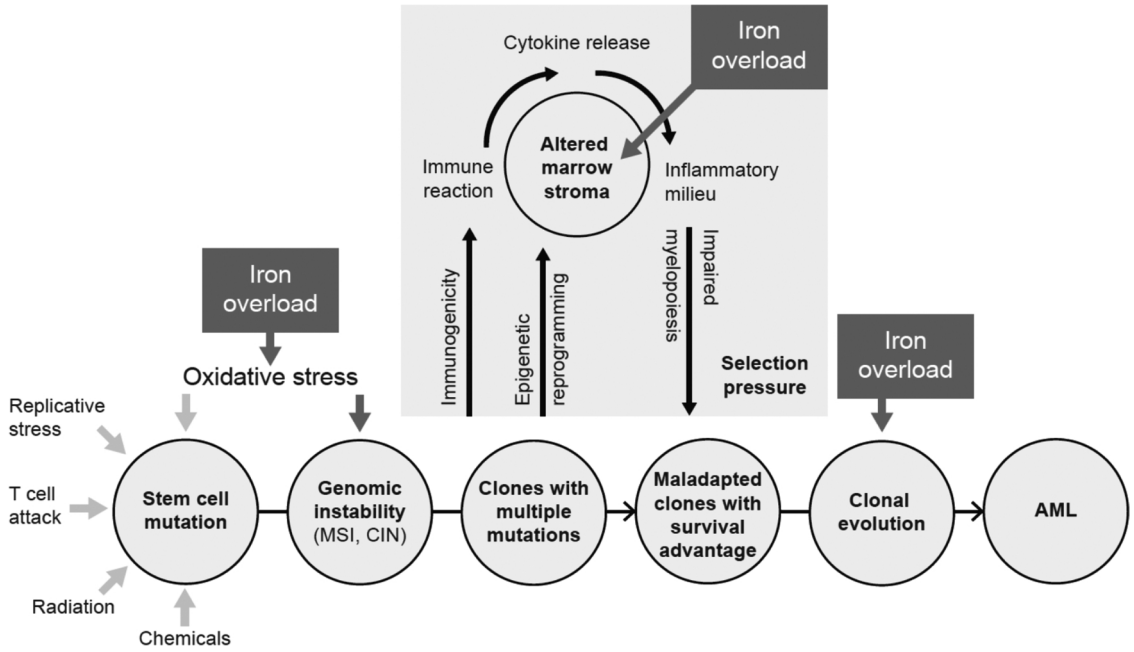

Fig. 4. Does iron overload play a role in the pathology of MDS?
Replicative stress, immune interactions, radiation, and chemicals induce mutations in hematopoietic stem cells that can be exacerbated by oxidative stress from iron overload. This results in genomic instability, leading to the acquisition of somatic mutations. Interactions with the bone marrow stroma lead to mesenchymal cell reprogramming, which supports the proliferation and survival of MDS cells. Cytokine release and the inflammatory milieu add to impairment of myelopoiesis and lead to selection pressure, clones with a survival advantage, clonal evolution, and progression to AML. Iron overload adds to cytokine alteration, inflammation, and clonal evolution. Iron chelation therapy or antioxidants may ameliorate these effects. AML, acute myeloid leukemia; CIN, chromosomal instability; DFX, deferasirox; MDS, myelodysplastic syndrome; MSI, microsatellite instability.

confer clinical benefit. Although observational studies found no increase in adverse events when DFX was administered with HMA, lenalidomide, or EPO, these studies did not focus on tolerability of combinations (Angelucci et al., 2014; Breccia et al., 2012; Maurillo et al., 2015). The combination of DFX and azacitidine is the subject of three clinical trials; two of these closed due to slow patient accrual, and results of the third are pending (ClinicalTrials.gov, 2017a, b; ClinicalTrials.gov, 2017c). The KALLISTO trial was designed to test whether a low dose of DFX in combination with EPO resulted in superior erythroid improvement rate compared with EPO alone. In this small study, the combination did not improve erythroid response, however, this trial closed after more than 2.5 years due to slow enrollment (n = 23) (Gattermann et al., 2017). As the beneficial effects on the cellular milieu conferred in MDS may differ with different chelators, consideration could be given to testing combinations. Of particular interest are observations that eltrombopag, which has iron chelating and immunomodulatory activity, can induce trilineage responses in aplastic anemia patients at doses too low to stimulate thrombopoiesis, and synergizes with other chelation agents to offload iron (Desmond et al., 2014; Olnes et al., 2012; Roth et al., 2012; Vlachodimitropoulou et al., 2017). Facilitated transport of chelators to specific tissues and organs is also being explored (Ma et al., 2009).

Chelators with anticancer activity are being developed, and whether chelators increase sensitivity to chemotherapeutic agents explored (Eberhard et al., 2009; Estrov et al., 1988; Leardi et al., 1998). Modification of existing chelators to increase their half-life is also underway (Hamilton et al., 2017). 'Masked' pro-chelators that have no significant iron chelating ability until the 'mask' is displaced by oxidative stress are in development; in a preclinical model, these protected cardiomyocytes against oxidative injury (Jansova et al., 2014). Free radical scavengers and antioxidants may prove useful (Cortelezzi et al., 2000; Ghoti et al., 2010). Other potential interventions include manipulation of the hepcidin–ferroportin system (Ramos et al., 2012; Sangkhae and Nemeth, 2017; Schmidt and Fleming, 2014).

Though there is as yet no evidence definitively linking IOL-induced oxidative stress to clinical endpoints in MDS, clinical data showing improvement in endpoints with ICT, including HI, are consistent with suppression of a toxic substance in a manner too rapid to be accounted for by removal of organ or total body iron. Although the current discussion has focused on MDS in the usual clinical setting, multiple endpoints around SCT are influenced by IOL and ICT (reviewed in (Leitch et al., 2017a)). Areas for future investigation of oxidative stress include: delineating which parameters or results of oxidative stress correlate with clinical endpoints in MDS and SCT; the duration of oxidative stress suppression required to optimize clinical endpoints; molecular pathways altered by oxidative stress and whether appropriate targeted therapies may be developed; whether IOL-induced oxidative stress suppression should be employed earlier than is usual practice in MDS; whether ICT can be effectively combined with other interventions to suppress oxidative stress; and the characteristics of patients with MDS predicting favorable response to these measures. It may be instructive in future clinical trials to measure longer lasting results of oxidative stress, such as 8-OH-dG or lipid peroxidation products rather than LPI (Gattermann and Rachmilewitz, 2011; Kikuchi et al., 2012; Pimkova et al., 2014).

Of note for future investigations, a recent study demonstrated that the differentiation program of MSCs from patients with MDS was disturbed; MDS cells altered ("reprogrammed") the gene expression of MSCs, leading to alterations in cytokine production and adhesion molecules important in supporting MDS progression. The survival of transplanted MDS cells was supported by MSCs derived from patients with MDS, or by MSCs reprogrammed by MDS cells. This model system lends itself to the study of molecular/cellular mechanisms of cellular survival and progression in MDS, including IOL, and to the study of potential treatments, including ICT (Medyouf et al., 2014). The observation that MDS colonies grow preferentially in hypoxic conditions lends itself to future investigations of MDS in general and of IOL in particular (Thompson et al., 2007). Questions and research tools for

Table 6
Iron overload, oxidative stress, impaired myelopoiesis, and iron chelation therapy-induced hematologic improvement: future directions.

Research agenda and tools
Research agenda: iron overload-induced oxidative stress
● Which parameters of oxidative stress correlate with clinical endpoints in MDS and SCT?
○ Measure long-lasting results of oxidative stress: 8-OH-dG; lipid peroxidation products
○ Further investigation of enhanced LPI association with clinical endpoints
○ Duration of oxidative stress suppression required to optimize clinical endpoints
○ Molecular pathways altered by oxidative stress – develop targeted therapies?
○ Should oxidative stress suppression be employed earlier than is usual in MDS?
○ Can ICT be effectively combined with other interventions to suppress oxidative stress?
● Determine which patient characteristics predict favorable response to these measures
Research tools
● MDS cells reprogram MSC gene expression, altering cytokines and adhesion molecules supporting MDS progression
● MDS colonies grow preferentially in hypoxic conditions
● These models allow analysis of MDS molecular/cellular mechanisms of survival and progression
○ dModels allow for preclinical study of potential treatments

8-OH-dG, 8-hydroxy-2′-deoxyguanosine; ICT, iron chelation therapy; LPI, labile plasma iron; MDS, myelodysplastic syndrome; MSC, mesenchymal stem cells; SCT, stem cell transplantation.

future investigation of iron overload, oxidative stress, impaired myelopoiesis and hematologic improvement with ICT are summarized in Table 6.

In conclusion, multiple clinical investigations in MDS have shown that IOL suppresses myelopoiesis and that ICT results in hematologic improvement in a substantial minority of patients. Accumulating preclinical evidence at the molecular, organelle, cellular, tissue, organ, and whole-body levels demonstrate, with considerable biological plausibility, that clinical endpoints in MDS including myelosuppression may be influenced by IOL, possibly via oxidative stress, and are attenuated using ICT. This evidence is sufficiently extensive to warrant serious consideration and future investigations aimed at determining how to best harness this phenomenon for the clinical benefit of MDS patients.

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HL and NG wrote, reviewed, revised and approved the manuscript.

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References

Abraham, I., Yami, M.A., Yun, S., Kim, H.J., Vincelette, N.D., McBride, A., MacDonald, K., 2017. Survival outcomes in iron chelated and non-chelated patients with lower-risk myelodysplastic syndromes: review and pooled analysis of observational studies. Leuk. Res. 57, 104–108.

Angelucci, E., Santini, V., Di Tucci, A.A., Quaresmini, G., Finelli, C., Volpe, A., Quarta, G., Rivellini, F., Sanpaolo, G., Cilloni, D., Salvi, F., Caocci, G., Molteni, A., Vallisa, D., Voso, M.T., Fenu, S., Borin, L., Latte, G., Alimena, G., Storti, S., Piciocchi, A., Fazi, P., Vignetti, M., Tura, S., 2014. Deferasirox for transfusion-dependent patients with myelodysplastic syndromes: safety, efficacy, and beyond (GIMEMA MDS0306 Trial). Eur. J. Haematol. 92, 527–536.

Angelucci, E., Li, J., Greenberg, P.L., Depei, W., Hou, M., Montaño Figueroa, E., Rodriguez, G.. Dong, Ghosh, X., Bornstein, J., O, Garcia-Manero, 2018. Safety and efficacy, including event-free survival, of deferasirox versus placebo in iron-overloaded patients with low- and Int-1-risk myelodysplastic syndromes (MDS): Outcomes from the randomized, double-blind Telesto study. Blood 132. abstract 234.

Badawi, M.A., Vickars, L.M., Chase, J.M., Leitch, H.A., 2010. Red blood cell transfusion independence following the initiation of iron chelation therapy in myelodysplastic syndrome. Adv. Hematol. 2010, 164045.

Balducci, L., 2006. Transfusion independence in patients with myelodysplastic syndromes: impact on outcomes and quality of life. Cancer 106, 2087–2094.

Banerjee, A., Mifsud, N.A., Bird, R., Forsyth, C., Szer, J., Tam, C., Kellner, S., Grigg, A., Motum, P., Bentley, M., Opat, S., Grigoriadis, G., 2015. The oral iron chelator deferasirox inhibits NF-kappaB mediated gene expression without impacting on proximal activation: implications for myelodysplasia and aplastic anaemia. Br. J. Haematol. 168, 576–582.

Bejar, R., 2017. Implications of molecular genetic diversity in myelodysplastic syndromes. Curr. Opin. Hematol. 24, 73–78.

Bennett, J.M., 2008. Consensus statement on iron overload in myelodysplastic syndromes. Am. J. Hematol. 83, 858–861.

Bennett, J.M., Catovsky, D., Daniel, M.T., Flandrin, G., Galton, D.A., Gralnick, H.R., Sultan, C., 1982. Proposals for the classification of the myelodysplastic syndromes. Br. J. Haematol. 51, 189–199.

Borgna-Pignatti, C., Rugolotto, S., De Stefano, P., Zhao, H., Cappellini, M.D., Del Vecchio, G.C., Romeo, M.A., Forni, G.L., Gamberini, M.R., Ghilardi, R., Piga, A., Cnaan, A., 2004. Survival and complications in patients with thalassemia major treated with transfusion and deferoxamine. Haematologica 89, 1187–1193.

Bowen, D., Wang, L., Frew, M., Kerr, R., Groves, M., 2003. Antioxidant enzyme expression in myelodysplastic and acute myeloid leukemia bone marrow: further evidence of a pathogenetic role for oxidative stress? Haematologica 88, 1070–1072.

Braun, T., Carvalho, G., Coquelle, A., Vozenin, M.C., Lepelley, P., Hirsch, F., Kiladjian, J.J., Ribrag, V., Fenaux, P., Kroemer, G., 2006. NF-kappaB constitutes a potential therapeutic target in high-risk myelodysplastic syndrome. Blood 107, 1156–1165.

Breccia, M., Finsinger, P., Loglisci, G., Federico, V., Santopietro, M., Colafigli, G., Petrucci, L., Salaroli, A., Serrao, A., Latagliata, R., Alimena, G., 2012. Deferasirox treatment for myelodysplastic syndromes: "real-life" efficacy and safety in a single-institution patient population. Ann. Hematol. 91, 1345–1349.

Brittenham, G.M., Griffith, P.M., Nienhuis, A.W., McLaren, C.E., Young, N.S., Tucker, E.E., Allen, C.J., Farrell, D.E., Harris, J.W., 1994. Efficacy of deferoxamine in preventing complications of iron overload in patients with thalassemia major. N. Engl. J. Med. 331, 567–573.

Broxmeyer, H.E., 1986. Acidic isoferritins as suppressor molecules. Blood 68, 796–798.

Broxmeyer, H.E., Cooper, S., Levi, S., Arosio, P., 1991. Mutated recombinant human heavy-chain ferritins and myelosuppression in vitro and in vivo: a link between ferritin ferroxidase activity and biological function. Proc. Natl. Acad. Sci. U. S. A. 88, 770–774.

Broxmeyer, H.E., Lu, L., Bicknell, D.C., Williams, D.E., Cooper, S., Levi, S., Salfeld, J., Arosio, P., 1986. The influence of purified recombinant human heavy-subunit and light-subunit ferritins on colony formation in vitro by granulocyte-macrophage and erythroid progenitor cells. Blood 68, 1257–1263.

Broxmeyer, H.E., Williams, D.E., Geissler, K., Hangoc, G., Cooper, S., Bicknell, D.C., Levi, S., Arosio, P., 1989. Suppressive effects in vivo of purified recombinant human H-subunit (acidic) ferritin on murine myelopoiesis. Blood 73, 74–79.

Buckstein, R., Alibhai, S., Lam, A., Zhang, L., Mamedov, A., Cheung, M.C., Callum, J., Piliotis, E., Tsiao, J., Lin, Y., Wells, R.A., 2009. Transfusion dependence and low hemoglobin have the greatest impact on quality of life (QOL) in MDS patients - a tertiary care cross sectional and longitudinal study. Blood 114, 986–987.

Callens, C., Coulon, S., Naudin, J., Radford-Weiss, I., Boissel, N., Raffoux, E., Wang, P.H., Agarwal, S., Tamouza, H., Paubelle, E., Asnafi, V., Ribeil, J.A., Dessen, P., Canioni, D., Chandesris, O., Rubio, M.T., Beaumont, C., Benhamou, M., Dombret, H., Macintyre, E., Monteiro, R.C., Moura, I.C., Hermine, O., 2010. Targeting iron homeostasis induces cellular differentiation and synergizes with differentiating agents in acute myeloid leukemia. J. Exp. Med. 207, 731–750.

Chai, X., Li, D., Cao, X., Zhang, Y., Mu, J., Lu, W., Xiao, X., Li, C., Meng, J., Chen, J., Li, Q., Wang, J., Meng, A., Zhao, M., 2015. ROS-mediated iron overload injures the hematopoiesis of bone marrow by damaging hematopoietic stem/progenitor cells in mice. Sci. Rep. 5, 10181.

Chee, C.E., Steensma, D.P., Wu, W., Hanson, C.A., Tefferi, A., 2008. Neither serum ferritin nor the number of red blood cell transfusions affect overall survival in refractory anemia with ringed sideroblasts. Am. J. Hematol. 83, 611–613.

Chen, C., Liu, Y., Liu, R., Ikenoue, T., Guan, K.L., Zheng, P., 2008. TSC-mTOR maintains quiescence and function of hematopoietic stem cells by repressing mitochondrial biogenesis and reactive oxygen species. J. Exp. Med. 205, 2397–2408.

Chen, J., Lu, W.Y., Zhao, M.F., Cao, X.L., Jiang, Y.Y., Jin, X., Xu, P., Yuan, T.T., Zhang, Y.C., Chai, X., Meng, J.X., Li, Q., Xiao, X., Mu, J., Li, D.G., Qi, A.P., 2017. Reactive oxygen species mediated T lymphocyte abnormalities in an iron-overloaded mouse model and iron-overloaded patients with myelodysplastic syndromes. Ann. Hematol. 96, 1085–1095.

Cheong, J.W., Kim, H.J., Lee, K.H., Yoon, S.S., Lee, J.H., Park, H.S., Kim, H.Y., Shim, H., Seong, C.M., Kim, C.S., Chung, J., Hyun, M.S., Jo, D.Y., Jung, C.W., Sohn, S.K., Yoon, H.J., Kim, B.S., Joo, Y.D., Park, C.Y., Min, Y.H., 2014. Deferasirox improves hematologic and hepatic function with effective reduction of serum ferritin and liver iron concentration in transfusional iron overload patients with myelodysplastic syndrome or aplastic anemia. Transfusion 54, 1542–1551.

Cheson, B.D., Greenberg, P.L., Bennett, J.M., Lowenberg, B., Wijermans, P.W., Nimer, S.D., Pinto, A., Beran, M., de Witte, T.M., Stone, R.M., Mittelman, M., Sanz, G.F., Gore, S.D., Schiffer, C.A., Kantarjian, H., 2006. Clinical application and proposal for modification of the International Working Group (IWG) response criteria in

myelodysplasia. Blood 108, 419–425.
Choi, S.O., Cho, Y.S., Kim, H.L., Park, J.W., 2007. ROS mediate the hypoxic repression of the hepcidin gene by inhibiting C/EBPalpha and STAT-3. Biochem. Biophys. Res. Commun. 356, 312–317.
Chung, Y.J., Robert, C., Gough, S.M., Rassool, F.V., Aplan, P.D., 2014. Oxidative stress leads to increased mutation frequency in a murine model of myelodysplastic syndrome. Leuk. Res. 38, 95–102.
ClinicalTrials.gov, 2017a. Azacitidine Plus Deferasirox (ICL670) in Higher Risk Myelodysplastic Syndromes (MDS). NCT02038816. https://clinicaltrials.gov/ct2/show/NCT02038816?term=deferasirox&cond=MDS&cntry=CA&rank=1.
ClinicalTrials.gov, 2017b. Phase II Study to Evaluate Overall Response in Patients With Higher Risk Myelodysplastic Syndromes (MDS) Treated With Azacitidine With or Without Deferasirox. NCT02159040. https://clinicaltrials.gov/ct2/show/NCT02159040?term=deferasirox%2C+azacitidine&cond=MDS&rank=1.
ClinicalTrials.gov, 2017c. A Study of Combined Deferasirox, Vitamin D and Azacytidine in High Risk MDS (GFM-EXVD-AZA). NCT01718366. https://clinicaltrials.gov/ct2/show/NCT01718366?term=deferasirox&cond=MDS&cntry=FR&rank=3.
Cortelezzi, A., Cattaneo, C., Sarina, B., Cristiani, S., Pomati, M., Silvestris, I., Motta, M., Ibatici, A., Gornati, G., Volpe, A.D., Maiolo, A.T., 2000. Efficacy of N-acetylcysteine and all-trans retinoic acid in restoring in vitro effective hemopoiesis in myelodysplastic syndromes. Leuk. Res. 24, 129–137.
Craven, S.E., French, D., Ye, W., de Sauvage, F., Rosenthal, A., 2005. Loss of Hspa9b in zebrafish recapitulates the ineffective hematopoiesis of the myelodysplastic syndrome. Blood 105, 3528–3534.
Cui, R., Gale, R.P., Zhu, G., Xu, Z., Qin, T., Zhang, Y., Huang, G., Li, B., Fang, L., Zhang, H., Pan, L., Hu, N., Qu, S., Xiao, Z., 2014. Serum iron metabolism and erythropoiesis in patients with myelodysplastic syndrome not receiving RBC transfusions. Leuk. Res. 38, 545–550.
Davis, B.A., O'Sullivan, C., Jarritt, P.H., Porter, J.B., 2004. Value of sequential monitoring of left ventricular ejection fraction in the management of thalassemia major. Blood 104, 263–269.
Davis, B.A., Porter, J.B., 2000. Long-term outcome of continuous 24-hour deferoxamine infusion via indwelling intravenous catheters in high-risk beta-thalassemia. Blood 95, 1229–1236.
de Souza, G.F., Barbosa, M.C., Santos, T.E., Carvalho, T.M., de Freitas, R.M., Martins, M.R., Goncalves, R.P., Pinheiro, R.F., Magalhaes, S.M., 2013. Increased parameters of oxidative stress and its relation to transfusion iron overload in patients with myelodysplastic syndromes. J. Clin. Pathol. 66, 996–998.
De Swart, L., Smith, A., Fenaux, P., Bowen, D., Sanz, G., Hellstrom-Lindberg, E., Symeonidis, A., Cermak, J., Germing, U., Stauder, R., Georgescu, O., MacKenzie, M., Malcovati, L., Skov Holm, M., Park, S., Beyne-Rauzy, O., Droste, J., De Witte, T., 2011. Transfusion-dependency is the most important prognostic factor for survival in 1000 newly diagnosed MDS patients with low- and intermediate-1 risk MDS in the European LeukemiaNet MDS Registry. Blood 118, 1195–1196.
de Swart, L., Smith, A., Johnston, T.W., Haase, D., Droste, J., Fenaux, P., Symeonidis, A., Sanz, G., Hellstrom-Lindberg, E., Cermak, J., Germing, U., Stauder, R., Georgescu, O., MacKenzie, M., Malcovati, L., Holm, M.S., Almeida, A.M., Madry, K., Slama, B., Guerci-Bresler, A., Sanhes, L., Beyne-Rauzy, O., Luno, E., Bowen, D., de Witte, T., 2015. Validation of the revised international prognostic scoring system (IPSS-R) in patients with lower-risk myelodysplastic syndromes: a report from the prospective European LeukaemiaNet MDS (EUMDS) registry. Br. J. Haematol. 170, 372–383.
Del Río Garma, J., Fernandez, L.C., Fonrodona, B.F.J., 1997. Desferrioxamine in the treatment of myelodysplastic syndromes. Haematologica 82, 639–640.
Della Porta, M.G., Kuendgen, A., Malcovati, L., Zipperer, E., Pascutto, C., Travaglino, E., Maffioli, M., Ambaglio, I., Invernizzi, R., Germing, U., Cazzola, M., 2008. Myelodysplastic syndrome (MDS)-specific comorbidity index for predicting the impact of extra-hematological comorbidities on survival of patients with MDS. Blood 112, 925–926.
Denu, J.M., Tanner, K.G., 1998. Specific and reversible inactivation of protein tyrosine phosphatases by hydrogen peroxide: evidence for a sulfenic acid intermediate and implications for redox regulation. Biochemistry 37, 5633–5642.
Desmond, R., Townsley, D.M., Dumitriu, B., Olnes, M.J., Scheinberg, P., Bevans, M., Parikh, A.R., Broder, K., Calvo, K.R., Wu, C.O., Young, N.S., Dunbar, C.E., 2014. Eltrombopag restores trilineage hematopoiesis in refractory severe aplastic anemia that can be sustained on discontinuation of drug. Blood 123, 1818–1825.
Di Tucci, A.A., Murru, R., Alberti, D., Rabault, B., Deplano, S., Angelucci, E., 2007. Correction of anemia in a transfusion-dependent patient with primary myelofibrosis receiving iron chelation therapy with deferasirox (Exjade, ICL670). Eur. J. Haematol. 78, 540–542.
Dixon, S.J., Stockwell, B.R., 2014. The role of iron and reactive oxygen species in cell death. Nat. Chem. Biol. 10, 9–17.
Eberhard, Y., McDermott, S.P., Wang, X., Gronda, M., Venugopal, A., Wood, T.E., Hurren, R., Datti, A., Batey, R.A., Wrana, J., Antholine, W.E., Dick, J., Schimmer, A.D., 2009. Chelation of intracellular iron with the antifungal agent ciclopirox olamine induces cell death in leukemia and myeloma cells. Blood 114, 3064–3073.
Efficace, F., Santini, V., La Nasa, G., Cottone, F., Finelli, C., Borin, L., Quaresmini, G., Di Tucci, A.A., Volpe, A., Cilloni, D., Quarta, G., Sanpaolo, G., Rivellini, F., Salvi, F., Molteni, A., Voso, M.T., Alimena, G., Fenu, S., Mandelli, F., Angelucci, E., 2016. Health-related quality of life in transfusion-dependent patients with myelodysplastic syndromes: a prospective study to assess the impact of iron chelation therapy. BMJ Support. Palliat. Care 6, 80–88.
Eliasson, P., Jonsson, J.I., 2010. The hematopoietic stem cell niche: low in oxygen but a nice place to be. J. Cell. Physiol. 222, 17–22.
Estrov, Z., Cohen, A., Gelfand, E.W., Freedman, M.H., 1988. Synergistic antiproliferative effects on HL-60 cells: deferoxamine enhances cytosine arabinoside, methotrexate, and daunorubicin cytotoxicity. Am. J. Pediatr. Hematol. 10, 288–291.
Fenaux, P., Mufti, G.J., Hellstrom-Lindberg, E., Santini, V., Gattermann, N., Germing, U., Sanz, G., List, A.F., Gore, S., Seymour, J.F., Dombret, H., Backstrom, J., Zimmerman, L., McKenzie, D., Beach, C.L., Silverman, L.R., 2010. Azacitidine prolongs overall survival compared with conventional care regimens in elderly patients with low bone marrow blast count acute myeloid leukemia. J. Clin. Oncol. 28, 562–569.
Gabutti, V., Piga, A., 1996. Results of long-term iron-chelating therapy. Acta Haematol. 95, 26–36.
Gattermann, N., 2008. Overview of guidelines on iron chelation therapy in patients with myelodysplastic syndromes and transfusional iron overload. Int. J. Hematol. 88, 24–29.
Gattermann, N., Coll, F., Jacobasch, L., Allameddine, A., DeBonnett, L., Azmon, A., Jin, J., 2017. Effect of deferasirox combined with erythropoietin versus erythropoietin alone on erythroid response in patients with low- and Int-1-risk myelodysplastic syndromes: results of the Phase II Kallisto trial. Blood 130, 2984.
Gattermann, N., Finelli, C., Della Porta, M., Fenaux, P., Stadler, M., Guerci-Bresler, A., Schmid, M., Taylor, K., Vassilieff, D., Habr, D., Marcellari, A., Roubert, B., Rose, C., 2012. Hematologic responses to deferasirox therapy in transfusion-dependent patients with myelodysplastic syndromes. Haematologica 97, 1364–1371.
Gattermann, N., Finelli, C., Porta, M.D., Fenaux, P., Ganser, A., Guerci-Bresler, A., Schmid, M., Taylor, K., Vassilieff, D., Habr, D., Domokos, G., Roubert, B., Rose, C., 2010a. Deferasirox in iron-overloaded patients with transfusion-dependent myelodysplastic syndromes: results from the large 1-year EPIC study. Leuk. Res. 34, 1143–1150.
Gattermann, N., Rachmilewitz, E.A., 2011. Iron overload in MDS-pathophysiology, diagnosis, and complications. Ann. Hematol. 90, 1–10.
Gattermann, N., Schmid, M., Guerci-Bresler, A., Della Porta, M., Taylor, K., Habr, D., Domokos, G., Roubert, B., Fenaux, P., 2010b. Correlation between deceased serum ferritin and improved liver transaminases during deferasirox (Exjade) treatment in iron-overloaded patients with myelodysplastic syndromes. Blood 114, 1462–1463.
Ghoti, H., Fibach, E., Merkel, D., Perez-Avraham, G., Grisariu, S., Rachmilewitz, E.A., 2010. Changes in parameters of oxidative stress and free iron biomarkers during treatment with deferasirox in iron-overloaded patients with myelodysplastic syndromes. Haematologica 95, 1433–1434.
Ghoti, H., Fibach, E., Westerman, M., Gordana, O., Ganz, T., Rachmilewitz, E.A., 2011. Increased serum hepcidin levels during treatment with deferasirox in iron-overloaded patients with myelodysplastic syndrome. Br. J. Haematol. 153, 118–120.
Greenberg, P., Cox, C., LeBeau, M.M., Fenaux, P., Morel, P., Sanz, G., Sanz, M., Vallespi, T., Hamblin, T., Oscier, D., Ohyashiki, K., Toyama, K., Aul, C., Mufti, G., Bennett, J., 1997. International scoring system for evaluating prognosis in myelodysplastic syndromes. Blood 89, 2079–2088.
Greenberg, P.L., Tuechler, H., Schanz, J., Sanz, G., Garcia-Manero, G., Sole, F., Bennett, J.M., Bowen, D., Fenaux, P., Dreyfus, F., Kantarjian, H., Kuendgen, A., Levis, A., Malcovati, L., Cazzola, M., Cermak, J., Fonatsch, C., Le Beau, M.M., Slovak, M.L., Krieger, O., Luebbert, M., Maciejewski, J., Magalhaes, S.M., Miyazaki, Y., Pfeilstocker, M., Sekeres, M., Sperr, W.R., Stauder, R., Tauro, S., Valent, P., Vallespi, T., van de Loosdrecht, A.A., Germing, U., Haase, D., 2012. Revised international prognostic scoring system for myelodysplastic syndromes. Blood 120, 2454–2465.
Gu, C., Gonzalez, J., Zhang, T., Kamel-Reid, S., Wells, R.A., 2013. The aryl hydrocarbon receptor nuclear translocator (ARNT) modulates the antioxidant response in AML cells. Leuk. Res. 37, 1750–1756.
Gu, S., Xv, Y., Fei, C., Xiao, C., Guo, J., Zhao, Y., Xv, F., Li, X., Chang, C., 2017. Labile plasma iron, more practical and more sensitive to iron overload in myelodysplastic syndromes. Hematology 22, 9–15.
Guariglia, R., Martorelli, M.C., Villani, O., Pietrantuono, G., Mansueto, G., D'Auria, F., Grieco, V., Bianchino, G., Lerose, R., Bochicchio, G.B., Musto, P., 2011. Positive effects on hematopoiesis in patients with myelodysplastic syndrome receiving deferasirox as oral iron chelation therapy: a brief review. Leuk. Res. 35, 566–570.
Hamilton, J.L., Ul-Haq, M.I., Creagh, A.L., Haynes, C.A., Kizhakkedathu, J.N., 2017. Iron binding and Iron removal efficiency of desferrioxamine based polymeric Iron chelators: influence of molecular size and Chelator density. Macromol. Biosci. 17.
Hartmann, J., Braulke, F., Sinzig, U., Wulf, G., Maas, J.H., Konietschke, F., Haase, D., 2013. Iron overload impairs proliferation of erythroid progenitors cells (BFU-E) from patients with myelodysplastic syndromes. Leuk. Res. 37, 327–332.
Hoeks, M., Yu, G., Langemeijer, S., Crouch, S., De Swart, L., Fenaux, P., Symeonidis, A., Cermak, J., Hellstrom-Lindberg, E., Sanz, G., Stauder, R., Skov-Holm, M., Mittelman, M., Madry, K., Malcovati, L., Tatic, A., Medina de Almeida, A., Germing, U., Savic, A., Gredelj-Šimec, N., Culligan, D., Itzykso, n.R., Guerci-Bresler, A., Slama, B., van Marrewijk, C., Droste, J., van Kraaij, M., Bowen, D.T., de Witte, T.M.M., Smith, A., 2017. Impact of treatment with iron chelation therapy in lower-risk MDS patients participating in the European Leukemianet MDS (EUMDS) Registry. Blood 130 abstract 425.
Hua, Y., Wang, C., Jiang, H., Wang, Y., Liu, C., Li, L., Liu, H., Shao, Z., Fu, R., 2017. Iron overload may promote alteration of NK cells and hematopoietic stem/progenitor cells by JNK and P38 pathway in myelodysplastic syndromes. Int. J. Hematol. 106, 248–257.
Improta, S., Della Cioppa, P., Esposito, M., Gagliardi, A., Lucania, A., Nitrato Izzo, G., Villa, M.R., Mastrullo, L., 2013. Iron chelation therapy improves haematological response in high-risk myelodysplastic patients treated with azacitidine. Leuk. Res. 39 (Suppl. 1), S52–53.
Jankowska, A.M., Gondek, L.P., Szpurka, H., Nearman, Z.P., Tiu, R.V., Maciejewski, J.P., 2008. Base excision repair dysfunction in a subgroup of patients with myelodysplastic syndrome. Leukemia 22, 551–558.
Jansova, H., Machacek, M., Wang, Q., Haskova, P., Jirkovska, A., Potuckova, E., Kielar, F., Franz, K.J., Simunek, T., 2014. Comparison of various iron chelators and prochelators as protective agents against cardiomyocyte oxidative injury. Free Radic. Biol. Med. 74, 210–221.

Jensen, P.D., Heickendorff, L., Pedersen, B., Bendix-Hansen, K., Jensen, F.T., Christensen, T., Boesen, A.M., Ellegaard, J., 1996. The effect of iron chelation on haemopoiesis in MDS patients with transfusional iron overload. Br. J. Haematol. 94, 288–299.
Jensen, P.D., Jensen, I.M., Ellegaard, J., 1992. Desferrioxamine treatment reduces blood transfusion requirements in patients with myelodysplastic syndrome. Br. J. Haematol. 80, 121–124.
Kantarjian, H., O'Brien, S., Ravandi, F., Cortes, J., Shan, J., Bennett, J.M., List, A., Fenaux, P., Sanz, G., Issa, J.P., Freireich, E.J., Garcia-Manero, G., 2008. Proposal for a new risk model in myelodysplastic syndrome that accounts for events not considered in the original International Prognostic Scoring System. Cancer 113, 1351–1361.
Kao, Y.R., Chen, J., Narayanagari, S.R., Todorova, T.I., Aivalioti, M.M., Ferreira, M., Ramos, P.M., Pallaud, C., Mantzaris, I., Shastri, A., Bussel, J.B., Verma, A., Steidl, U., Will, B., 2018. Thrombopoietin receptor-independent stimulation of hematopoietic stem cells by eltrombopag. Sci. Transl. Med. 10.
Kennedy, J.A., Ebert, B.L., 2017. Clinical implications of genetic mutations in myelodysplastic syndrome. J. Clin. Oncol. 35, 968–974.
Kikuchi, S., Kobune, M., Iyama, S., Sato, T., Murase, K., Kawano, Y., Takada, K., Ono, K., Kaneko, Y., Miyanishi, K., Sato, Y., Hayashi, T., Takimoto, R., Kato, J., 2012. Improvement of iron-mediated oxidative DNA damage in patients with transfusion-dependent myelodysplastic syndrome by treatment with deferasirox. Free Radic. Biol. Med. 53, 643–648.
Kobune, M., Kikuchi, S., Iyama, S., Takada, K., Murase, K., Ono, K., Horiguchi, H., Kamihara, Y., Hashimoto, A., Tatekoshi, A., Hayashi, T., Sato, T., Miyanishi, K., Sato, Y., Takimoto, R., Kato, J., 2013. Iron chelation therapy could rapidly reduce oxidative DNA damage in CD34+ hematopoietic cells before decrease of serum ferritin level. Blood 122 Abstract 959.
Kochhar, H., Leger, C.S., Leitch, H.A., 2015. Durable red blood cell transfusion independence in a patient with an MDS/MPN overlap syndrome following discontinuation of iron chelation therapy. Case Rep. Hematol. 2015, 253294.
Kohgo, Y., Ikuta, K., Ohtake, T., Torimoto, Y., Kato, J., 2008. Body iron metabolism and pathophysiology of iron overload. Int. J. Hematol. 88, 7–15.
Korn, C., Mendez-Ferrer, S., 2017. Myeloid malignancies and the microenvironment. Blood 129, 811–822.
Koumoutsea, E.V., Koonyosying, P., Porter, J.B., Cooper, N., Psaila, B., Sola-Visner, M., 2016. Eltrombopag (ELT) reverses iron mediated suppression of insulin secretion in pancreatic cells by chelating iron and decreasing ROS. Blood 128 abstract 1278.
Koumoutsea, E.V., Porter, J.B., Cooper, N., Psaila, B., Sola-Visner, M., 2015. A Potential novel application of eltrombopag: a combination agent to enhance iron chelation therapy. Blood 127.
Latagliata, R., Montagna, C., Porrini, R., Di Veroli, A., Leonetti, S.C., Niscola, P., Ciccone, F., Spadea, A., Breccia, M., Maurillo, L., Rago, A., Spirito, F., Cedrone, M., De Muro, M., Montanaro, M., Andriani, A., Bagnato, A., Montefusco, E., Alimena, G., 2016. Chelation efficacy and erythroid response during deferasirox treatment in patients with myeloproliferative neoplasms in fibrotic phase. Eur. J. Haematol. 96, 643–649.
Leardi, A., Caraglia, M., Selleri, C., Pepe, S., Pizzi, C., Notaro, R., Fabbrocini, A., De Lorenzo, S., Musico, M., Abbruzzese, A., Bianco, A.R., Tagliaferri, P., 1998. Desferioxamine increases iron depletion and apoptosis induced by ara-C of human myeloid leukaemic cells. Br. J. Haematol. 102, 746–752.
Leimberg, J.M., Konijn, A.M., Fibach, E., 2003. Developing human erythroid cells grown in transferrin-free medium utilize iron originating from extracellular ferritin. Am. J. Hematol. 73, 211–212.
Leitch, H.A., 2007. Improving clinical outcome in patients with myelodysplastic syndrome and iron overload using iron chelation therapy. Leuk. Res. 31 (Suppl. 3), S7–9.
Leitch, H.A., Fibach, E., Rachmilewitz, E., 2017a. Toxicity of iron overload and iron overload reduction in the setting of hematopoietic stem cell transplantation for hematologic malignancies. Crit. Rev. Oncol. Hematol. 113, 156–170.
Leitch, H.A., Leger, C.S., Goodman, T.A., Wong, K.K., Wong, D.H.C., Ramadan, K.M., Rollins, M.D., Barnett, M.J., Galbraith, P.F., Vickars, L.M., 2008. Improved survival in patients with myelodysplastic syndrome receiving iron chelation therapy. Clin. Leuk. 2, 205–211.
Leitch, H.A., Parmar, A., Wells, R.A., Chodirker, L., Zhu, N., Nevill, T.J., Yee, K.W.L., Leber, B., Keating, M.M., Sabloff, M., St Hilaire, E., Kumar, R., Delage, R., Geddes, M., Storring, J.M., Kew, A., Shamy, A., Elemary, M., Lenis, M., Mamedov, A., Ivo, J., Francis, J., Zhang, L., Buckstein, R., 2017b. Overall survival in lower IPSS risk MDS by receipt of iron chelation therapy, adjusting for patient-related factors and measuring from time of first red blood cell transfusion dependence: an MDS-CAN analysis. Br. J. Haematol. 179, 83–97.
Leitch, H.A., Thachil, J., 2016. Iron physiology, iron overload, and the porphyrias. In: Steensma, D.P. (Ed.), American Society of Hematology Self-Assessment Program.
List, A., Dewald, G., Bennett, J., Giagounidis, A., Raza, A., Feldman, E., Powell, B., Greenberg, P., Thomas, D., Stone, R., Reeder, C., Wride, K., Patin, J., Schmidt, M., Zeldis, J., Knight, R., 2006. Lenalidomide in the myelodysplastic syndrome with chromosome 5q deletion. N. Engl. J. Med. 355, 1456–1465.
List, A.F., Baer, M.R., Steensma, D.P., Raza, A., Esposito, J., Martinez-Lopez, N., Paley, C., Feigert, J., Besa, E., 2012. Deferasirox reduces serum ferritin and labile plasma iron in RBC transfusion-dependent patients with myelodysplastic syndrome. J. Clin. Oncol. 30, 2134–2139.
Lyons, R.M., Marek, B.J., Paley, C., Esposito, J., McNamara, K., Richards, P.D., DiBella, N., Garcia-Manero, G., 2017. Relation between chelation and clinical outcomes in lower-risk patients with myelodysplastic syndromes: registry analysis at 5 years. Leuk. Res. 56, 88–95.
Ma, M.T., Karas, J.A., White, J.M., Scanlon, D., Donnelly, P.S., 2009. A new bifunctional chelator for copper radiopharmaceuticals: a cage amine ligand with a carboxylate functional group for conjugation to peptides. Chem. Commun. 3237–3239.
Mainous 3rd, A.G., Tanner, R.J., Hulihan, M.M., Amaya, M., Coates, T.D., 2014. The impact of chelation therapy on survival in transfusional iron overload: a meta-analysis of myelodysplastic syndrome. Br. J. Haematol. 167, 720–723.
Malcovati, L., Germing, U., Kuendgen, A., Della Porta, M.G., Pascutto, C., Invernizzi, R., Giagounidis, A., Hildebrandt, B., Bernasconi, P., Knipp, S., Strupp, C., Lazzarino, M., Aul, C., Cazzola, M., 2007. Time-dependent prognostic scoring system for predicting survival and leukemic evolution in myelodysplastic syndromes. J. Clin. Oncol. 25, 3503–3510.
Malcovati, L., Porta, M.G., Pascutto, C., Invernizzi, R., Boni, M., Travaglino, E., Passamonti, F., Arcaini, L., Maffioli, M., Bernasconi, P., Lazzarino, M., Cazzola, M., 2005. Prognostic factors and life expectancy in myelodysplastic syndromes classified according to WHO criteria: a basis for clinical decision making. J. Clin. Oncol. 23, 7594–7603.
Marlein, C.R., Zaitseva, L., Piddock, R.E., Robinson, S.D., Edwards, D.R., Shafat, M.S., Zhou, Z., Lawes, M., Bowles, K.M., Rushworth, S.A., 2017. NADPH oxidase-2 derived superoxide drives mitochondrial transfer from bone marrow stromal cells to leukemic blasts. Blood 130, 1649–1660.
Matatall, K.A., Jeong, M., Chen, S., Sun, D., Chen, F., Mo, Q., Kimmel, M., King, K.Y., 2016. Chronic infection depletes hematopoietic stem cells through stress-induced terminal differentiation. Cell Rep. 17, 2584–2595.
Matatall, K.A., Shen, C.C., Challen, G.A., King, K.Y., 2014. Type II interferon promotes differentiation of myeloid-biased hematopoietic stem cells. Stem Cell 32, 3023–3030.
Maurillo, L., Breccia, M., Buccisano, F., Voso, M.T., Niscola, P., Trape, G., Tatarelli, C., D'Addosio, A., Latagliata, R., Fenu, S., Piccioni, A.L., Fragasso, A., Aloe Spiriti, M.A., Refrigeri, M., Criscuolo, M., Musto, P., Venditti, A., 2015. Deferasirox chelation therapy in patients with transfusion-dependent MDS: a' real-world' report from two regional Italian registries: gruppo Romano Mielodisplasie and Registro Basilicata. Eur. J. Haematol. 95, 52–56.
Medyouf, H., Mossner, M., Jann, J.C., Nolte, F., Raffel, S., Herrmann, C., Lier, A., Eisen, C., Nowak, V., Zens, B., Mudder, K., Klein, C., Oblander, J., Fey, S., Vogler, J., Fabarius, A., Riedl, E., Roehl, H., Kohlmann, A., Staller, M., Haferlach, C., Muller, N., John, T., Platzbecker, U., Metzgeroth, G., Hofmann, W.K., Trumpp, A., Nowak, D., 2014. Myelodysplastic cells in patients reprogram mesenchymal stromal cells to establish a transplantable stem cell niche disease unit. Cell Stem Cell 14, 824–837.
Messa, E., Biale, L., Castiglione, A., Lunghi, M., Bonferroni, M., Salvi, F., Allione, B., Ferrero, D., Calabrese, C., De Gobbi, M., Nicoli, P., Gioia, D., Levis, A., Saglio, G., Cilloni, D., 2017. Erythroid response during iron chelation therapy in a cohort of patients affected by hematologic malignancies and aplastic anemia with transfusion requirement and iron overload: a FISM Italian multicenter retrospective study. Leuk. Lymphoma 58, 2752–2754.
Messa, E., Carturan, S., Maffe, C., Pautasso, M., Bracco, E., Roetto, A., Messa, F., Arruga, F., Defilippi, I., Rosso, V., Zanone, C., Rotolo, A., Greco, E., Pellegrino, R.M., Alberti, D., Saglio, G., Cilloni, D., 2010. Deferasirox is a powerful NF-kappaB inhibitor in myelodysplastic cells and in leukemia cell lines acting independently from cell iron deprivation by chelation and reactive oxygen species scavenging. Haematologica 95, 1308–1316.
Messa, E., Cilloni, D., Messa, F., Arruga, F., Roetto, A., Saglio, G., 2008a. Deferasirox treatment improved the hemoglobin level and decreased transfusion requirements in four patients with the myelodysplastic syndrome and primary myelofibrosis. Acta Haematol. 120, 70–74.
Messa, E., Cilloni, D., Messa, F., Arruga, F., Roetto, A., Saglio, G., 2008b. Deferasirox treatment improved the hemoglobin level and decreased transfusion requirements in four patients with the myelodysplastic syndrome and primary myelofibrosis. Acta Haematol. 120, 70–74.
Meunier, M., Ancelet, S., Lefebvre, C., Arnaud, J., Garrel, C., Pezet, M., Wang, Y., Faure, P., Szymanski, G., Duployez, N., Preudhomme, C., Biard, D., Polack, B., Cahn, J.Y., Moulis, J.M., Park, S., 2017. Reactive oxygen species levels control NF-kappaB activation by low dose deferasirox in erythroid progenitors of low risk myelodysplastic syndromes. Oncotarget 8, 105510–105524.
Molteni, A., Riva, M., Pellizzari, A., Borin, L., Freyrie, A., Greco, R., Ubezio, M., Bernardi, M., Fariciotti, A., Nador, G., Nichelatti, M., Ravano, E., Morra, E., 2013. Hematological improvement during iron-chelation therapy in myelodysplastic syndromes: the experience of the "Rete Ematologica Lombarda". Leuk. Res. 37, 1233–1240.
Mossner, M., Stohr, A., Nolte, F., Jann, J.-C., Fey, S., Nowak, V., Oblander, J., Pressler, J., Baldus, C.D., Schultze, T.J., Neumann, M., Hoffman, W.-K., Nowak, D., 2014. Gene expression of the erythroid regulator erythroferrone (ERFE) is highly deregulated in CD71+ erythroprogenitor cells of patients with myelodysplastic syndromes and demonstrates prognostic relevance. Blood 124 abstract 4620.
Muckenthaler, M.U., Rivella, S., Hentze, M.W., Galy, B., 2017. A red carpet for iron metabolism. Cell 168, 344–361.
Musto, P., Maurillo, L., Simeon, V., Poloni, C., Finelli, C., Clissa, C., Balleari, E., Ricco, A., D'Arco, A.M., Cortelezzi, A., Tarantini, G., Villani, O., Mansueto, G., Criscuolo, C., Breccia, M., Niscola, P., Sanna, A., Autore, F., Voso, M.T., Fenu, S., Venditti, A., Santini, V., Levis, A., 2014. Iron-chelating therapy with deferasirox in higher risk myelodysplastic syndromes: a retrospective, multicenter, Italian study. Haematologica Abstract 4061.
Nathan, C., 2003. Specificity of a third kind: reactive oxygen and nitrogen intermediates in cell signaling. J. Clin. Invest. 111, 769–778.
Nathan, C., Cunningham-Bussel, A., 2013. Beyond oxidative stress: an immunologist's guide to reactive oxygen species. Nat. Rev. Immunol. 13, 349–361.
Nathan, C., Ding, A., 2010. SnapShot: reactive oxygen intermediates (ROI). Cell 140 (951–951), e952.
Neukirchen, J., Fox, F., Kundgen, A., Nachtkamp, K., Strupp, C., Haas, R., Germing, U., Gattermann, N., 2012. Improved survival in MDS patients receiving iron chelation therapy - a matched pair analysis of 188 patients from the Dusseldorf MDS registry. Leuk. Res. 36, 1067–1070.
Nolte, F., Hochsmann, B., Giagounidis, A., Lubbert, M., Platzbecker, U., Haase, D., Luck,

A., Gattermann, N., Taupitz, M., Baier, M., Leismann, O., Junkes, A., Schumann, C., Hofmann, W.K., Schrezenmeier, H., 2013. Results from a 1-year, open-label, single arm, multi-center trial evaluating the efficacy and safety of oral deferasirox in patients diagnosed with low and int-1 risk myelodysplastic syndrome (MDS) and transfusion-dependent iron overload. Ann. Hematol. 92, 191–198.
Novotna, B., Bagryantseva, Y., Siskova, M., Neuwirtova, R., 2009. Oxidative DNA damage in bone marrow cells of patients with low-risk myelodysplastic syndrome. Leuk. Res. 33, 340–343.
Ohyashiki, J.H., Kobayashi, C., Hamamura, R., Okabe, S., Tauchi, T., Ohyashiki, K., 2009. The oral iron chelator deferasirox represses signaling through the mTOR in myeloid leukemia cells by enhancing expression of REDD1. Cancer Sci. 100, 970–977.
Oliva, E.N., Ronco, F., Marino, A., Alati, C., Pratico, G., Nobile, F., 2010. Iron chelation therapy associated with improvement of hematopoiesis in transfusion-dependent patients. Transfusion 50, 1568–1570.
Olivieri, N.F., Brittenham, G.M., 1997. Iron-chelating therapy and the treatment of thalassemia. Blood 89, 739–761.
Olnes, M.J., Scheinberg, P., Calvo, K.R., Desmond, R., Tang, Y., Dumitriu, B., Parikh, A.R., Soto, S., Biancotto, A., Feng, X., Lozier, J., Wu, C.O., Young, N.S., Dunbar, C.E., 2012. Eltrombopag and improved hematopoiesis in refractory aplastic anemia. N. Engl. J. Med. 367, 11–19.
Park, S.J., Han, C.W., 2008. Complete hematopoietic recovery after continuous iron chelation therapy in a patient with severe aplastic anemia with secondary hemochromatosis. J. Korean Med. Sci. 23, 320–323.
Parmar, K., Mauch, P., Vergilio, J.A., Sackstein, R., Down, J.D., 2007. Distribution of hematopoietic stem cells in the bone marrow according to regional hypoxia. Proc. Natl. Acad. Sci. U. S. A. 104, 5431–5436.
Paubelle, E., Zylbersztejn, F., Alkhaeir, S., Suarez, F., Callens, C., Dussiot, M., Isnard, F., Rubio, M.T., Damaj, G., Gorin, N.C., Marolleau, J.P., Monteiro, R.C., Moura, I.C., Hermine, O., 2013. Deferasirox and vitamin D improves overall survival in elderly patients with acute myeloid leukemia after demethylating agents failure. PLoS One 8, e65998.
Pietras, E.M., 2017. Inflammation: a key regulator of hematopoietic stem cell fate in health and disease. Blood 130, 1693–1698.
Pileggi, C., Di Sanzo, M., Mascaro, V., Marafioti, M.G., Costanzo, F.S., Pavia, M., 2017. Role of serum ferritin level on overall survival in patients with myelodysplastic syndromes: results of a meta-analysis of observational studies. PLoS One 12, e0179016.
Pimkova, K., Chrastinova, L., Suttnar, J., Stikarova, J., Kotlin, R., Cermak, J., Dyr, J.E., 2014. Plasma levels of aminothiols, nitrite, nitrate, and malondialdehyde in myelodysplastic syndromes in the context of clinical outcomes and as a consequence of iron overload. Oxid. Med. Cell. Longev. 2014, 416028.
Platzbecker, U., Germing, U., Gotze, K.S., Kiewe, P., Mayer, K., Chromik, J., Radsak, M., Wolff, T., Zhang, X., Laadem, A., Sherman, M.L., Attie, K.M., Giagounidis, A., 2017a. Luspatercept for the treatment of anaemia in patients with lower-risk myelodysplastic syndromes (PACE-MDS): a multicentre, open-label phase 2 dose-finding study with long-term extension study. Lancet Oncol. 18, 1338–1347.
Platzbecker, U., Symeonidis, A., Oliva, E.N., Goede, J.S., Delforge, M., Mayer, J., Slama, B., Badre, S., Gasal, E., Mehta, B., Franklin, J., 2017b. A phase 3 randomized placebo-controlled trial of darbepoetin alfa in patients with anemia and lower-risk myelodysplastic syndromes. Leukemia 31, 1944–1950.
Prus, E., Fibach, E., 2011. Uptake of non-transferrin iron by erythroid cells. Anemia 2011, 945289.
Pullarkat, V., Sehgal, A., Li, L., Meng, Z., Lin, A., Forman, S., Bhatia, R., 2012. Deferasirox exposure induces reactive oxygen species and reduces growth and viability of myelodysplastic hematopoietic progenitors. Leuk. Res. 36, 966–973.
Raaijmakers, M.H., 2014. Disease progression in myelodysplastic syndromes: do mesenchymal cells pave the way? Cell Stem Cell 14, 695–697.
Raaijmakers, M.H., Mukherjee, S., Guo, S., Zhang, S., Kobayashi, T., Schoonmaker, J.A., Ebert, B.L., Al-Shahrour, F., Hasserjian, R.P., Scadden, E.O., Aung, Z., Matza, M., Merkenschlager, M., Lin, C., Rommens, J.M., Scadden, D.T., 2010. Bone progenitor dysfunction induces myelodysplasia and secondary leukaemia. Nature 464, 852–857.
Ramos, E., Ruchala, P., Goodnough, J.B., Kautz, L., Preza, G.C., Nemeth, E., Ganz, T., 2012. Minihepcidins prevent iron overload in a hepcidin-deficient mouse model of severe hemochromatosis. Blood 120, 3829–3836.
Rassool, F.V., Gaymes, T.J., Omidvar, N., Brady, N., Beurlet, S., Pla, M., Reboul, M., Lea, N., Chomienne, C., Thomas, N.S., Mufti, G.J., Padua, R.A., 2007. Reactive oxygen species, DNA damage, and error-prone repair: a model for genomic instability with progression in myeloid leukemia? Cancer Res. 67, 8762–8771.
Remacha, A.F., Arrizabalaga, B., Villegas, A., Duran, M.S., Hermosin, L., de Paz, R., Garcia, M., Diez Campelo, M., Sanz, G., 2015. Evolution of iron overload in patients with low-risk myelodysplastic syndrome: iron chelation therapy and organ complications. Ann. Hematol. 94, 779–787.
Ren, X., Dorrington, K.L., Maxwell, P.H., Robbins, P.A., 2000. Effects of desferrioxamine on serum erythropoietin and ventilatory sensitivity to hypoxia in humans. J. Appl. Physiol. 89, 680–686.
Rose, C., Brechignac, S., Vassilief, D., Pascal, L., Stamatoullas, A., Guerci, A., Larbaa, D., Dreyfus, F., Beyne-Rauzy, O., Chaury, M.P., Roy, L., Cheze, S., Morel, P., Fenaux, P., Myelodysplasies, at.G.G.Fd., 2010. Does iron chelation therapy improve survival in regularly transfused lower risk MDS patients? A multicenter study by the GFM. Leuk. Res. 34, 864–870.
Rose, C., Fitoussi, O., Gyan, E., Hacini, M., Amé, S., Corront, B., Beyne-Rauzy, O., Adiko, D.I., Loppinet, E.-A., Ali-Ammar, N., Wattel, E., Dreyfus, F., Cheze, S., 2016. Prospective evaluation of the effect of deferasirox on hematologic response in transfusion-dependent patients with low-risk MDS and iron overload: the Rythmex study. Blood 128 abstract 2008.
Roth, M., Will, B., Simkin, G., Narayanagari, S., Barreyro, L., Bartholdy, B., Tamari, R., Mitsiades, C.S., Verma, A., Steidl, U., 2012. Eltrombopag inhibits the proliferation of leukemia cells via reduction of intracellular iron and induction of differentiation. Blood 120, 386–394.
Ruddell, R.G., Hoang-Le, D., Barwood, J.M., Rutherford, P.S., Piva, T.J., Watters, D.J., Santambrogio, P., Arosio, P., Ramm, G.A., 2009. Ferritin functions as a proinflammatory cytokine via iron-independent protein kinase C zeta/nuclear factor kappaB-regulated signaling in rat hepatic stellate cells. Hepatology 49, 887–900.
Saigo, K., Kono, M., Takagi, Y., Takenokuchi, M., Hiramatsu, Y., Tada, H., Hishita, T., Misawa, M., Imoto, S., Imashuku, S., 2013. Deferasirox reduces oxidative stress in patients with transfusion dependency. J. Clin. Med. Res. 5, 57–60.
Saigo, K., Takenokuchi, M., Hiramatsu, Y., Tada, H., Hishita, T., Takata, M., Misawa, M., Imoto, S., Imashuku, S., 2011. Oxidative stress levels in myelodysplastic syndrome patients: their relationship to serum ferritin and haemoglobin values. J. Int. Med. Res. 39, 1941–1945.
Sallmyr, A., Fan, J., Rassool, F.V., 2008. Genomic instability in myeloid malignancies: increased reactive oxygen species (ROS), DNA double strand breaks (DSBs) and error-prone repair. Cancer Lett. 270, 1–9.
Sanford, D., Hsia, C.C., 2015. A case of transfusion independence in a patient with myelodysplastic syndrome using deferasirox, sustained for two years after stopping therapy. Curr. Oncol. 22, e128–e132.
Sangkhae, V., Nemeth, E., 2017. Regulation of the iron homeostatic hormone hepcidin. Adv. Nutr. 8, 126–136.
Santini, V., Girelli, D., Sanna, A., Martinelli, N., Duca, L., Campostrini, N., Cortelezzi, A., Corbella, M., Bosi, A., Reda, G., Olivieri, O., Cappellini, M.D., 2011. Hepcidin levels and their determinants in different types of myelodysplastic syndromes. PLoS One 6, e23109.
Sanz, G., Nomdedeu, B., Such, E., Bernal, T., Belkaid, M., Ardanaz, T., Marco, V., Pedro, C., Ramos, F., Consuelo del Canizo, M., Luno, E., Cobo, F., Carbonell, F., Gomez, V., Munoz, J.A., Luz Amigo, M., Bailen, M.J., Bueno, J., Requena, M.J., Bargay, J., Sanchez, J., Senent, L., Arenillas, L., De Paz, R., Xicoy, B., Duarte, R., Cervera, J., 2008. Independent impact of iron overload and transfusion dependency on survival and leukemic evolution in patients with myelodysplastic syndrome. Blood 112, 238–239.
Sattler, M., Winkler, T., Verma, S., Byrne, C.H., Shrikhande, G., Salgia, R., Griffin, J.D., 1999. Hematopoietic growth factors signal through the formation of reactive oxygen species. Blood 93, 2928–2935.
Schanz, J., Tuchler, H., Sole, F., Mallo, M., Luno, E., Cervera, J., Granada, I., Hildebrandt, B., Slovak, M.L., Ohyashiki, K., Steidl, C., Fonatsch, C., Pfeilstocker, M., Nosslinger, T., Valent, P., Giagounidis, A., Aul, C., Lubbert, M., Stauder, R., Krieger, O., Garcia-Manero, G., Faderl, S., Pierce, S., Le Beau, M.M., Bennett, J.M., Greenberg, P., Germing, U., Haase, D., 2012. New comprehensive cytogenetic scoring system for primary myelodysplastic syndromes (MDS) and oligoblastic acute myeloid leukemia after MDS derived from an international database merge. J. Clin. Oncol. 30, 820–829.
Schmidt, P.J., Fleming, M.D., 2014. Modulation of hepcidin as therapy for primary and secondary iron overload disorders: preclinical models and approaches. Hematol. Oncol. Clin. North Am. 28, 387–401.
Shao, L., Li, H., Pazhanisamy, S.K., Meng, A., Wang, Y., Zhou, D., 2011. Reactive oxygen species and hematopoietic stem cell senescence. Int. J. Hematol. 94, 24–32.
Smeets, M.E., Vreugdenhil, G., Holdrinet, R.S., 1996. Improvement of erythropoiesis during treatment with deferiprone in a patient with myelofibrosis and transfusional hemosiderosis. Am. J. Hematol. 51, 243–244.
Steensma, D.P., 2009. Myelodysplasia paranoia: iron as the new radon. Leuk. Res. 33, 1158–1163.
Swerdlow, S.H., Campo, E., Harris, N.L., Jaffe, E.S., Pileri, S.A., Stein, H., Thiel, J., Vardiman, J.W., 2008. WHO classification of tumours of haematopoietic and lymphoid tissues. WHO Press, Lyon.
Tai-Nagara, I., Matsuoka, S., Ariga, H., Suda, T., 2014. Mortalin and DJ-1 coordinately regulate hematopoietic stem cell function through the control of oxidative stress. Blood 123, 41–50.
Takizawa, H., Boettcher, S., Manz, M.G., 2012. Demand-adapted regulation of early hematopoiesis in infection and inflammation. Blood 119, 2991–3002.
Taoka, K., Kumano, K., Nakamura, F., Hosoi, M., Goyama, S., Imai, Y., Hangaishi, A., Kurokawa, M., 2012. The effect of iron overload and chelation on erythroid differentiation. Int. J. Hematol. 95, 149–159.
Tataranni, T., Agriesti, F., Mazzoccoli, C., Ruggieri, V., Scrima, R., Laurenzana, I., D'Auria, F., Falzetti, F., Di Ianni, M., Musto, P., Capitanio, N., Piccoli, C., 2015. The iron chelator deferasirox affects redox signalling in haematopoietic stem/progenitor cells. Br. J. Haematol. 170, 236–246.
Tefferi, A., Stone, R.M., 2009. Iron chelation therapy in myelodysplastic syndrome - Cui bono? Leukemia 23, 1373.
Telfer, P.T., Prestcott, E., Holden, S., Walker, M., Hoffbrand, A.V., Wonke, B., 2000. Hepatic iron concentration combined with long-term monitoring of serum ferritin to predict complications of iron overload in thalassaemia major. Br. J. Haematol. 110, 971–977.
Thompson, J.E., Conlon, J.P., Yang, X., Sanchez, P.V., Carroll, M., 2007. Enhanced growth of myelodysplastic colonies in hypoxic conditions. Exp. Hematol. 35, 21–31.
Vinchi, F., Costa da Silva, M., Sparla, R., Weidner, H., Rauner, M., Tolosano, E., Platzbecker, U., Muckenthaler, M.U., 2017. Red blood cell transfusions shape macrophages towards an anti-inflammatory functional phenotype and impair the immune esponse to infectious cues. Blood abstract 227.
Vlachodimitropoulou, E., Chen, Y.L., Garbowski, M., Koonyosying, P., Psaila, B., Sola-Visner, M., Cooper, N., Hider, R., Porter, J., 2017. Eltrombopag: a powerful chelator of cellular or extracellular iron (III) alone or combined with a second chelator. Blood 130, 1923–1933.
Walter, D., Lier, A., Geiselhart, A., Thalheimer, F.B., Huntscha, S., Sobotta, M.C., Moehrle, B., Brocks, D., Bayindir, I., Kaschutnig, P., Muedder, K., Klein, C., Jauch, A.,

Schroeder, T., Geiger, H., Dick, T.P., Holland-Letz, T., Schmezer, P., Lane, S.W., Rieger, M.A., Essers, M.A., Williams, D.A., Trumpp, A., Milsom, M.D., 2015. Exit from dormancy provokes DNA-damage-induced attrition in haematopoietic stem cells. Nature 520, 549–552.
Wermke, M., Eckoldt, J., Gotze, K.S., Klein, S.A., Bug, G., de Wreede, L.C., Kramer, M., Stolzel, F., von Bonin, M., Schetelig, J., Laniado, M., Plodeck, V., Hofmann, W.K., Ehninger, G., Bornhauser, M., Wolf, D., Theurl, I., Platzbecker, U., 2018. Enhanced labile plasma iron and outcome in acute myeloid leukaemia and myelodysplastic syndrome after allogeneic haemopoietic cell transplantation (ALLIVE): a prospective, multicentre, observational trial. Lancet Haematol. 5, e201–e210.
Wong, C.A.C., Wong, S.A.Y., Leitch, H.A., 2018. Iron overload in lower international prognostic scoring system risk patients with myelodysplastic syndrome receiving red blood cell transfusions: relation to infections and possible benefit of iron chelation therapy. Leuk. Res. 67, 75–81.
Wong, S.A., Leitch, H.A., 2018. Iron chelation therapy in lower IPSS risk myelodysplastic syndromes; which subtypes benefit? Leuk. Res. 64, 24–29.
Yamamoto, M., Tanaka, H., Toki, Y., Hatayama, M., Ito, S., Addo, L., Shindo, M., Sasaki, K., Ikuta, K., Ohtake, T., Fujiya, M., Torimoto, Y., Kohgo, Y., 2016. Iron-induced epigenetic abnormalities of mouse bone marrow through aberrant activation of aconitase and isocitrate dehydrogenase. Int. J. Hematol. 104, 491–501.
Zeidan, A.M., Hendrick, F., Friedmann, E., Baer, M.R., Gore, S.D., Sasane, M., Paley, C., Davidoff, A.J., 2015. Deferasirox therapy is associated with reduced mortality risk in a medicare population with myelodysplastic syndromes. J. Comp. Eff. Res. 4, 327–340.
Zhang, C.C., Sadek, H.A., 2014. Hypoxia and metabolic properties of hematopoietic stem cells. Antioxid. Redox Signal. 20, 1891–1901.
Zhang, Y., Zhai, W., Zhao, M., Li, D., Chai, X., Cao, X., Meng, J., Chen, J., Xiao, X., Li, Q., Mu, J., Shen, J., Meng, A., 2015. Effects of iron overload on the bone marrow microenvironment in mice. PLoS One 10, e0120219.
Zheng, Q.Q., Zhao, Y.S., Guo, J., Zhao, S.D., Song, L.X., Fei, C.M., Zhang, Z., Li, X., Chang, C.K., 2017. Iron overload promotes erythroid apoptosis through regulating HIF-1a/ROS signaling pathway in patients with myelodysplastic syndrome. Leuk. Res. 58, 55–62.
Zhu, Y., Li, X., Chang, C., Xu, F., He, Q., Guo, J., Tao, Y., Liu, Y., Liu, L., Shi, W., 2016. SF3B1-mutated myelodysplastic syndrome with ring sideroblasts harbors more severe iron overload and corresponding over-erythropoiesis. Leuk. Res. 44, 8–16.
Zipperer, E., Post, J.G., Herkert, M., Kundgen, A., Fox, F., Haas, R., Gattermann, N., Germing, U., 2013. Serum hepcidin measured with an improved ELISA correlates with parameters of iron metabolism in patients with myelodysplastic syndrome. Ann. Hematol. 92, 1617–1623.

Dr. Leitch is a Hematologist at St. Paul's Hospital in Vancouver, Canada and Clinical Professor in the Department of Medicine at the University of British Columbia. Dr. Leitch has a clinical and research interest in transfusional iron overload in acquired anemias such as the myelodysplastic syndromes (MDS), and a longstanding interest in lymphoproliferative and other blood disorders in the setting of HIV infection. She is Director of Hematology/Oncology Research at St. Paul's Hospital, a reviewer of scientific papers for multiple medical journals, and an active member of the UBC Department of Medicine Research Advisory Committee, the Canadian Consortium on MDS (CCMDS), the Canadian Hematology Society (CHS) and the American Society of Hematology (ASH).

Dr. Gattermann is Professor of Medicine at the Department of Hematology, Oncology, and Clinical Immunology, and Managing Director of the University Cancer Center at Heinrich-Heine-University (HHU), Düsseldorf, Germany. His research focus is on myelodysplastic syndromes, covering epidemiology, pathogenesis, prognosis, and therapy. He is particularly interested in problems of iron metabolism ranging from transfusional iron overload to the pathomechanism of mitochondrial iron overload in sideroblastic anemia. Dr. Gattermann is a member of the German Society of Hematology and Oncology (DGHO), the European LeukemiaNet, the American Society of Hematology (ASH), and the ASH Scientific Committee on Iron and Heme.