Somatic mutations are one of the major drivers in the pathogenesis of myelodysplastic syndrome (MDS), some of which are associated with the clinical phenotype. Red blood cell (RBC) transfusions are an important component of supportive care in patients with lower‐risk MDS (LR‐MDS), but are associated with increased iron deposits and ferritin levels leading to iron overload, contributing to morbidity and mortality (Malcovati *et al*, [**2011**](https://onlinelibrary.wiley.com/doi/full/10.1111/bjh.15655#bjh15655-bib-0005)). Increased ferritin levels have been reported in about 50% of patients at initial MDS diagnosis, across all different subtypes, prior to transfusion support, and play a significant prognostic role in survival (Voso *et al*, [**2013a**](https://onlinelibrary.wiley.com/doi/full/10.1111/bjh.15655#bjh15655-bib-0009)). Iron accumulation in the mitochondria is the main feature of the specific MDS subtype ‘with ring sideroblasts’ (MDS‐RS), which is defined by the presence of either ≥15% RS in the bone marrow, or by the detection of mutations of the splicing factor SF3B1 in presence of ≥5% ring sideroblasts (Arber *et al*, [**2016**](https://onlinelibrary.wiley.com/doi/full/10.1111/bjh.15655#bjh15655-bib-0002)). SF3B1 mutations have been detected in 70–80% of MDS‐RS.

Due to the deleterious prognostic role of iron accumulation in MDS, iron‐chelating therapy (ICT) is indicated in LR‐MDS with ferritin levels over 1000 μg/l and/or a history of at least 20 prior RBC transfusions (Malcovati *et al*, [**2013**](https://onlinelibrary.wiley.com/doi/full/10.1111/bjh.15655#bjh15655-bib-0006)). The oral iron chelator deferasirox (DFX), has been associated with prolonged survival in MDS, although this remains controversial (Gattermann *et al*, [**2012**](https://onlinelibrary.wiley.com/doi/full/10.1111/bjh.15655#bjh15655-bib-0004); Angelucci *et al*, [**2014**](https://onlinelibrary.wiley.com/doi/full/10.1111/bjh.15655#bjh15655-bib-0001)). Several reports and clinical studies have shown that haematological improvement (HI) may occur during ICT in MDS (10–20% of cases)in the erythroid series, neutrophils and platelets (Gattermann *et al*, [**2012**](https://onlinelibrary.wiley.com/doi/full/10.1111/bjh.15655#bjh15655-bib-0004) and Angelucci *et al*, [**2014**](https://onlinelibrary.wiley.com/doi/full/10.1111/bjh.15655#bjh15655-bib-0001)).

We were interested in the molecular mechanisms associated with HI (according to Cheson *et al*, [**2006**](https://onlinelibrary.wiley.com/doi/full/10.1111/bjh.15655#bjh15655-bib-0003)) during iron chelating treatment in MDS. DFX was administered according to guidelines in patients with MDS or primary myelofibrosis (PMF). Patients were consecutively enrolled in the multicentre prospective translational study ‘Identification of erythroid response mechanisms in patients with MDS undergoing iron‐chelation therapy’, approved by the EC of San Luigi Gonzaga Hospital Orbassano, Italy (Approval N. 14/2013). Informed consent was obtained from all patients, according to the Declaration of Helsinki.

The mutational status of critical genes was assessed in 58 LR‐MDS patients and 2 PMF patients undergoing DFX treatment. Table [**1**](https://onlinelibrary.wiley.com/doi/full/10.1111/bjh.15655#bjh15655-tbl-0001) shows patient characteristics. Nineteen patients (31·7%) achieved HI during DFX treatment (10 HI‐erythroid, 1 trilinear improvement and 8 patients became transfusion independent). This apparently high HI rate to DFX may be related to a selection bias in the translational study and may not be representative of the real haematological response rates in MDS (Gattermann *et al*, [**2012**](https://onlinelibrary.wiley.com/doi/full/10.1111/bjh.15655#bjh15655-bib-0004); and Angelucci *et al*, [**2014**](https://onlinelibrary.wiley.com/doi/full/10.1111/bjh.15655#bjh15655-bib-0001)). Mutational profiles were then compared to those of 41 patients who did not achieve HI.

**Table 1.**Patient characteristics

|  | **Total (*n* = 60)** | **Patients without haematological response (*n* = 41, 68·3%)** | **Patients with haematological response (*n* = 19, 31·7%)** | ***P*‐value** |
| --- | --- | --- | --- | --- |
| Age, years | | | | |
| Median (range) | 72 (33–89) | 72 (55–89) | 72 (33–88) | 0·8576 |
| Diagnosis | | | | |
| RA | 15 (25·0%) | 10 | 5 |  |
| RARS | 9 (15·0%) | 6 | 3 |
| RCMD | 23 (38·3%) | 16 | 7 |
| RAEB | 10 (16·7%) | 8 | 2 |
| CMML | 1 (1·7%) | 1 | 0 |
| PMF | 2 (3·3%) | 0 | 2 |
| Karyotype (*n* = 56) | | | | |
| Normal | 47 | 34 | 13 |  |
| Del 20q | 1 | 1 | 0 |
| Del 7 | 2 | 1 | 1 |
| ‐Y | 1 | 1 | 0 |
| Trisomy 8 | 2 | 1 | 1 |
| 5q‐ | 1 | 0 | 1 |
| Complex | 2 | 1 | 1 |
| BM blasts, % | | | | |
| Median (range) | 3·93 (1–18) | 3·86 (1–18) | 4·09 (1–16) | 0·8375 |
| Hb, g/l | | | | |
| Median (range) | 82·5 (30–108) | 85 (70–108) | 77·5 (30–105) | 0·0913 |
| Neutrophil count, 109/l | | | | |
| Median (range) | 2·3 (0·5–8·7) | 2·1 (0·5–7) | 2·7 (0·5–8·7) | 0·2830 |
| Platelet count, 109/l | | | | |
| Median (range) | 213 (35–695) | 224 (35–695) | 191 (40–486) | 0·3761 |
| IPSS‐R (*n* = 55) | | | | |
| Very‐low | 3 | 2 | 1 |  |
| Low | 27 | 17 | 10 |
| Intermediate | 17 | 13 | 4 |
| High | 6 | 4 | 2 |
| Very‐high | 2 | 1 | 1 |
| Ferritin pre‐DFX, μg/l | | | | |
| Median (range) | 2209 (649–5080) | 2091 (693–4335) | 2437 (649–5080) | 0·3254 |
| Ferritin post‐DFX, μg/l | | | | |
| Median (range) | 1625 (199–3900) | 1573 (400–3900) | 1724 (199–3800) | 0·7070 |
| Ratio Ferritin post‐/pre‐DFX | | | | |
| Median (range) | 0·86 (0·17–2·75) | 0·93 (0·17–2·75) | 0·75 (0·19–1·17) | 0·3322 |

* BM, bone marrow; CMML, chronic myelomonocytic leukaemia; DFX, Deferasirox; Hb, haemoglobin; IPSS‐R, revised international prognostic scoring system; PMF, primary myelofibrosis; RA, refractory anaemia; RAEB, refractory anaemia with excess of blasts; RARS, refractory anaemia with ring sideroblasts; RCMD, refractory cytopenia with multilineage dysplasia.

At the time of MDS diagnosis, DNA was extracted from bone marrow mononuclear cells using the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany). Thirty genes, frequently mutated in myeloid malignancies, were screened for somatic mutations in 15 of the patients (7 responsive and 8 resistant), using next generation sequencing (NGS) and Myeloid Solution (SOPHiA GENETICS, Saint‐Sulpice, Switzerland). The resulting captured libraries were further processed on a HiSeq® sequencing platform (Illumina, San Diego, CA, USA). Generated FASTQ sequencing files were then uploaded on the SOPHiA DDM® platform for analysis by SOPHiA technology. Only mutations identified as highly or potentially pathogenic by the SOPHiA DDM® platform were considered for analysis. The sensitivity of this NGS method is about 1%. Associations between the prevalence of mutations and patient characteristics were studied using the Fisher's exact test. Non‐parametric *t*‐tests were performed in case of non‐Gaussian distribution (GraphPad Prism 6, GraphPad Software, San Diego, CA, USA).

Treatment with DFX lead to a significant decrease in ferritin levels after 6 months when compared to basal levels (1625 μg/l vs. 2209 μg/l, *P*= 0·0011, Table [**1**](https://onlinelibrary.wiley.com/doi/full/10.1111/bjh.15655#bjh15655-tbl-0001)). However, there was no significant association between HI and patient characteristics (Table [**1**](https://onlinelibrary.wiley.com/doi/full/10.1111/bjh.15655#bjh15655-tbl-0001)). Fifty‐three somatic mutations, were identified in 14 of 15 patients (93·3%) at a median variant allele frequency of 25·97% (range 1·13–99·14%). The median number of mutations per patient was 3·53 (range, 0–6). The most commonly mutated genes were: *ASXL1*in 9 of 15 patients (60%), and *RUNX1*,*DNMT3A*,*SF3B1*and *TET2*in 4 of 15 patients (27% each) (Fig [**1**](https://onlinelibrary.wiley.com/doi/full/10.1111/bjh.15655#bjh15655-fig-0001)). In this analysis, no single gene mutation was predictive of HI, but mutation number was lower in DFX responders, as compared to resistant patients (mean 2·4 vs. 4·5 mutations/patient, respectively; *P*= 0·0232). The mutational profile of the 2 PMF patients (both responders) was similar to that of MDS patients with HI following DFX.

**Figure 1**

[**Open in figure viewer**](https://onlinelibrary.wiley.com/doi/full/10.1111/bjh.15655)[**PowerPoint**](https://onlinelibrary.wiley.com/action/downloadFigures?id=bjh15655-fig-0001&doi=10.1111%2Fbjh.15655)

Distribution of mutations identified by next generation sequencing in MDS patients treated with the iron‐chelator Deferasirox. Grey boxes represent mutated patients (light grey for responders and dark grey for resistant); white boxes indicate wild‐type patients. The numbers in boxes indicates the number of mutations identified in the same gene. Myelodysplastic syndrome (MDS ) subtypes, including RA , RARS , RCMD and RAEB are reported. The case series also included two patients with PMF . *ABL 1*,*BRAF*,*CALR*,*ETV 6*,*FLT 3*,*HRAS*,*IDH 1*,*IDH 2*,*KIT*,*NPM 1*,*NRAS*,*PTPN 11*,*SRSF 2*and *WT 1*genes were also studied, but no mutations were found. PMF , primary myelofibrosis; RA , refractory anaemia; RAEB , refractory anaemia with excess of blasts; RARS , refractory anaemia with ring sideroblasts; RCMD , refractory cytopenia with multilineage dysplasia.

We then screened an additional 45 patients, also enrolled in the translational study**,**for mutations in the gene hotspot regions of spliceosome machinery enzymes (*SF3B1*,*SRSF2*,*U2AF1*) and epigenetic regulators (*IDH1*,*IDH2*and *DNMT3A*), using Sanger sequencing, as previously reported (Voso *et al*, [**2013b**](https://onlinelibrary.wiley.com/doi/full/10.1111/bjh.15655#bjh15655-bib-0010)). In the additional cohort, *SF3B1*was the most commonly mutated gene (19/45 patients, 42·2%), but there was no association between mutations and HI (14/33 resistant patients, 42% vs. 5/12 responsive patients, 42%). When considering the entire cohort of 60 patients, we did not find any association between HI and mutations in the spliceosome machinery. As previously reported (Malcovati *et al*, [**2015**](https://onlinelibrary.wiley.com/doi/full/10.1111/bjh.15655#bjh15655-bib-0007)), *SF3B1*mutations were prevalent in refractory anaemia with RS (8 out of 9 patients, 89%), but HI rate to DFX in this patient subgroup was similar to that of all other patients (3/9, 33% vs. 16/51, 31%). On the other hand, *SF3B1*mutations were associated with higher platelet counts and a lower proportion of bone marrow blast at initial MDS diagnosis, as compared to wild type patients (*P*= 0·0092, and *P*= 0·0350, respectively).

Our data are in line with the overall positive prognostic profile of a lower mutation burden in MDS, which has been associated with delayed leukaemic transformation and prolonged survival, and in our study predicted for HI after iron‐chelating treatment (Papaemmanuil *et al*, [**2013**](https://onlinelibrary.wiley.com/doi/full/10.1111/bjh.15655#bjh15655-bib-0008)). In this setting, a low number of somatic mutations in bone marrow progenitors contributes to the positive effects of ICT regarding the overall haematopoietic function in MDS. Iron accumulation in RS and mutations of the spliceosome machinery are not predictive of HI after ICT, thereby supporting the different pathogenic mechanism and role of mitochondrial iron.

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**Author contributions**

EF: performed the research, analysed the data and wrote the paper; CC, GF, SL: performed the research; PN, EB, AM, CF, LF, MC, FS, FB, LM: Enrolled patients; FLC, DC: Designed the research study and enrolled patients; MTV: