Received Date : 20-Jun-2016

Accepted Date : 08-Aug-2016

Article type : Original Article

MRI assessment of iron load Cardiac iron load and function in transfused patients treated with deferasirox (the MILE study)

P Joy Ho¹, Lay Tay², Juliana Teo³, Paula Marlton⁴, Andrew Grigg⁵, Tim St Pierre⁶, Greg Brown⁷, Caro-Anne Badcock⁸, Robert Traficante⁸, Othon L Gervasio⁹ and Donald K Bowden¹⁰

¹*Institute of Haematology, Royal Prince Alfred Hospital; University of Sydney, Sydney;*

²*Dept. of Haematology, Royal Adelaide Hospital, Adelaide;* ³*Dept. of Haematology, The Children's Hospital at Westmead, Sydney;* ⁴*Dept. of Haematology, Princess Alexandra Hospital, Brisbane;* *School of Medicine University of Queensland;* ⁵*Dept. of Clinical Haematology, Royal Melbourne Hospital, Melbourne;* ⁶*University of Western Australia, Perth;* ⁷*Dept. of Radiology, Royal Adelaide Hospital, Adelaide;* ⁸*Statistical Revelations Pty Ltd, Melbourne;* ⁹*Novartis Pharmaceuticals, Sydney;* ¹⁰*Thalassaemia Services Victoria, Monash Medical Centre, Melbourne, Australia*

Correspondence: Prof P. Joy Ho, Institute of Haematology, Royal Prince Alfred Hospital, Sydney, phone 61 2 95158031, fax 61 2 95156698, joy.ho@sswahs.nsw.gov.au

Acknowledgments: This study was sponsored by Novartis Pharma AG. Financial support for medical editorial assistance was provided by Novartis Pharmaceuticals.

Running title (max 40 characters): Deferasirox MILE study of iron overload

Tables/Figures: 3/23 (max 6 tables/figures)

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: [10.1111/ejh.12793](https://doi.org/10.1111/ejh.12793)

This article is protected by copyright. All rights reserved

Abstract word count: 196 (max 200 words)

Word count: 3566

References: 50 (max 50 references)

Keywords: cardiac iron, hepatic iron, deferasirox, MRI iron assessment, transfusional siderosis

Abstract

Objectives: In this study, To assess the effect of iron chelation therapy with deferasirox on cardiac iron and function was assessed in patients with transfusion-dependent thalassemia major, sickle-cell disease (SCD) and myelodysplastic syndromes (MDS).

Methods: This phase IV, single-arm, open-label study over 53 weeks evaluated change in cardiac and liver iron load with deferasirox (up to 40mg/kg/day), measured by magnetic resonance imaging (MRI).

Results: Cardiac iron load (measured by myocardial T2*) significantly improved ($P=0.002$) overall for the full analysis set ($N=46$ evaluable patients; $n=36$ thalassemia major, $n=4$ SCD, $n=6$ MDS). Results were significant for patients with normal and moderate baseline cardiac iron ($P=0.017$ and $P=0.015$, respectively) but not in the five patients with severe cardiac iron load. Liver iron concentration (LIC) was significantly reduced decreased overall (mean LIC 10.4 to 8.2mg Fe/g dry tissue [dw]; $P=0.024$), particularly in those with baseline LIC >7 mg Fe/g dw (19.9 to 15.6mg Fe/g dw; $P=0.002$). Furthermore, myocardial T2* significantly increased in patients with LIC <7 mg Fe/g dw but not in those with a higher LIC. Safety was analyses were consistent with previous reports.

Conclusions: Once-daily deferasirox over 1 year significantly increased myocardial T2* and reduced LIC. This confirms that single-agent deferasirox is effective in the maintenance and reduction management of cardiac iron levels, especially for patients with myocardial T2* >10 ms. Clinicaltrials.gov identifier:NCT00673608.

Introduction

Cardiac failure is a major cause of death in patients with β thalassemia major and iron overload (1-4). A 10-year follow-up of patients with β thalassemia major demonstrated that cardiac failure-free survival correlated with serum ferritin and compliance with

chelation (5). Earlier studies have shown that iron-induced cardiomyopathy caused 71% of deaths in patients with β thalassemia major (6), while more recent investigations have revealed an improved prognosis, likely to be as a result of changes in iron chelation, iron monitoring and improvement in general care (7). The introduction of oral chelators such as deferasirox and deferiprone has led to an increase in adherence of patients to their therapy as compared with subcutaneous infusions of deferoxamine (DFO) (8). In contrast, the impact of iron overload on cardiac morbidity in patients with myelodysplastic syndromes (MDS) and myeloproliferative disease (MPD) is not well established, but there is increasing interest in the effect of iron overload and the role of iron chelation in these patients as survival improves with the advent of more effective therapies (9-13).

In recent years, magnetic resonance imaging (MRI) has been increasingly used in the monitoring of myocardial iron loading in transfusion-dependent patients (14-16). MRI for myocardial T2* measurements has shown excellent inter-scanner and inter-examination reproducibility, making MRI suitable for longitudinal monitoring (15, 17-19). Multiple studies have demonstrated that patients with low myocardial T2* have a significantly greater risk of resting systolic dysfunction and need for cardiac-related treatments than patients with T2* in the normal range (14, 20-22).

We designed a protocol (the MILE study) in which the primary endpoint was to assess the effect of iron chelation with deferasirox (up to 40 mg/kg/day) on cardiac iron and function, measured by changes in myocardial T2* by MRI in two cohorts of iron-overloaded, transfusion-dependent patients: those with hemoglobinopathies (β thalassemia major and sickle cell disease [SCD]) and a second cohort of patients with other anemias including MDS and MPD. In addition, the study employed MRI evaluation of left ventricular ejection fraction (LVEF) (23, 24) and measurement of liver iron concentration (LIC) by R2-MRI assessment (FerriScan[®], Resonance Health (25)).

Patients and methods

Eligibility criteria

Patients with hemoglobinopathies, MDS or other inherited or acquired anemias, ≥ 18 years and weighing >40 kg were eligible. Further inclusion criteria were a lifetime

minimum of ≥ 20 units of packed red blood cell transfusions, serum ferritin $> 500 \mu\text{g/L}$ for patients with hemoglobinopathies or $> 1000 \mu\text{g/L}$ for patients with other anemias, and normal or minimally abnormal cardiac function ($\text{LVEF} \geq 50\%$ measured by echocardiography or gated heart pool scan).

Patients were excluded if they had high-risk MDS, myocardial $\text{T2}^* < 5 \text{ ms}$, uncontrolled systemic hypertension, mean levels of alanine aminotransferase (ALT) $> 5 \times$ upper limit of normal (ULN) and serum creatinine above the age-appropriate ULN or creatinine clearance below 60 mL/min or significant proteinuria as indicated by a urinary protein/creatinine ratio $\geq 1.0 \text{ mg/mg}$ in a non-first void urine sample at baseline.

Study design

The MILE study is a Phase IV two-cohort single-arm open-label study. Patients were stratified into two cohorts: hemoglobinopathies and other anemias (eg MDS). MRI assessment of myocardial T2^* and LIC were measured at baseline (screening), and at Week 53 of the study (end of study; EOS). Myocardial T2^* measurements were repeated after 6 months in patients with baseline T2^* between 5 and 10 ms. Safety markers (serum creatinine, liver function tests and adverse events) and serum ferritin were measured monthly.

The recommended deferasirox starting dose was 20 mg/kg/day . Dose adjustments of 5 to 10 mg/kg were made between 10 and 30 mg/kg/day , according to the individual patient's response assessed by serum ferritin and therapeutic goals (maintenance or reduction of iron burden). In patients not adequately controlled with doses of 30 mg/kg/day , doses of up to 40 mg/kg/day were used in this study. Dose reductions (or interruption when required) were made according to safety markers (renal and hepatic function), or when serum ferritin was $< 1000 \mu\text{g/L}$ according to the study protocol.

The study protocol was approved by local ethics committees of all the participating centers. The study was conducted in accordance with the Declaration of Helsinki and ICH guidelines for good clinical practice. All patients provided written informed consent.

Assessments

Results across all indications were pooled and further analyzed by subgroup as defined in the statistical analysis plan. The primary endpoint was the change in cardiac iron load

from baseline to 53 weeks (EOS), defined as the natural log ratio of myocardial T2* at 53 weeks to baseline, $\ln(T2^*_{53}/T2^*_0)$.

For each measurement, a single short axis mid-ventricular slice (10 mm thickness) was imaged with a single breath-hold ECG triggered segmented multi-echo spoiled gradient echo sequence, using Philips Achieva 2.5 and Siemens AVANTO syngo B15 1.5-T scanners. On both scanner types, the repetition time was determined by the heart rate, the flip angle was set at 20°, and the field of view was fixed at 400 x 300 mm with a 256 x 96 acquisition matrix to give controlled voxel dimensions of 10 x 3.2 x 1.55 mm. The resulting data acquisition period was 15 to 20 seconds. Eight different gradient echo times were obtained. On the Siemens scanners the echo times (TE) were 2.59 – 1.7 ms (Δ TE 2.23 ms) with a bandwidth of 815Hz/pixel. On Philips scanners the TE values were 1.7 to 20.8 ms with a bandwidth of 751 Hz/pixel. Three multi-echo datasets of good quality were obtained at each examination.

Secondary efficacy endpoints were the change in cardiac function (LVEF measured by cardiac MRI) (26), R2-MRI-assessed LIC (FerriScan®) (25) and serum ferritin levels during 53 weeks of treatment.

The safety and tolerability of deferasirox were also assessed.

Statistical methods

Safety analyses were performed for all subjects in the full analysis population, which included patients with hemoglobinopathies and MDS who had at least one dose of the study drug. Efficacy analyses were performed for all subjects in the full analysis population who had baseline and EOS data. A generalized linear model framework was used for analyses that included factors for patient cohort (transfused patients with hemoglobinopathies or other anemias), time (Month 0 or Month 12), baseline category and corresponding interaction terms to investigate changes over time overall, by patient cohort, and by baseline category and patient cohort.

The sample size was originally calculated based on the standard deviation (SD) of the change in $\ln(T2^*)$ (0.47) from previously published reports (27) in order to detect a change in myocardial T2* comparing baseline and EOS assessments of the overall patient population. A planned per-protocol sample size reassessment was undertaken

when 42 patients had follow-up data (the $\ln(T2^*)$ was calculated for each result and the change in $\ln(T2^*)$ estimated for each patient; change in $\ln(T2^*)$ (0.26)). This sample size reassessment based on the SD allowed a reduction in the total number of patients required to assess the change in myocardial $T2^*$ (from 118 to a minimum of 30 patients). Additional investigations comparing cohorts, subgroups defined by baseline categories and the interactions were exploratory in nature. Confidence intervals (CIs) are provided for all subgroup and cohort results to clearly reflect the uncertainty in the results as a result of the small sample size. *P*-values have been provided but need to be interpreted with caution, especially if the number of patients is less than 12.

Results

Patient characteristics

Fifty-three patients were enrolled. Baseline patient demographics are shown in Table 1.

Myocardial iron and cardiac function

The primary endpoint, change in cardiac iron load measured by myocardial $T2^*$, was evaluable for 46 patients in the full analysis set (36 thalassemia major, four SCD, six MDS patients), while seven patients were not evaluable for this endpoint as a result of a lack of post-baseline measurements for myocardial $T2^*$. An overall increase in the geometric myocardial $T2^*$ from 21.9 to 24.1 ms was observed (ie a relative increase of 10% [95% CI: 4%, 16%]; *P*=0.002; Figure 1a). This result was heavily influenced by the increase for the largest cohort (patients with hemoglobinopathies, *n*=42; increase of 10% [95% CI: 3%, 17%]; *P*=0.004).

The most pronounced improvement was seen in patients with moderate cardiac siderosis (baseline $T2^*$ 10–20 ms; *n*=8, all in the hemoglobinopathies cohort), with an increase from 13.2 to 15.5 ms (relative increase of 18% [95% CI: 3%, 34%]; *P*=0.015; Figure 1a). No significant change was observed for patients with severe cardiac siderosis (baseline $T2^*$ between 5 and 10 ms; *n*=5; $T2^*$ increased from 7.3 to 7.6 ms, a relative increase of 4% (95% CI: –12%, 24%; Figure 1a).

Descriptive statistics revealed that in the subgroup of patients with severe cardiac siderosis (who showed little change in myocardial $T2^*$), one patient had low baseline LIC (2.8 mg Fe/g dry tissue) but received a mean dose of deferasirox <30 mg/kg/day. The other four patients who received mean doses >30 mg/kg/day had LIC baseline values

>25 mg Fe/g dry tissue (mean of 37 mg Fe/g dry tissue). An increase in myocardial T2* was seen for patients with MDS (n=6) from 29.3 to 32.7 ms (a relative increase of 12% [95% CI: -5%, 31%]; $P=0.2$).

To determine whether LIC had an impact on the degree of cardiac iron reduction, patients were stratified according to their baseline LIC and the changes in myocardial T2* assessed according to these subgroups. This showed that patients with a lower baseline LIC had a greater reduction in cardiac iron than those with high LIC. The subgroup with LIC <7 mg Fe/g dry tissue (n=25) had a statistically significant increase in myocardial T2* at the EOS, while the subgroup with a higher LIC at baseline (≥ 7 mg Fe/g dry tissue, n=21) did not show a statistically significant change in myocardial T2* (Table 2).

All patients had baseline LVEF values within the normal range, including those with severe cardiac siderosis (baseline myocardial T2* between 5 and 10 ms). Overall, there was no significant change in LVEF throughout the study (64.9% at baseline to 63.6% at EOS; $P=0.07$). The differences were not statistically significant by analysis of baseline T2* (5–10 ms, 10–20 ms and >20 ms subgroups; mean baseline LVEF >60% for all subgroups; Figure 1b). For patients with severe cardiac siderosis (baseline myocardial T2* between 5 and 10 ms), LVEF remained within the normal range, and there was no change between the measurements at baseline and 12 months ($P=0.6$).

LIC and serum ferritin and dosing of deferasirox

LIC

Statistically significant reductions in LIC were observed for the overall patient population ($P=0.024$), especially for those with baseline LIC ≥ 7 mg Fe/g dry tissue ($P=0.002$; Figure 2). The mean doses of deferasirox (SD) for the overall patient group and subgroups according to the baseline LIC were as follows: all patients, 26.9 mg/kg/day (10.49); <7 mg Fe/g dry tissue, 25.6 mg/kg/day (9.50); and ≥ 7 mg Fe/g dry tissue, 28.5 mg/kg/day (11.54). Reductions in the mean LIC were also found for the hemoglobinopathies subgroup (n=40, reduction of 16%, borderline significance $P=0.066$), with a statistically significant reduction for patients with high LIC levels at baseline (ie LIC ≥ 7 mg Fe/g dry tissue; n=16, reduction of 20%; $P=0.007$). The mean LIC in the MDS group was 9.78 (1.67) mg Fe/g dry tissue at baseline, and 5.23 (1.93) mg Fe/g dry tissue at the EOS (reduction of 4.13 mg Fe/g dry tissue [95% CI: -67% to -

21%], $P=0.0044$; exploratory analysis). For patients with baseline LIC <7 mg Fe/g dry tissue (overall patient group, hemoglobinopathies and MDS), deferasirox maintained the mean hepatic levels of iron within the target range (Figure 2).

Serum ferritin

Serum ferritin reductions in the overall patient population and hemoglobinopathies subgroup were not statistically significant (-5.5 $\mu\text{g/L/week}$, $P=0.08$ and -2.6 $\mu\text{g/L/week}$, $P=0.4$, respectively). When SCD patients ($n=4$) in whom sickling events causing an acute phase reaction may have confounded serum ferritin measurements were excluded from this analysis, there was a statistically significant reduction in serum ferritin (-7.16 $\mu\text{g/L/week}$; $P=0.038$). A statistically significant reduction in serum ferritin was seen in the MDS subgroup ($P=0.004$).

Dosing

Doses of deferasirox ≥ 30 mg/kg/day resulted in greater reduction in LIC and increase in myocardial $T2^*$ in patients in the overall patient population (statistically significant for both parameters; $P=0.037$ and 0.001 respectively; Table 3). Doses between 20 and 30 mg/kg/day induced the maintenance of LIC and $T2^*$ levels. Despite a trend for reduction in serum ferritin levels over time, this was not statistically significant in any of the three dose subgroups.

Safety

Ten patients discontinued the study prematurely: consent withdrawn ($n=2$), adverse events (diarrhea, $n=2$; elevated serum creatinine, $n=2$), protocol violation ($n=3$; study dose lower than that recommended by the protocol at enrollment) and unsatisfactory therapeutic effect ($n=1$). Serious adverse events (SAEs) occurred in seven patients, and included diarrhea, rectal hemorrhage, vomiting, chest pain, pyrexia, sickle cell anemia with crisis, lobar pneumonia, neutropenic sepsis, wrist fracture, bone pain, musculoskeletal pain, mania, and renal colic. Diarrhea and vomiting were the only SAEs considered related to treatment. All seven patients had a complete recovery from these events.

No statistically significant increases changes were seen in serum creatinine, ALT and aspartate aminotransferase (AST) levels at EOS compared to baseline for the overall patient population (Figure 3), or when patients were stratified by subgroup

(hemoglobinopathies/MDS). ALT was the only parameter that showed a statistically significant reduction over time; this was only evident in the MDS subgroup ($P=0.01$).

The change in safety markers for patients receiving a mean dose of deferasirox ≥ 30 mg/kg/day did not show statistical difference in comparison with patients receiving <30 mg/kg/day, when EOS values were compared to baseline ($P=0.4$, 0.2 and 0.7 for serum creatinine, ALT and AST, respectively [results extracted from the statistical model based on the overall patient population]).

Discussion

The Phase IV MILE study evaluated the efficacy and safety of deferasirox therapy at doses up to 40 mg/kg/day in patients with a variety of transfusion-dependent anemias. Of 46 evaluable patients, including 36 with thalassemia major, four with SCD and six with MDS, once-daily deferasirox resulted in a significant increase in myocardial T2* ($P=0.002$), meeting the primary objective of the study, and maintained LVEF within the normal range. Despite the improvement in prognosis for haemoglobinopathy patients in recent years (7, 28), myocardial siderosis remains an important cause of morbidity and mortality. Clinical data on the efficacy of different treatment regimes, including our experience here, adds to a growing body of data (21, 22, 27, 29-35). An improved understanding of the role of cardiac iron loading and the effectiveness of iron chelation in other transfusion-dependent anemias such as MDS is also pertinent (10, 36, 37), because of increases in overall life expectancy due to the advent of new therapies. It is clear that iron loading in MDS and malignant diseases occurs with different kinetics and has a different impact as compared with the haemoglobinopathies, and the mechanisms continue to be investigated (36, 37). Within the limitations of a small number of MDS patients in this study, MRI analysis was performed on both cardiac and liver iron load and was correlated with data on serum ferritin, the primary mode of iron monitoring in clinical practice.

In patients with thalassemia major, deferasirox treatment for up to 18 months has been shown to increase myocardial T2* in patients with mild, moderate and severe cardiac siderosis (21, 27, 34). In contrast, subgroup analyses in our study showed a statistically significant increase in myocardial T2* for patients with baseline T2* >10 ms (in both 10–20 ms and >20 ms subgroups), however no significant change was detected for patients with baseline myocardial T2* between 5 and 10 ms ($n=5$). In this small subgroup of five

patients with severe cardiac iron load, a subtherapeutic dose of deferasirox or a high baseline LIC may have contributed to the maintenance rather than reduction of cardiac iron levels during the study. High levels of liver iron have been associated with reduced clearance of cardiac iron (34). Four out of the five patients who received mean deferasirox doses >30 mg/kg/day had baseline LIC values >25 mg Fe/g dry tissue, with a mean of 37 mg Fe/g dry tissue. Our findings contrast with that of Wood *et al.* (34) who described an improvement in myocardial T2* over 18 months in 13 patients with severe cardiac iron overload (mean baseline myocardial T2* <10 ms), treated with deferasirox at doses of up to 40 mg/kg/day; but as in our study, they also identified a high baseline LIC as a predictor of unfavorable labile plasma iron or cardiac response (35).

Reduction in cardiac iron may be greater in patients with low LIC (and, conversely, high LIC may slow down the rate of cardiac iron reduction) (34, 35). Our results support an impact of LIC on clearance of cardiac iron, as patients with a low baseline LIC had a statistically significant increase in myocardial T2*, whereas patients with a higher LIC showed no significant change in myocardial T2*, thus emphasizing the importance of assessing and controlling total body iron. This effect is likely to be related to the effect of liver and total body iron on labile plasma iron (LPI). It has been demonstrated that the labile iron pool markedly increases at high LIC (10). Poor control of total body and liver iron can lead to long periods of exposure to LPI, which can predispose patients to increased cardiac iron uptake. This was supported by the findings of Wood *et al.*, where cardiac non-responders to deferasirox clearly had higher LPI levels while, as noted above, high initial LIC was found to be a strong predictor of both abnormal LPI and suboptimal cardiac response (34, 35). In a previously reported study of 652 thalassemia patients, myocardial T2* was identified as the better predictor of the onset of cardiac dysfunction over 12 months as compared with LIC or serum ferritin; although fewer than 3% of these patients received deferasirox (17). Clearly there remains some heterogeneity in the data among these studies on cardiac iron, especially with respect to the impact of different levels of iron loading. These data also highlight the need for long-term iron chelation therapy to remove myocardial iron.

While the efficacy of deferasirox in treating and preventing cardiac iron overload in thalassemia major patients has been well described in prospective studies of up to 3 years duration (22, 30-35), there are relatively fewer published data on the efficacy of deferasirox in other transfusion-dependent diseases, such as MDS (10, 38, 39). There is

some controversy on the evidence for myocardial iron loading in patients with MDS (10, 37, 40, 41). However, transfused patients with MDS have been shown to experience a higher rate of cardiac events compared with those not receiving transfusions (82.4% versus 67.1%, $P<0.001$) (42). Although only a small number of MDS patients were enrolled in the present study ($n=6$) – all with myocardial $T2^*$ above 20 ms at baseline (consistent with previous reports (37, 40, 43)) and LVEF measurements within the normal range – chelation with deferasirox increased myocardial $T2^*$ in five patients. This was accompanied by a significant reduction in mean (SD) LIC; 9.78 (1.67) mg Fe/g dry tissue at baseline, and 5.23 (1.93) mg Fe/g dry tissue at the EOS (a change of -4.13 mg Fe/g dry tissue [95% CI: -67% to -21%], $P=0.0044$; exploratory analysis). The implications of this study on the treatment of MDS patients are limited by the small enrollment numbers, though the clinical significance of these outcomes for MDS patients warrants further investigation in larger studies.

A significant reduction in hepatic siderosis was observed for all patients with baseline values ≥ 7 mg Fe/g dry tissue, while mean LIC remained within the target range for patients with baseline LIC < 7 mg Fe/g dry tissue, for whom the main treatment goal is maintenance of hepatic iron levels. LIC was assessed using MRI, a widely accepted non-invasive method with high sensitivity and reproducibility (44, 45). Difficulties in access and cost may limit the use of MRI in some jurisdictions and, therefore, trends in serum ferritin are often used as the sole variable for monitoring body iron. However, in our study, despite the significant reduction in mean LIC, the reduction in serum ferritin levels did not reach statistical significance. ~~only statistically significant for the MDS subgroup~~. It should be noted that this study was not powered to detect differences in serum ferritin. Furthermore, additional analysis showed that the exclusion of data from the SCD patients, who have greater intra- and inter-patient variation in serum ferritin levels likely due to the impact of acute phase reaction of sickling on serum ferritin measurements, led to a statistically significant reduction in serum ferritin for the overall group. These findings are consistent with the inherent inaccuracy of serum ferritin as a sole measure of iron load in patients with SCD as reported previously (46, 47) and support the use of MRI to monitor tissue iron burden, as recommended by the Sick Cell Society (48).

A clear dose-response relationship was also demonstrated in our study. Mean doses of deferasirox ≥ 30 mg/kg/day induced a decrease in LIC and an increase in myocardial $T2^*$

for the overall patient population, while doses of between 20 and 30 mg/kg/day maintained total body iron levels throughout the study. Thus, in patients with elevated cardiac or liver iron, doses of at least 30 mg/kg/day are required to achieve a reduction in iron levels.

Safety analyses were consistent with previous reports (36, 49, 50) and indicate an acceptable deferasirox safety profile. Deferasirox did not induce statistically significant changes in serum creatinine, ALT or AST in the overall patient population from baseline to EOS. A statistically significant decrease in ALT was observed in the MDS subgroup, in agreement with previously reported data from the large-scale EPIC study (36). The present analysis also concurred with previous findings that the adverse event profile at deferasirox doses over 30 mg/kg/day was consistent with lower doses (49, 50).

In conclusion, these results provide further supportive data showing that once-daily single-agent deferasirox is an effective therapy for the reduction and maintenance of cardiac iron levels in transfusion-dependent patients (study primary endpoint), especially for patients with myocardial T2* >10 ms. Deferasirox also had an acceptable safety profile. These results add to the growing body of data on the use of deferasirox to reduce total body iron across a range of transfusion-dependent anemias, and highlight considerations for physicians to achieve optimal management of iron-overloaded patients.

Acknowledgements

The authors thank Iris Alhassid, Jane Lawson (Novartis Pharmaceuticals Australia) and Gillian Ryan (formerly of Novartis Pharmaceuticals Australia) for ongoing clinical support. We also thank Dr Ann Solterbeck, for the statistical support (Statistical Revelations Pty Ltd, Melbourne); Joanne Zacharopoulos, Mary Nteris and Barbara Hicks for medical editorial assistance (INC Research); Rebecca Helson for editorial assistance (Mudskipper Business Ltd); and Dr Stephen Worthley for the LVEF assessments (Royal Adelaide Hospital). The MILE study and work presented here were sponsored and funded by Novartis Pharmaceuticals Corporation. Financial support for editorial assistance was provided by Novartis Pharmaceuticals Corporation.

Authorship contributions

P Joy Ho served as the principal investigator of this trial, recruiting patients to the study. All authors contributed to data interpretation and review of the data, and provided their comments on this manuscript. All authors approved the final manuscript.

Disclosures of conflicts of interest

P Joy Ho: Honoraria, Invited speaker and Membership on advisory committee, Novartis

Lay Tay: Membership on advisory committee, Novartis

Juliana Teo: Research funding, Novartis

Paula Marlton: Membership on advisory committee, Novartis

Andrew Grigg: Invited speaker and membership on an advisory committee, Novartis

Tim St Pierre: Consultancy, equity ownership, membership on an entity's Board of Directors or advisory committees and speaker's bureau, Resonance Health. Consultancy; research funding and speaker's bureau, Novartis

Greg Brown: Consultancy and invited speaker, Novartis

Caro-Anne Badcock: Consultancy, Novartis

Robert Traficante: Consultancy, Novartis

Othon L Gervasio: Employment, Novartis

Donald K Bowden: Honoraria, Research funding and speaker's bureau, Novartis

References

1. Borgna-Pignatti C, Rugolotto S, De Stefano P, Zhao H, Cappellini MD, Del Vecchio GC, Romeo MA, Forni GL, Gamberini MR, Ghilardi R, Piga A, Cnaan A. Survival and complications in patients with thalassemia major treated with transfusion and deferoxamine. *Haematologica* 2004;**89**:1187-1193.
2. Borgna-Pignatti C, Cappellini MD, De Stefano P, Del Vecchio GC, Forni GL, Gamberini MR, Ghilardi R, Piga A, Romeo MA, Zhao H, Cnaan A. Cardiac morbidity and mortality in deferoxamine- or deferiprone-treated patients with thalassemia major. *Blood* 2006;**107**:3733-3737.
3. Brittenham GM, Griffith PM, Nienhuis AW, McLaren CE, Young NS, Tucker EE, Allen CJ, Farrell DE, Harris JW. Efficacy of deferoxamine in preventing complications of iron overload in patients with thalassemia major. *N Engl J Med* 1994;**331**:567-573.

4. Zurlo MG, De Stefano P, Borgna-Pignatti C, Di Palma A, Piga A, Melevendi C, Di Gregorio F, Burattini MG, Terzoli S. Survival and causes of death in thalassaemia major. *Lancet* 1989;**2**:27-30.
5. Olivieri NF, Brittenham GM. Iron-chelating therapy and the treatment of thalassemia. *Blood* 1997;**89**:739-761.
6. Borgna-Pignatti C, Rugolotto S, De Stefano P, Piga A, Di Gregorio F, Gamberini MR, Sabato V, Melevendi C, Cappellini MD, Verlato G. Survival and disease complications in thalassemia major. *Ann NY Acad Sci* 1998;**850**:227-231.
7. Modell B, Khan M, Darlison M, Westwood MA, Ingram D, Pennell DJ. Improved survival of thalassaemia major in the UK and relation to T2* cardiovascular magnetic resonance. *J Cardiovasc Magn Reson* 2008;**10**:42.
8. Trachtenberg FL, Gerstenberger E, Xu Y, Mednick L, Sobota A, Ware H, Thompson AA, Neufeld EJ, Yamashita R. Relationship among chelator adherence, change in chelators, and quality of life in thalassemia. *Qual Life Res* 2014;**23**:2277-2288.
9. Greenberg PL. Myelodysplastic syndromes: iron overload consequences and current chelating therapies. *J Natl Compr Canc Netw* 2006;**4**:91-96.
10. Jensen PD, Jensen FT, Christensen T, Eiskjaer H, Baandrup U, Nielsen JL. Evaluation of myocardial iron by magnetic resonance imaging during iron chelation therapy with deferrioxamine: indication of close relation between myocardial iron content and chelatable iron pool. *Blood* 2003;**101**:4632-4639.
11. Leitch HA, Leger CS, Goodman TA, Wong KK, Wong DH, Ramadan KM, Rollins MD, Barnett MJ, Galbraith PF, Vickars LM. Improved survival in patients with myelodysplastic syndrome receiving iron chelation therapy. *Clin Leukemia* 2008;**2**:205-211.
12. Malcovati L, Della Porta MG, Pascutto C, Invernizzi R, Boni M, Travaglino E, Passamonti F, Arcaini L, Maffioli M, Bernasconi P, Lazzarino M, Cazzola M. Prognostic factors and life expectancy in myelodysplastic syndromes classified according to WHO criteria: a basis for clinical decision making. *J Clin Oncol* 2005;**23**:7594-7603.
13. Rose C, Brechignac S, Vassilief D, Pascal L, Stamatoullas A, Guerci-Bresler A, Larbaa D, Dreyfus F, Beyne-Rauzy O, Chaury MP, Roy L, Cheze S, Morel P, Fenaux P. Does

iron chelation therapy improve survival in regularly transfused lower risk MDS patients?
A multicenter study by the GFM. *Leuk Res* 2010;**34**:864-870.

14. Anderson LJ, Holden S, Davis B, Prescott E, Charrier CC, Bunce NH, Firmin DN, Wonke B, Porter J, Walker JM, Pennell DJ. Cardiovascular T2-star (T2*) magnetic resonance for the early diagnosis of myocardial iron overload. *Eur Heart J* 2001;**22**:2171-2179.
15. Ghugre NR, Enriquez CM, Coates TD, Nelson MD, Jr., Wood JC. Improved R2* measurements in myocardial iron overload. *J Magn Reson Imaging* 2006;**23**:9-16.
16. Tanner MA, He T, Westwood MA, Firmin DN, Pennell DJ. Multi-center validation of the transferability of the magnetic resonance T2* technique for the quantification of tissue iron. *Haematologica* 2006;**91**:1388-1391.
17. Kirk P, Roughton M, Porter JB, Walker JM, Tanner MA, Patel J, Wu D, Taylor J, Westwood MA, Anderson LJ, Pennell DJ. Cardiac T2* magnetic resonance for prediction of cardiac complications in thalassemia major. *Circulation* 2009;**120**:1961-1968.
18. Westwood MA, Anderson LJ, Firmin DN, Gatehouse PD, Lorenz CH, Wonke B, Pennell DJ. Interscanner reproducibility of cardiovascular magnetic resonance T2* measurements of tissue iron in thalassemia. *J Magn Reson Imaging* 2003;**18**:616-620.
19. Westwood MA, Firmin DN, Gildo M, Renzo G, Stathis G, Markissia K, Vasili B, Pennell DJ. Intercentre reproducibility of magnetic resonance T2* measurements of myocardial iron in thalassaemia. *Int J Cardiovasc Imaging* 2005;**21**:531-538.
20. Patton N, Brown G, Leung M, Bavishi K, Taylor J, Lloyd J, Lee SH, Tay L, Worthley S. Observational study of iron overload as assessed by magnetic resonance imaging (MRI) in an adult population of transfusion dependent patients with beta thalassaemia: significant association between low cardiac T2* < 10 ms and the occurrence of cardiac events. *Intern Med J* 2010;**40**:419-426.
21. Pathare A, Taher A, Daar S. Deferasirox (Exjade®) significantly improves cardiac T2* in heavily iron-overloaded patients with beta-thalassemia major. *Ann Hematol* 2010;**89**:405-409.
22. Pennell DJ, Porter JB, Cappellini MD, Chan LL, El-Beshlawy A, Aydinok Y, Ibrahim H, Li CK, Viprakasit V, Elalfy MS, Kattamis A, Smith G, Habr D, Domokos G, Roubert B,

Taher A. Deferasirox for up to 3 years leads to continued improvement of myocardial T2* in patients with beta-thalassemia major. *Haematologica* 2012;**97**:842-848.

23. Grothues F, Smith GC, Moon JC, Bellenger NG, Collins P, Klein HU, Pennell DJ. Comparison of interstudy reproducibility of cardiovascular magnetic resonance with two-dimensional echocardiography in normal subjects and in patients with heart failure or left ventricular hypertrophy. *Am J Cardiol* 2002;**90**:29-34.
24. Bellenger NG, Davies LC, Francis JM, Coats AJ, Pennell DJ. Reduction in sample size for studies of remodeling in heart failure by the use of cardiovascular magnetic resonance. *J Cardiovasc Magn Reson* 2000;**2**:271-278.
25. St Pierre TG, Clark PR, Chua-anusorn W, Fleming AJ, Jeffrey GP, Olynyk JK, Potrakul P, Robins E, Lindeman R. Noninvasive measurement and imaging of liver iron concentrations using proton magnetic resonance. *Blood* 2005;**105**:855-861.
26. Wong DT, Leung MC, Richardson JD, Puri R, Bertaso AG, Williams K, Meredith IT, Teo KS, Worthley MI, Worthley SG. Cardiac magnetic resonance derived late microvascular obstruction assessment post ST-segment elevation myocardial infarction is the best predictor of left ventricular function: a comparison of angiographic and cardiac magnetic resonance derived measurements. *Int J Cardiovasc Imaging* 2012;**28**:1971-1981.
27. Pennell D, Porter JB, Cappellini MD, Li C-K, Aydinok Y, ., Chan LL, Kattamis A, Smith G, Habr D, Domokos G, Hmissi A, Taher A. Efficacy and safety of deferasirox (Exjade®) in reducing cardiac iron in patients with β -thalassemia major: results from the cardiac substudy of the EPIC trial. *Blood* 2008;**112**(11):abst 3873.
28. Elmariah H, Garrett ME, De Castro LM, Jonassaint JC, Ataga KI, Eckman JR, shley-Koch AE, Telen MJ. Factors associated with survival in a contemporary adult sickle cell disease cohort. *Am J Hematol* 2014;**89**:530-535.
29. Pennell DJ, Berdoukas V, Karagiorga M, Ladis V, Piga A, Aessopos A, Gotsis ED, Tanner MA, Smith GC, Westwood MA, Wonke B, Galanello R. Randomized controlled trial of deferiprone or deferoxamine in beta-thalassemia major patients with asymptomatic myocardial siderosis. *Blood* 2006;**107**:3738-3744.
30. Pennell DJ, Porter JB, Cappellini MD, El-Beshlawy A, Chan LL, Aydinok Y, Elalfy MS, Sutcharitchan P, Li CK, Ibrahim H, Viprakasit V, Kattamis A, Smith G, Habr D,

Domokos G, Roubert B, Taher A. Efficacy of deferasirox in reducing and preventing cardiac iron overload in β -thalassemia. *Blood* 2010;**115**:2364-2371.

31. Pennell DJ, Porter JB, Cappellini MD, Chan LL, El-Beshlawy A, Aydinok Y, Ibrahim H, Li CK, Viprakasit V, Elalfy MS, Kattamis A, Smith G, Habr D, Domokos G, Roubert B, Taher A. Continued improvement in myocardial T2* over 2 years of deferasirox therapy in β -thalassemia major patients with cardiac iron overload. *Haematologica* 2011;**96**:48-54.
32. Pennell DJ, Porter JB, Piga A, Lai Y, El-Beshlawy A, Belhoul KM, Elalfy M, Yesilipek A, Kilinc Y, Lawniczek T, Habr D, Weisskopf M, Zhang Y, Aydinok Y. A 1-year randomized controlled trial of deferasirox versus deferoxamine for myocardial iron removal in beta-thalassemia major (CORDELIA). *Blood* 2014;**123**:1447-1454.
33. Pennell DJ, Porter JB, Piga A, Lai YR, El-Beshlawy A, Elalfy M, Yesilipek A, Kilinc Y, Habr D, Musallam KM, Shen J, Aydinok Y. Sustained improvements in myocardial T2* over 2 years in severely iron-overloaded patients with beta thalassemia major treated with deferasirox or deferoxamine. *Am J Hematol* 2015;**90**:91-96.
34. Wood JC, Kang BP, Thompson A, Giardina P, Harmatz P, Glynos T, Paley C, Coates TD. The effect of deferasirox on cardiac iron in thalassemia major: impact of total body iron stores. *Blood* 2010;**116**:537-543.
35. Wood JC, Glynos T, Thompson A, Giardina P, Harmatz P, Kang BP, Paley C, Coates TD. Relationship between labile plasma iron, liver iron concentration and cardiac response in a deferasirox monotherapy trial. *Haematologica* 2011;**96**:1055-1058.
36. Gattermann N, Finelli C, Della Porta M, Fenaux P, Ganzer A, Guerci-Bresler A, Schmid M, Taylor K, Vassilief D, Habr D, Domokos G, Roubert B, Rose C. Deferasirox in iron-overloaded patients with transfusion-dependent myelodysplastic syndromes: Results from the large 1-year EPIC study. *Leuk Res* 2010;**34**:1143-1150.
37. Konen E, Ghoti H, Goitein O, Winder A, Kushnir T, Eshet Y, Rachmilewitz E. No evidence for myocardial iron overload in multitransfused patients with myelodysplastic syndrome using cardiac magnetic resonance T2* technique. *Am J Hematol* 2007;**82**:1013-1016.
38. Glanville J, Eleftheriou P, Porter J. MRI evidence of cardiac iron accumulation in myelodysplasia and unusual anemias. *Blood* 2006;**108**(11):abst 1553.

39. Virtanen JM, Remes KJ, Itala-Remes MA, Saunavaara JP, Komu ME, Partanen AM, Parkkola RK. The relationship between cardiac and liver iron evaluated by MR imaging in haematological malignancies and chronic liver disease. *Blood Cancer J* 2012;**2**:e49.
40. Chacko J, Pennell DJ, Tanner MA, Hamblin TJ, Wonke B, Levy T, Thomas PW, Killick SB. Myocardial iron loading by magnetic resonance imaging T2* in good prognostic myelodysplastic syndrome patients on long-term blood transfusions. *Br J Haematol* 2007;**138**:587-593.
41. Di Tucci AA, Matta G, Deplano S, Gabbas A, Depau C, Derudas D, Caocci G, Agus A, Angelucci E. Myocardial iron overload assessment by T2* magnetic resonance imaging in adult transfusion dependent patients with acquired anemias. *Haematologica* 2008;**93**:1385-1388.
42. Goldberg SL, Chen E, Corral M, Guo A, Mody-Patel N, Pecora AL, Laouri M. Incidence and clinical complications of myelodysplastic syndromes among United States medicare beneficiaries. *J Clin Oncol* 2010;**28**:2847-2852.
43. Roy NB, Myerson S, Schuh AH, Bignell P, Patel R, Wainscoat JS, McGowan S, Marchi E, Atoyebi W, Littlewood T, Chacko J, Vyas P, Killick SB. Cardiac iron overload in transfusion-dependent patients with myelodysplastic syndromes. *Br J Haematol* 2011;**154**:521-524.
44. St Pierre TG, El-Beshlawy A, Elalfy M, Al JA, Al ZK, Daar S, Habr D, Kriemler-Krahn U, Taher A. Multicenter validation of spin-density projection-assisted R2-MRI for the noninvasive measurement of liver iron concentration. *Magn Reson Med* 2014;**71**:2215-2223.
45. Wood JC, Ghugre N. Magnetic resonance imaging assessment of excess iron in thalassemia, sickle cell disease and other iron overload diseases. *Hemoglobin* 2008;**32**:85-96.
46. Brittenham GM, Cohen AR, McLaren CE, Martin MB, Griffith PM, Nienhuis AW, Young NS, Allen CJ, Farrell DE, Harris JW. Hepatic iron stores and plasma ferritin concentration in patients with sickle cell anemia and thalassemia major. *Am J Hematol* 1993;**42**:81-85.
47. Vichinsky E, Onyekwere O, Porter J, Swerdlow P, Eckman J, Lane P, Files B, Hassell K, Kelly P, Wilson F, Bernaudin F, Forni GL, Okpala I, Ressayre-Djaffer C, Alberti D, Holland J, Marks P, Fung E, Fischer R, Mueller BU, Coates T. A randomized

comparison of deferasirox versus deferoxamine for the treatment of transfusional iron overload in sickle cell disease. *Br J Haematol* 2007;**136**:501-508.

48. Sickle Cell Society. Standards for the clinical care of adults with sickle cell disease in the UK. 2008. Available at:
<http://www.sicklecellsociety.org/app/webroot/files/files/CareBook.pdf>.
49. Cappellini MD, Porter JB, El-Beshlawy A, Li C-K, Seymour JF, Elalfy M, Gattermann N, Giraudier S, Lee J-W, Chan LL, Lin K-H, Rose C, Taher A, Thein SL, Viprakasit V, Habr D, Domokos G, Roubert B, Kattamis A, on behalf of the EPIC study investigators. Tailoring iron chelation by iron intake and serum ferritin: the prospective multicenter EPIC study of deferasirox in 1744 patients with various transfusion-dependent anemias. *Haematologica* 2010;**95**:557-566.
50. Taher A, Cappellini MD, Vichinsky E, Galanello R, Piga A, Lawniczek T, Clark J, Habr D, Porter JB. Efficacy and safety of deferasirox doses of >30 mg/kg per d in patients with transfusion-dependent anaemia and iron overload. *Br J Haematol* 2009;**147**:752-759.

Table 1: Patient characteristics

Characteristic	Thalassemia/SCD, n=42	MDS n=9	BMT n=1	DBA n=1	All n=53
Median age	28 (16–75)	65 (53–79)	55	16	30 (16–79)
Sex (Female:Male)	26:16	5:4	1:0	0:1	32:21
Transfusions per 28 days (units PC)	3.3 (±0.75)	3.3 (±1.88)	3.4	2.8	3.3 (±1.00)
Baseline serum ferritin (µg/L)	1807 (±1085)	2176 (±855)	2081	355	1847 (±1051)
Baseline LIC (mg Fe/g dry tissue) [†]	10.2 (±13.18)	9.8 (±1.69)	22.5	2.2	10.2 (±11.90)
Baseline myocardial T2* (ms) [‡]	23.9 (±10.53)	29.5 (±3.38)	30.2	19.7	24.8 (±9.80)
Naïve to deferasirox	6 (14%)	6 (67%)	1 (100%)	–	13 (25%)
Mean dose at enrollment (mg/kg/day)	25.9 (±8.38)	23.3 (±5.77)	0	10.0	25.4 (±8.45)
<20	6 (17%)	–	–	1 (100%)	7 (18%)

Characteristic	Thalassemia/SCD, n=42	MDS n=9	BMT n=1	DBA n=1	All n=53
20 to <30	9 (25%)	2 (67%)	–	–	11 (28%)
≥30	21 (58%)	1 (33%)	–	–	22 (55%)
Mean dose during study (mg/kg/day)	29.7 (±8.94)	19.0 (±8.85)	6.1	5.0	26.9 (±10.49)

Values are based on descriptive statistics. BMT and DBA patient data are excluded from the 'Other' cohort and hence from all formal statistical analyses, as a result of the differences between the disease states, cohort size (n=2) and mean dose, considered to be sub-therapeutic for total body iron reduction (6.1 and 5 mg/kg/day for each patient, respectively).

SCD, sickle cell disease; MDS, myelodysplastic syndromes; BMT, bone marrow transplant; DBA, Diamond–Blackfan anemia.

[†]LIC normal range: 0.17–1.8 mg/g dry weight; target range in transfusion-dependent patients with hemoglobinopathies: 3–7 mg/g dry weight (5).

[‡]Myocardial T2* >20 ms: cardiac iron is not apparent; 10–20 ms: moderate iron overload; <10 ms: severe iron overload (5, 14, 17).

Table 2: Change in myocardial T2* according to baseline LIC

Baseline LIC	Myocardial T2*	Myocardial T2*	Ratio [†] (95% CI)	P value [‡]
	at baseline	at EOS		
All (n=46)	21.92	24.10	1.10 (4%, 16%)	0.002 [§]
Low LIC; <7 mg Fe/g dry tissue (n=25)	25.09	28.08	1.12 (4%, 21%)	0.005 [§]
High LIC; ≥7 mg Fe/g dry tissue (n=21)	18.67	20.09	1.08 (–1%, 17%)	0.087

[†]Results taken from the model $\log(T2^*) = \text{timepoint} + \text{cohort} + \text{baseline LIC category} + \text{timepoint} \times \text{baseline LIC category}$ interaction. The 95% CIs represent the relative change in myocardial T2* from baseline to EOS; [‡]The P-value for testing the null hypothesis that the ratio is equal to 1;

[§]Indicates statistically significant changes in myocardial T2* from baseline to EOS.

EOS, end of study; CI, confidence interval; LIC, liver iron concentration.

Table 3: Effect of deferasirox dose on serum ferritin, LIC and myocardial T2*

Average dose (mg/kg/day) (% patients on dose)	Change (P-value)		
	Serum ferritin [†] (µg/L/week)	LIC [‡]	Myocardial T2* [‡]
<20 (18%)	–1.38 (0.9; NS)	+1% (1.0; NS)	+3% (0.8; NS)
20–30 (28%)	–9.32 (0.1; NS)	–17% (0.3; NS)	+6% (0.2; NS)
≥30 (55%)	–4.76 (0.3; NS)	–20% (0.037[§])	+14% (0.001[§])

[†]Serum ferritin values show change in µg/L per week.

[‡]LIC and T2* show relative change at end of study from baseline.

[§]Statistically significant (P≤0.05).

LIC, liver iron concentration; NS, non significant ($P>0.05$).

Figure legends

Figure 1: Change in a) myocardial T2* and b) LVEF after 12 months

a) Results are presented as the adjusted means on back-transformed T2* values $\pm$ 95% confidence intervals after the analysis model. [†]Statistically significant. b) Results are presented as the adjusted means $\pm$ standard error of the mean after the analysis model.

LVEF, left ventricular ejection fraction; NS, non significant.

Figure 2: Change in liver iron concentration after 12 months

Results are presented as the adjusted means $\pm$ standard error of the mean after the analysis model.

[†]Statistically significant.

LIC, liver iron concentration; NS, non significant.

Figure 3: Serum creatinine, ALT and AST levels at EOS compared with baseline (overall patient population)

Individual values based on descriptive statistics, mean $\pm$ standard error of the mean. *P* value based on estimated trend (trend not shown). The horizontal axis has not been fitted linearly according to time (ie month).

ALT, alanine aminotransferase; AST, aspartate aminotransferase; EOS, end of study; NS, non significant.

Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:

Ho, PJ;Tay, L;Teo, J;Marlton, P;Grigg, A;St Pierre, T;Brown, G;Badcock, C-A;Traficante, R;Gervasio, OL;Bowden, DK

Title:

Cardiac iron load and function in transfused patients treated with deferasirox (the MILE study)

Date:

2017-02-01

Citation:

Ho, P. J., Tay, L., Teo, J., Marlton, P., Grigg, A., St Pierre, T., Brown, G., Badcock, C. -A., Traficante, R., Gervasio, O. L. & Bowden, D. K. (2017). Cardiac iron load and function in transfused patients treated with deferasirox (the MILE study). EUROPEAN JOURNAL OF HAEMATOLOGY, 98 (2), pp.97-105. <https://doi.org/10.1111/ejh.12793>.

Persistent Link:

<http://hdl.handle.net/11343/291749>