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Iron-chelating therapy with deferasirox in transfusion-dependent, higher risk myelodysplastic syndromes: a retrospective, multicentre study

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Summary

Iron chelation is controversial in higher risk myelodysplastic syndromes (HR-MDS), outside the allogeneic transplant setting. We conducted a retrospective, multicentre study in 51 patients with transfusion-dependent, intermediate-to-very high risk MDS, according to the revised international prognostic scoring system, treated with the oral iron chelating agent deferasirox (DFX). Thirty-six patients (71%) received azacitidine concomitantly. DFX was given at a median dose of 1000 mg/day (range 375–2500 mg) for a median of 11 months (range 0·4–75). Eight patients (16%) showed grade 2–3 toxicities (renal or gastrointestinal), 4 of whom (8%) required drug interruption. Median ferritin levels decreased from 1709 μg/l at baseline to 1100 μg/l after 12 months of treatment ($P = 0·02$). Seventeen patients showed abnormal transaminase levels at baseline, which improved or normalized under DFX treatment in eight cases. One patient showed a remarkable haematological improvement. At a median follow up of 35·3 months, median overall survival was 37·5 months. The results of this first survey of DFX in HR-MDS are comparable, in terms of safety and efficacy, with those observed in lower-risk MDS. Though larger, prospective studies are required to demonstrate real clinical benefits, our data suggest that DFX is feasible and might be considered in a selected cohort of HR-MDS patients.

Keywords: iron chelation, deferasirox, myelodyslastic syndromes, International Prognostic Scoring System, revised-International Prognostic Scoring System.

First published online 17 April 2017
doi: 10.1111/bjh.14621

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Received 21 September 2016; accepted for publication 27 December 2016
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This paper was presented in part at the XX EHA Congress, Stockholm, June 2014.

Iron-chelating therapy (ICT) is an accepted treatment for iron overload in transfusion-dependent patients with lower-risk myelodysplastic syndromes (MDS). According to the guidelines, patients who have received 20–30 red blood cell (RBC) transfusions and/or have serum ferritin (SF) levels of 1000–2500 μg/l, are candidates for ICT (Santini *et al*, 2010; Malcovati *et al*, 2013; NCCN, 2016). Conversely, with the important exception of patients eligible for allogeneic stem cell transplantation (AlloSCT) and who have been shown to benefit from pre- and post-transplant ICT (Armand *et al*, 2007, 2011; Alessandrino *et al*, 2010, 2011; Sivgin *et al*, 2013; Michallet *et al*, 2017; Jaekel *et al*, 2016), this treatment, although not formally contraindicated, is not generally recommended in higher-risk MDS (HR-MDS). Main limiting factors are the expected shorter overall survival (OS) of these patients, and the potential increased risk of renal or hepatic impairment/failure and gastro-intestinal bleeding (NCCN, 2016). As a consequence, limited data are currently available on the use of ICT, particularly the oral chelator deferasirox (DFX), in HR-MDS not eligible for AlloSCT.

It is well known that transfusion dependence and the consequent iron overload (as measured by SF levels and number of RBC transfusions received) are well correlate with reduced OS and a higher probability of leukaemic transformation in MDS patients (Cazzola & Malcovati, 2005; Malcovati *et al*, 2005, 2006). Of interest, about one-third of patients analysed in those studies had an intermediate-2 or high International Prognostic Scoring System (IPSS) score (Greenberg *et al*, 1997) including, in particular, subjects with poor risk karyotype, thus suggesting that the negative prognostic effect of iron overload could affect also HR-MDS. In another retrospective Spanish study conducted in 2241 patients with MDS, OS was significantly better among 123 patients with refractory anaemia with excess blasts (RAEB; 78 RAEB-1 and 45 RAEB-2), in those with SF levels <1000 μg/l (Sanz *et al*, 2008).

The introduction of azacitidine into clinical practice has significantly changed the course of disease in HR-MDS, by improving survival in a significant proportion of these patients (Fenaux *et al*, 2009). In this new scenario, some guidelines now suggest that: "... iron chelation should be considered for transfusion-dependent patients with int-2 and high IPSS risk while responding to therapies able to modify their life expectancy" (Santini *et al*, 2010). Interestingly, recent reports indicate that SF levels and the number of transfusions received may represent independent prognostic factors for response, OS and leukaemic transformation in HR-MDS receiving azacitidine (Komrokji *et al*, 2011; Itzykson *et al*, 2011; García *et al*, 2012; Tsang & Leitch, 2016).

On the other hand, our understanding of pathophisiology of iron metabolism has greatly increased in the last decade and we are now able to better predict iron toxicity mechanisms and targets (Porter & Garbowski, 2014). In particular, from a biological point of view, *in vitro* studies suggest that iron overload induces genotoxic/mutagenic effects on both the mitochondrial and nuclear DNA of haematopoietic cells, increasing genomic instability and favouring progression of a pre-leukaemic status to frank acute myeloid leukaemia (AML) (Porter *et al*, 2016; Gao *et al*, 2009; Chan *et al*, 2010); in this setting, redox signalling seems to play a significant role (Chung *et al*, 2014; De Souza *et al*, 2015).

In the presence of iron overload, after transferrin saturation, plasma iron is present as non-transferrin-bound iron (NTBI). A component of NTBI, called labile plasma iron

(LPI), is a potent redox-active form capable of entering cells and inducing cellular iron overload (Cabantchik *et al*, 2005). Interestingly, NTBI and LPI can be found in patient plasma outside conditions of clear iron overload, as in patients receiving chemotherapy (Dürken *et al*, 1997).

Recently, it has been postulated that LPI can also determine increased apoptosis of developing precursors in the bone marrow, leading to ineffective erythropoiesis (Prus & Fibach, 2011). This has been confirmed by *in vitro* studies on human samples from MDS patients showing that iron overload suppresses the proliferation of erythroid progenitor cells, particularly burst-forming unit erythroid (BFU-E) cells (Hartmann *et al*, 2013). Furthermore, it has been demonstrated in mice models that iron overload could impair haematopoiesis not only by damaging haematopoietic stem cells, but also their microenvironment by different mechanisms (Zhang *et al*, 2015; Chai *et al*, 2015). Interestingly, in many of the above described conditions, agents targeting iron homeostasis (Taoka *et al*, 2012; Le & Richardson, 2004; Jiang *et al*, 2005; Guo *et al*, 2006; Eberhard *et al*, 2009; Callens *et al*, 2010; Roth *et al*, 2012), particularly DFX (Tataranni *et al*, Banerjee *et al*, 2015; Kikuchi *et al*, 2012; Pullarkat *et al*, 2012; Messa *et al*, 2010; Ghoti *et al*, 2010), have been reported to exert *in vitro* possible protective effects. Finally, iron overload due to transfusions may reduce the efficacy of neutrophils, macrophages and NK cells, favouring infections, which may be fatal complications of more intensive treatments in patients with higher-risk MDS (Goldberg *et al*, 2010). Based on these considerations, ICT might have a rationale in HR-MDS, at least in selected patients. Therefore, we evaluated use, efficacy, tolerability and other clinical effects of DFX, currently the most used iron-chelating agent for lower risk MDS (Breccia & Alimena, 2013; Temraz *et al*, 2014; Shenoy *et al*, 2014), in HR-MDS treated in clinical practice, outside of clinical trials.

Patients and methods

This was a retrospective, multicentre, non interventional study, based on clinical data collected from HR-MDS patients conducted across 14 Italian haematology centres that contribute to the two major national MDS registries (FISM, Fondazione Italiana Sindromi Mielodisplastiche; GROM, Gruppo Romano Mielodisplasie) and to GIMEMA (Gruppo Italiano Malattie Ematologiche dell'Adulto) MDS Working Party. Investigators were asked to refer all consecutive patients with intermediate, high or very high R-IPSS risk MDS (Greenberg *et al*, 2012) registered in their database and treated with DFX in daily practice. All data were pooled and only patients with a complete and adequately monitored dataset [age, sex, haemoglobin, white blood cell and platelet counts, parameters of hepatic (alanine transaminase [ALT]/aspartate transaminase [AST]) and renal (serum creatinine) function, number of RBC units received, revised World Health Organization (WHO) diagnosis (Arber *et al*, 2016) and revised IPSS (R-IPSS) assessment, SF levels before and after ICT (at least two controls), previous and concomitant treatments, DFX doses employed, DFX-related adverse events observed and a follow-up of at least 1 year after starting DFX, for surviving patients] were analysed. The study was approved by the Unique Regional Ethic Committee of Basilicata. According to Italian law and due to the retrospective, non-interventional nature of the survey, informed consent from single patients was not required.

Continuous variables were reported as means and standard deviations or medians and range. Ferritin levels were analysed using a one-way non-parametric test (Kruskall–Wallis) and Dunn's multiple comparison test was also performed. Categorical variables were reported as count and percentage. Median OS and 95% confidence interval (CI), from diagnosis or beginning of DFX therapy to last follow-up, was calculated and the survival curves were estimated and plotted with the Kaplan–Meier method. Statistical significance was defined as $P < 0{\cdot}05$. Analyses were performed using package R version 3.2.3 (The R Project for Statistical Computing, Vienna, Austria).

Results

After an initial enrolment of 58 patients, representing 13·7% (range 4·5–33%) of 423 HR-MDS patients observed in the participating Centres during the study period, the analysis was restricted to 51 patients with a complete dataset of clinical information and, in particular, properly classified as having intermediate, high or very high MDS according to the R-IPSS (Greenberg *et al*, 2012). The reasons why the investigators chose to treat their patients with DFX were: (i) candidates for AlloSCT ($n = 8$, 16%), (ii) a very high transfusion burden ($n = 17$, 33%), (iii) stable or responsive disease under life-extending therapies (life expectancy > 1 year) ($n = 24$, 47%), (iv) other or unspecified reasons ($n = 2$, 4%). These patients were considered unsuitable for deferoxamine mainly because of the possible increased risk of infections and poor compliance due to the parenteral administration of this drug.

Table I summarizes demographic, clinical and laboratory data. Thirty-four patients were males and 17 females; median age was 65 years (range 36–83). According to the 2016 updated WHO classification (Arber *et al*, 2016), patients were: MDS with single lineage dysplasia (MDS-SLD, $n = 1$); MDS with multilineage dysplasia (MDS-MLD, $n = 2$); MDS with excess blast type 1 (MDS-EB-1, $n = 11$); MDS with excess blast type 2 (MDS-EB-2, $n = 30$); otherwise unclassifiable MDS (MDS-U, $n = 7$). R-IPSS was intermediate in 7 patients, high in 29 patients and very high in 15 patients.

Median time from diagnosis to DFX treatment was 11·1 months (range 0–84·9). The median number of RBC transfusions received before starting DFX was 23 (range 2–60), while the median SF and Hb levels were 1709 μg/l (range 460–7293) and 82 g/l (range 65–108), respectively.

Thirty-five patients had previously received recombinant erythropoietin (n = 6, 11·7%), azacitidine (n = 16, 31·4%) or both (n = 13, 25·5%).

DFX was administered orally at the median dose of 1000 mg/day (range 375–2500 mg), for a median time of 11 months (range 0·4–75). The initial daily dose/kg was 5 mg in 4 patients (8%,) 10 mg/kg/day in 21 patients (42%), 20 mg/kg/day in 24 patients (48%) and 30 mg/kg/day in 1 patient (2%) (mean dose: 14·8 ± 5·9 mg/kg). The dose was reduced in 10 patients due to intolerance and increased in 4 patients, due to inefficacy.

Patients were strictly monitored, according to current guidelines, for possible toxicities due to DFX, particularly with respect to renal, hepatic, ocular and auditory functions (Santini *et al*, 2010; Malcovati *et al*, 2013). Seven patients (13·7%) developed grade 2 (5 renal, 2 gastrointestinal) and one patient (1·9%) grade 3 (gastrointestinal) toxicity. DFX was stopped in 7 patients (three renal and one gastrointestinal toxicities, three because of leukaemic evolution). One patient stopped DFX after reaching normal SF values.

In evaluable patients, median ferritin levels decreased from 1709 μg/l at baseline (n = 51, range 460–7293), to 1421 μg/l after 1 month of DFX treatment (n = 42, 443–8513), to 1382 μg/l at 6 months (n = 32, 439–10·112) and to 1100 μg/l at 12 months (n = 22, 198–4·282) (Fig 1) (Kruskall–Wallis test P = 0·047; Dunn's multiple comparison test P = 0·02 for comparison between pre-chelation and after-12 months values). Under DFX treatment, eight out of 17 patients (47%) improved or normalized their ALT/AST levels, which were increased at baseline.

Thirty-nine patients continued or started concomitant therapies with lenalidomide (n = 1, 1·9%), recombinant erythropoietin (n = 2, 3·9%), azacitidine (n = 25, 49%) or recombinant erythropoietin and azacitidine (n = 11, 21·6%) during DFX therapy; at least temporary clinical benefits from these treatments occurred in 22 patients. Four patients successfully received allogeneic stem cell transplantation after DFX therapy.

The median number of RBC transfusions per week received before starting DFX and at last follow-up remained essentially unchanged (data not shown). However, after 6 weeks of treatment, one patient achieved a complete haematological response according to International Working Group (IWG) criteria (Cheson *et al*, 2006), without receiving any other active treatment in addition to DFX when this response occurred. Initial response lasted 1 year, was lost when DFX was interrupted due to the normal ferritin levels

Table I. Clinical characteristic of patients.

Patients	
Male/Female	34/17
Median age (range), years	65 (36–83)
WHO 2016 classification (Arber *et al*, 2016)	
MDS-SLD	1
MDS-MLD	2
MDS-EB-1	11
MDS-EB-2	30
MDS-U*	7
R-IPSS	
Intermediate risk	7
High-risk	29
Very high-risk	15
Other characteristics at start of DFX	
Median time from diagnosis (range), months	11·1 (0–84·9)
Median number of RBC transfusions received (range)	23 (2–60)
Median serum ferritin level (range), μg/l	1·709 (460–7·293)
Median Hb (g/l) levels (range)	82 (65–108)
Median serum creatinine levels (range), μmol/l	75·14 (73·38–131·72)
Normal/abnormal AST/ALT	34/17
Previous treatments	
Recombinant erythropoietin	6
Azacitidine	16
Recombinant erythropoietin and azacitidine	13

ALT, Serum alanine aminotransferase; AST, Serum aspartate aminotransferase; DFX, Deferasirox; Hb, Haemoglobin; MDS-EB-1, MDS with excess blast type-1 (WHO 2008**: refractory anaemia with excess blast type 1); MDS-EB-2, MDS with excess blast type-2 (WHO 2008**: refractory anaemia with excess blast type 2); MDS-MLD, MDS with multi- lineage dysplasia (WHO 2008**: refractory cytopenia with multi-lineage dysplasia); MDS-SLD, MDS with single lineage dysplasia (WHO 2008**: refractory aneamia); MDS-U, MDS unclassifiable; MDS, Myelodysplastic syndromes: R-IPSS, Revised International Prognostic Scoring System; RBC, Red blood cells; WHO, World Health Organization.

*MDS-U included, among others, 3 patients with secondary, therapy-related MDS and 1 MDS with bone marrow fibrosis.

**WHO (2008) classification: Swerdlow *et al* (2008).

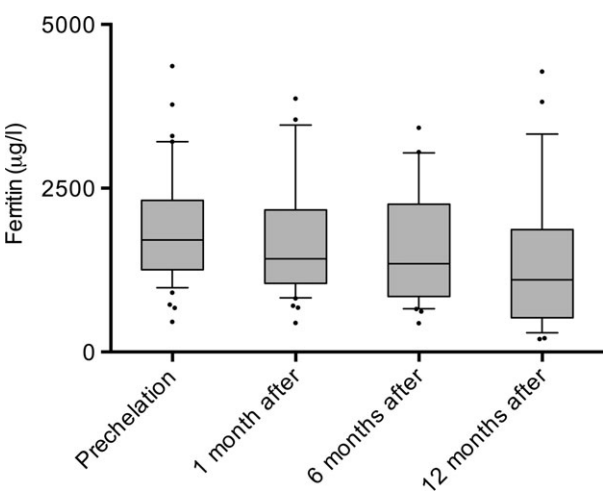

Fig 1. Monitoring of ferritin serum levels (μg/l) during deferasirox therapy in 51 higher risk myelodysplastic syndromes (HR-MDS) patients. Kruskall–Wallis test: P = 0·0478; Dunn's multiple comparison test: P = 0·0205, in the comparison between pre-chelation and after 12 months of treatment levels.

being reached, and occurred again at DFX re-start, for about 11 additional months, after which the disease progressed and the patient died.

At a median follow-up of 35·3 months from diagnosis and 21·4 months after the start of ICT, median OS was 37·5 (95% CI 24·7–51·3) and 24·4 (95% CI 14·6–31·8) months, respectively (Fig 2), with 19 patients (37%) evolved into AML.

Discussion

In the absence of randomized studies (Meerpohl *et al*, 2014) and the final results of the phase III TELESTO trial comparing DFX vs placebo in lower risk, transfusion-dependent MDS (NCT00940602), the exact role of ICT still remains an unsettled and debated issue in MDS (Merkel & Nagler, 2014; Steensma & Gattermann, 2013; de Witte, 2013; Neukirchen, 2014). However, recent prospective trials (List *et al*, 2012; Cermak *et al*, 2013; Nolte *et al*, 2013; Angelucci *et al*, 2014; Cheong *et al*, 2014; Kohgo *et al*, 2015) and retrospective analyses (Breccia *et al*, 2012; Gattermann *et al*, 2012a; Improta *et al*, 2013; Remacha *et al*, 2015; Maurillo *et al*, 2015) support the use of ICT with DFX in lower-risk transfusion-dependent MDS as a useful tool to reduce iron overload (both SF and LPI) and to improve/preserve organ functions. More importantly, observational studies (Leitch *et al*, 2008; Rose *et al*, 2010; Neukirchen *et al*, 2012; Leitch *et al*, 2012; Lyons *et al*, 2014; Delforge *et al*, 2014; Remacha *et al*, 2015; Zeidan *et al*, 2015) and a metanalysis (Mainous *et al*, 2014) have also reported a survival advantage for lower risk MDS patients who receive adequate ICT, in particular DFX, compared to patients who do not; this observation, however, has not yet been confirmed by a prospective study.

As already mentioned, outside the setting of AlloSCT (which is applicable to a small number of these patients), consistent data specifically focusing on the use DFX in HR-MDS are lacking. In two real-life studies with deferasirox (eXtend and eXjange) Gattermann *et al* (2012a) reported 20 MDS patients with intermediate-2/high IPSS risk. In another observational, single institution study with DFX, Breccia *et al* (2012) included 8 MDS patients who evolved from low/intermediate-1 to intermediate-2 IPSS risk under DFX and 8 with intermediate-2 risk at the start of deferasirox treatment. We previously reported 14 patients with intermediate-2 and 2 patients with high risk IPSS in another retrospective, multicentre study on the use of DFX in MDS (Maurillo *et al*, 2015). Finally, Cheong *et al* (2014) used DFX to treat with DFX 8 patients with intermediate-2 or high risk MDS. However, no details on the clinical outcome of these patients were reported in these studies. In the present paper, we have specifically addressed, for the first time, to the best of our knowledge, the role of DFX in HR-MDS patients. Despite the limited number of patients and the retrospective nature of the study, we show that such treatment is feasible (the rate of interruptions due to toxicity was only 8%, which is less than that usually observed in lower risk patients), is effective in lowering SF levels and, probably, in improving hepatic function. Particularly, DFX could be safely administered, without adding toxicity, in HR-MDS patients receiving or planned to receive other active therapies, including AlloSCT, and above all, azacitidine. This is an important and useful information, as iron overload represents a significant prognostic factor in these patients. For example, at the Moffit Cancer Center in Tampa, USA, 139 intermediate-2 or high risk IPSS MDS patients, more than half of whom were treated with azacitidine, were evaluated for iron overload (Komrokji *et al*, 2011). After a median follow up of 34·9 months, age > 60 years, MD Anderson risk group, and SF > 1000 μg/l were independent prognostic factors for both OS and AML transformation at multivariate analysis, although this effect was not significant after adjusting for the number of RBC units received. High SF at baseline also had a negative impact on response and OS in MDS patients treated with azacitidine in a Spanish experience (García *et al*, 2012). Furthermore,

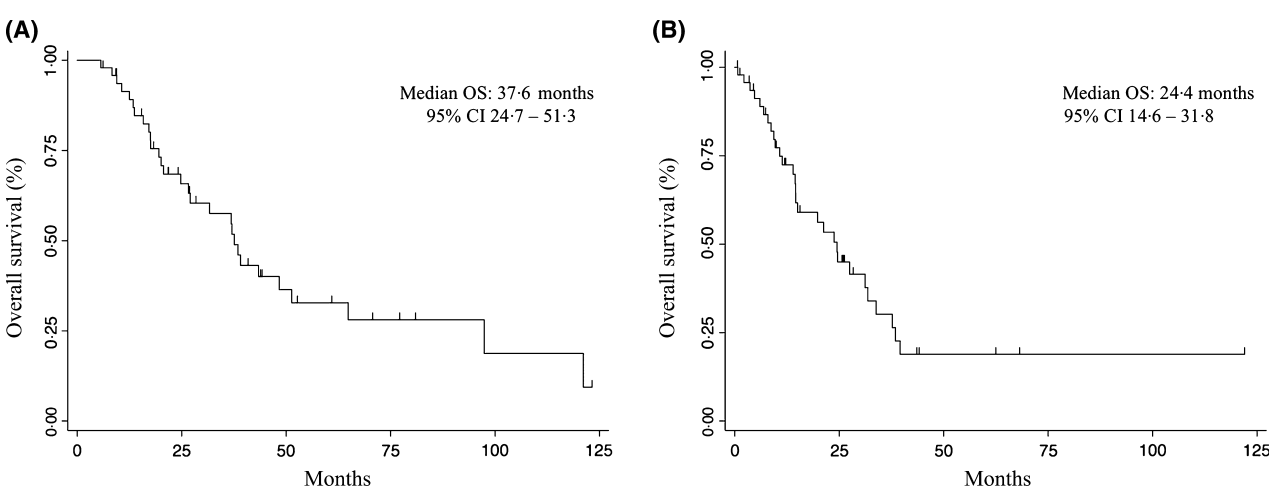

Fig 2. Overall survival curves of 51 higher risk myelodysplastic syndromes patients treated with deferasirox: (A) from diagnosis; (B) from the start of defereasirox. 95% CI, 95% confidence interval; OS, overall survival.

transfusion-dependence (>4 RBC units/8 weeks) at baseline negatively affected survival for MDS patients treated with azacitidine in another French study (Itzykson *et al*, 2011), while increased SF levels after azacitidine therapy were found to correlate with lower probability of response to this treatment (Tsang & Leitch, 2016). Finally, iron overload due to transfusions may facilitate severe bacterial and fungal infections in patients treated with azacitidine, probably by interfering with the immune system (Goldberg *et al*, 2010). All these observations raise the question of whether ICT could improve the clinical results obtained in transfusion-dependent patients with HR-MDS, especially in those treated with azacitidine (or other hypomethylating agents), an issue that warrants further and specific investigation.

Among the possible advantages of ICT in MDS, evidence is emerging that a non-negligible percentage of lower risk patients (probably 10–15%) treated with DFX may experience improvement in blood counts (mainly haemoglobin, less frequently white blood cells or platelets) (Messa *et al*, 2008; Cilloni *et al*, 2011; Guariglia *et al*, 2011; Gattermann *et al*, 2012b; List *et al*, 2012; Molteni *et al*, 2013; Angelucci *et al*, 2014; Breccia *et al*, 2015); the mechanism(s) and relationship(s) to the chelating affect of DFX of this phenomenon are under investigation (Tataranni *et al*, 2015; Messa *et al*, 2010). As occasionally already reported in HR-MDS patients (Messa *et al*, 2008; Cilloni *et al*, 2011; Guariglia *et al*, 2011; Breccia *et al*, 2012), in our series one patient (2%) showed a significant haematological improvement under DFX, a proportion that is less than that observed in previous studies on lower risk MDS. The type of response, however, was indeed impressive, as this patient achieved a rapid and significant tri-lineage improvement, with prolonged complete abolition of both RBC and platelet transfusions. Thus, the possible role of DFX in inducing haematological responses also in HR-MDS needs to be further evaluated.

Due to the heterogeneous characteristics and the limited number of patients included in the study, we cannot draw any conclusion about the possible long-term effects of DFX in HR-MDS. However, the reported OS of 37·5 months may be considered relevant for this group of patients, though they probably represent a positive selection by investigators and other active treatments employed should also be taken into consideration. Interestingly, a retrospective study including 7212 primary untreated MDS patients initially classified by major MDS prognostic risk scoring systems recently demonstrated that hazards regarding mortality and AML diminished over time from diagnosis in HR-MDS, whereas they remained stable in lower-risk patients (Pfeilstöcker *et al*, 2016). Indeed, after approximately 3·5 years, hazards in the separate risk groups became similar and were essentially equivalent after 5 years. These data, which underline the changing time-dependent performance of prognostic scores, should be considered in clinical decision making; in particular, they reinforce the choice of using ICT in these originally HR-MDS patients, definable as "long survivors".

Two randomized, phase 2 trials comparing azacitidine alone vs azacitidine plus DFX (NCT02159040 and NCT02038816) and one non-randomized phase 1/2 study by the Groupe Francophone des Myelodysplasies, which evaluates the addition of vitamin D to the two-drug combination (NCT01718366) are currently ongoing in HR-MDS. They will better address whether the combination of azacitidine and DFX is safe (mainly in terms of infections) and able to improve, along with SF and LPI levels, the response rate, time to leukaemic evolution and OS in this setting of patients. In one of these studies (NCT02038816), the role of azacitidine and DFX combination on haematopioetic stem cells is also under investigation by studying intracellular reactive oxygen species (ROS), erythroid colony-forming units, markers of DNA damage and specific signalling pathways.

In conclusion, we have described here the first experience with DFX in HR-MDS patients outside of clinical trials, reporting results that are substantially comparable, in terms of safety and efficacy, with those observed in lower risk patients. Prospective studies will determine definitely the potential benefits of ICT in HR-MDS, in a changing scenario characterized by the widespread use of hypomethylating agents, the consequent longer survival of patients and the perspectives related to the introduction of new prognostic factors and new active agents, able to modify the natural history of these diseases.

Acknowledgements

This paper was supported by funds from the Italian Ministry of Health, Current Research, to IRCCS-CROB, Rionero in Vulture, Italy.

Author contributions

PM and LM designed the research study; VS analysed the data and performed statistical analysis; PM, LM and VS wrote the paper; AP, CF, EB, AR, FR, AC, GT, OV, GM, MB, PN, AS, CC, MTV, SF, AV and VSa contributed to patient enrolment and collected the data; MRM, DS and GMar were responsible for database management; PM, EA and AL coordinated the study. All authors substantially contributed to the interpretation of the data, reviewed the draft critically and approved the final version.

Conflicts of interests

Regarding possible COI with the present paper, PM has received honoraria from Novartis and Celgene, EA is chair of the steering committee of TELESTO trial (Novartis) and coordinator of the safety board of Luspatercept use in Tularemia (Celgene). All of the other authors did not declare any conflicts of interest.

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