

Randomized Controlled Trial of Deferiprone or Deferoxamine in Beta-Thalassemia Major Patients with Asymptomatic Myocardial Siderosis

Pennell DJ,^{1,2} Berdoukas V,³ Karagiorga M,³ Ladis V,⁴ Piga A,⁵ Aessopos A,⁶ Gotsis ED,⁷ Tanner MA,² Smith GC,² Westwood MA,^{1,2} Wonke B,⁸ Galanello R.⁹

1. National Heart and Lung Institute, Imperial College, London UK
2. Royal Brompton Hospital, London UK
3. Aghia Sophia Children's Hospital, Athens, Greece
4. Aghia Sophia Children's Hospital, University of Athens, Greece
5. Centro Microcitemie, University of Torino, Italy
6. Laikon Hospital, University of Athens, Greece
7. Institute Euromedica-Encephalos, Athens, Greece
8. Whittington Hospital, London, UK
9. Ospedale Regionale per le Microcitemie, Cagliari, Italy

This trial is registered in www.clinicaltrials.gov as NCT00105495.

This trial was sponsored by Apotex Research Inc.

Several of the authors (DJP, VB, AP, SG, RG) have declared a financial interest in Apotex whose product, deferiprone, was studied in the present work.

Several of the authors (DJP, AP, RG) have declared a financial interest in Novartis whose product, deferoxamine, was studied in the present work.

Key words: Heart, thalassemia, magnetic resonance, iron, chelation, deferiprone, deferoxamine, desferrioxamine, randomized controlled trial

Address for correspondence:

Dr Dudley Pennell
Professor of Cardiology
Royal Brompton Hospital
Sydney Street
London SW3 6NP
UK
Tel: +44 20 7351 8810
Fax: +44 20 7351 8816
Email: d.pennell@ic.ac.uk

ABSTRACT

Background Most deaths in beta-thalassemia major result from cardiac complications due to iron overload. Differential effects on myocardial siderosis may exist between different chelators.

Methods A randomized controlled trial was performed in 61 patients previously maintained on subcutaneous deferoxamine. The primary endpoint was the change in myocardial siderosis (myocardial T2*) over 1 year in patients maintained on subcutaneous deferoxamine or those switched to oral deferiprone monotherapy.

Results The dose of deferiprone was 92 mg/kg/day and deferoxamine was 43 mg/kg for 5.7 days/week. Compliance was $94 \pm 5.3\%$ and $93 \pm 9.7\%$ ($p=0.81$) respectively. The improvement in myocardial T2* was significantly greater for deferiprone than deferoxamine (27% vs 13%, $p=0.023$). Left ventricular ejection fraction increased significantly more in the deferiprone group (3.1% vs 0.3% absolute units, $p=0.0034$). The changes in liver iron (-0.93 vs -1.54mg/g dw, $p=0.40$), and serum ferritin (-181 vs -466ug/L, $p=0.16$) respectively were not significantly different between groups. The most frequent adverse events were transient gastrointestinal symptoms for deferiprone patients, and local reactions at the infusion site for deferoxamine. There were no episodes of agranulocytosis.

Conclusions Deferiprone monotherapy was significantly more effective than deferoxamine over 1 year in improving asymptomatic myocardial siderosis in beta-thalassemia major.

INTRODUCTION

Iron induced heart failure and arrhythmia are the commonest causes of death in beta thalassemia major, accounting for up to 71% of deaths in older series,^{1,2,3} and 67% of deaths in a more recent report, despite improvements in overall survival.⁴ There is a need therefore to identify myocardial siderosis early, and improve treatments for heart complications. Myocardial siderosis can be identified by cardiovascular magnetic resonance (CMR) measurement of myocardial T2*, which is highly sensitive to tissue iron concentration, and this has been validated in the UK,^{5,6} and independently in the USA.⁷ Patients having increased myocardial siderosis have been shown to be at increased risk of left ventricular (LV) systolic and diastolic dysfunction,^{5,7,8,9} arrhythmias,⁷ and heart failure.^{5,10} In one series, patients presenting with heart failure had a mean T2* of 5.1 ms,¹¹ which is substantially below the normal lower limit of 20ms,⁵ and another series showed 89% of patients presenting in heart failure having a T2* <10ms.¹⁰ Myocardial T2* in normals is not affected by impaired left ventricular function, age, or infarction.¹² The T2* technique is fast,¹³ and is highly reproducible between different MR scanners.^{5,14,15,16,17} T2* calibration has been achieved for the liver in humans,^{5,18} in the heart in animals,¹⁹ and will soon be available for the human heart.²⁰ Myocardial T2* can currently be used therefore to determine changes in human myocardial iron, but not the absolute myocardial iron concentration. The T2* technique shows improvement in myocardial siderosis in response to intravenous deferoxamine treatment in acute heart failure,¹¹ and a case controlled study found significantly less myocardial siderosis and improved LV ejection fraction (EF) in thalassemia patients treated with deferiprone compared with deferoxamine.²¹ These data suggest that myocardial T2* has value for comparing the cardiac efficacy of chelators. We therefore compared these 2 treatments as monotherapy in a prospective, multi-center, randomized controlled trial, using

the change over 1 year in myocardial T2* as the primary outcome measure, to determine whether deferiprone had superior cardiac efficacy compared with deferoxamine.

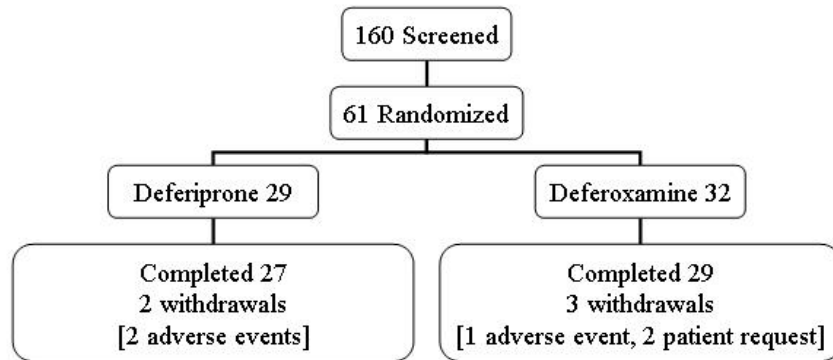
METHODS

Patient recruitment

This open-label trial was conducted in 4 centers in Italy and Greece. All patients had homozygous beta-thalassemia major, were regularly transfused and chelated with subcutaneous deferoxamine monotherapy, and had no symptoms of heart failure prior to screening. The mean dose of deferoxamine in the randomized patients prior to entry into the trial was 39 ± 8 mg/kg/day for 5.7 days/week (equivalent to 32 mg/kg/day). Patients aged >18 years were screened (N=160) and selected if the myocardial T2* was abnormal (<20ms), but not severe (<8ms), and if the LV EF was >56%. No patient with symptomatic heart failure was eligible for the trial. This rationale excluded patients with no significant cardiac siderosis, and allowed patients with severe myocardial siderosis or LV dysfunction to receive best current clinical management. Sixty-one patients were randomized (figure 1). The reasons for screening failure (n=99) were usually a myocardial T2* outside the required range (<8ms in 11%; >20ms in 76%). Additional less common exclusions were LV EF <56% (2%); liver enzymes >3 times upper limit (3%); unsuitable psychological condition (1%); age <18 or >36 years (8%); claustrophobia (3%); pre-transfusion Hb <9g/dL (3%); refused or unable to participate (7%). Eighteen patients failed >1 criterion. Each center's ethics committee approved the study and patients gave signed informed consent. Secondary trial endpoints included cardiac volumes and function, liver iron concentration (LIC), and serum ferritin concentrations.

Figure 1. Screening, randomization, completion and withdrawal patient numbers.

Figure 1



Cardiovascular magnetic resonance

The T2* sequence was installed in Athens (GE CVi) and Cagliari (GE Signa Echo-speed). Validation at each site included scanning of phantoms of known T2* value, the scanning of 5 patients at each site twice for local reproducibility, and rescanning within 3 days after flying to London for comparison with a reference scanner (Siemens Sonata). We predefined a limit of acceptability of 15% variation in T2* for each site compared with London. The variability compared with London was 9.7% in Athens, and 1.6% in Cagliari. Site interstudy variability was 3.5% and 2.4% respectively. These results,¹⁵ and full details of the T2* MR sequence have been published.¹³ Myocardial T2* was analysed using dedicated software (Thalassemia-Tools; Cardiovascular Imaging Solutions, London, UK) with consistent regions of interest in the ventricular septum which avoid susceptibility artefacts,^{5,22} as previously described.^{13,14} Cardiac volumes were acquired using contiguous steady state free precession short axis cines

from base to apex,²³ a technique with excellent reproducibility.²⁴ Measurements were scheduled for 3-10 days after transfusions. A core laboratory in London analyzed all CMR scans using dedicated software (CMRtools; Cardiovascular Imaging Solutions, London, UK). All measurements were made in London by 2 reviewers in consensus who were blinded to treatment allocation. Trial analyses were not fed back to the centers during the trial

Other iron measurements

LIC was assessed at baseline and 12 months using a superconducting quantum interference device (SQUID).²⁵ All the measurements were centralized at the Turin University facility (Model 5700 3-Channel SQUID, Tristan Technologies).²⁶ Prior to the start of the trial, SQUID was considered the best non-invasive technique for measuring liver iron. Serum ferritin was measured at baseline and 3 monthly. Serum was separated, labeled and stored frozen at -20°C until shipped to a central laboratory, where it was measured by microparticle enzyme immunoassay (Abbott AXSYM System).

Safety assessments

Patients were monitored weekly for absolute neutrophil count (ANC) and any adverse events. Serum alanine transaminase (ALT) levels were measured quarterly, serum zinc levels were measured at baseline, and 6 monthly, and serum creatinine levels were measured at baseline and 12 months.

Statistical analysis

The statistical analysis plan was defined prior to breaking the randomization code and locking of the database of the study. All continuous parameters were analyzed using the 2-sample t-test, using a p-value of 0.05 as the threshold for statistical significance. Since tissue iron is

linearly related to the inverse of T2*, this measure was log-transformed prior to analysis to linearize the relationship and provide an unbiased estimate of relative change from baseline for both treatments. Data are presented as mean \pm standard deviation (SD), except for the T2* data which use the geometric mean (antilog of the mean of the log data) \pm the coefficient of variation (CV) defined as $\sqrt{[e^{\text{MSE}} - 1]}$, where MSE is the mean square error (equivalent to the variance of the mean in log scale). Proportions of patients between the treatment groups were compared by Fisher's exact test. Trend analysis over time for ferritin and ALT data was performed using repeated measures ANOVA (MIXED in SAS). Sample size calculations indicated that in the single center setting, 32 patients would show a 5% difference in myocardial T2* between drugs for a type 1 error of 0.05 and a power of 80%. This sample size was increased to 60 to allow for reproducibility deterioration in the multicenter setting, and to allow for 20% drop-outs. All analyses presented are based on intention-to-treat. Last observation carried forward was used to fill the missing data for withdrawn patients in the efficacy analyses. Deferiprone compliance was measured using the Medication Event Monitoring System device, and calculated as the percent of openings with an interval >4 hours recorded, divided by number of doses prescribed. Deferoxamine compliance was calculated as the percentage of completed infusions, as determined by the Crono pumps, divided by the number of infusions prescribed. Statistical analysis was performed using SAS System for PC, Release 8.2.

RESULTS

Patient characterization

The baseline characteristics are shown in table 1. The groups were well matched for the primary endpoint of myocardial siderosis. Matching was good for most other measures

including liver iron and transfusional iron input, but significant differences were present for the serum ferritin, hemoglobin and white cell count.

Table 1 Descriptive statistics of the treatment groups at baseline. Data are shown as number (%), or mean \pm SD, except for the myocardial T2* which is shown as geometric mean and coefficient of variation (CV). Na- not applicable.

	Deferiprone	Deferoxamine	P value
Number randomized	29 (48%)	32 (52%)	na
Age (years)	25.1 \pm 3.8	26.2 \pm 4.7	0.33
Gender			
Male	15 (52%)	16 (50%)	1.0
Female	14 (48%)	16 (50%)	
Ethnicity			
Greek	16 (55%)	18 (56%)	1.0
Italian	13 (45%)	14 (44%)	
Race			
Caucasian	29 (100%)	32 (100%)	1.0
Heart measures:			
Myocardial T2* (ms)	13.0 (CV 32%)	13.3 (CV 30%)	0.77
End-diastolic volume (mL)	134 \pm 32	132 \pm 23	0.81
End-systolic volume (mL)	43 \pm 14	41 \pm 13	0.51
Ejection fraction (%)	69.7 \pm 5.4	68.4 \pm 4.9	0.34
Iron measures:			
Liver iron concentration (ug/L)	6.16 \pm 6.0	6.32 \pm 5.8	0.92
Serum ferritin (ug/L)	1791 \pm 1029	2795 \pm 2441	0.039
Number (%) with serum ferritin >2500ug/L	5 (17%)	13 (41%)	0.055
Blood measures:			

Transfusional iron input (mL/kg/year)	152±43.4	144 ±44.4	0.52
Hemoglobin (g/L)	105 ±12.0	113 ±11.9	0.023
Total white blood cell count (x10 ⁹ /L)	7.86 ±3.39	11.5 ±9.42	0.047
Corrected white blood cell count (x10 ⁹ /L)	7.68 ±2.96	9.79 ±4.49	0.033
Absolute neutrophil count (x10 ⁹ /L)	4.26 ±1.62	5.10 ±2.28	0.11
Platelets (x10 ⁹ /L)	318 ±129	318 ±159	0.99
Liver measures:			
Alanine transaminase (U/L)	37.7 ±32.1	52.2 ±33.8	0.093
Hepatitis C positive			
Yes	18 (62%)	16 (50%)	0.44
No	11 (38%)	16 (50%)	
HIV positive			
Yes	0 (0%)	0 (0%)	na
No	29 (100%)	32 (100%)	
Splenuctomy			
Yes	4 (14%)	11 (34%)	0.079
No	25 (86%)	21 (66%)	
Other biochemistry:			
Creatinine (umol/L)	71.0 ±12.5	69.1 ±12.8	0.56
Zinc (umol/L)	13.0 ±3.6	13.3 ±2.3	0.78
Weight (kg)	57.7 ±7.9	60.6 ±13.2	0.30

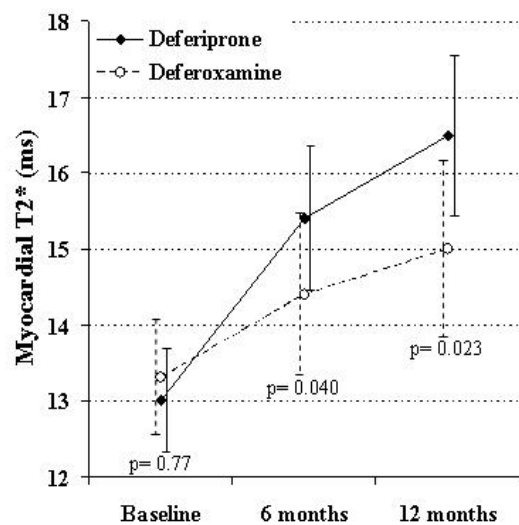
The target dose for subcutaneous deferoxamine was 50mg/kg/day for ≥ 5 days per week. The actual dose prescribed for deferoxamine was 43 mg/kg for 5.7 days/week (equivalent to 35 mg/kg/day for 7 days per week). This is equal to the recommended dose of 35 mg/kg/day for stable patients with similar ferritin levels from the product data sheet, and slightly higher than the 40 mg/kg/day for 5 days/week from clinical recommendations,²⁷ although lower than the maximal dose sometimes used in clinical practice. Oral deferiprone was initiated at 75 mg/kg/day and increased to the target of 100 mg/kg/day. The actual prescribed dose of deferiprone was 92 mg/kg/day. Compliance was similar (deferiprone 94 \pm 5.3%; deferoxamine 93 \pm 9.7%; $p=0.81$). Five patients withdrew; 3 taking deferoxamine: deterioration of cardiac function (1); personal reasons (2); and 2 taking deferiprone: elevated liver enzymes (2) which in one case was probably caused by cytomegalovirus hepatitis with increased IgM levels to CMV that fell, but with negative PCR.

*Myocardial T2**

Myocardial T2* rose with deferiprone at 6 and 12 months to 15.4ms (+18%; CV 38%; $p<0.001$) and 16.5ms (+27%; CV 38%; $p<0.001$). The myocardial T2* rose with deferoxamine at 6 and 12 months to 14.4ms (+9%; CV 37%, $p=0.0034$) and 15.0ms (+13%; CV 39%; $p<0.001$). The difference in the change between drugs was significant at 6 months (ratio of geometric mean 1.09; $p=0.040$), and at 12 months (ratio 1.12; $p=0.023$; figure 2). A rise in T2* and EF was seen in 19 (66%) deferiprone patients and 14 (45%) deferoxamine patients.

Figure 2. The change in myocardial T2* at 6 and 12 months was significantly greater in the patients taking deferiprone rather than deferoxamine. In this analysis are included 29 deferiprone patients and 31 deferoxamine patients (1 deferoxamine patient was excluded because T2* and ejection fraction were not measured at 6 and 12 months, and the 4 drop-outs were included using last observation carried over). The vertical axis shows the geometric mean of T2*. Standard error bars are shown.

Figure 2



Because of the baseline differences between groups in serum ferritin, white cell count and hemoglobin, further analysis of these factors and their interaction with the outcome of the primary endpoint was performed. The pre-defined statistical plan called for parametric analysis of the serum ferritin, but on inspection of the data, we found 2 extreme outliers in the deferoxamine group with ferritin values of 9259, and 9300ug/L, and Shapiro-Wilk's test indicated that the group was not normally distributed ($P < 0.001$). Accordingly, a log transformation was applied and this was successful in normalizing the ferritin data ($p = 0.76$).

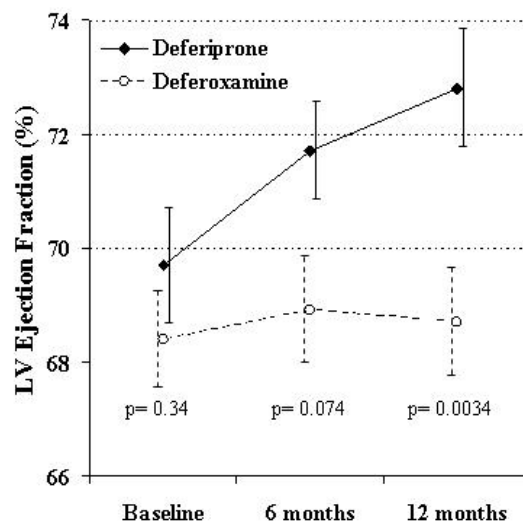
In addition, the differences in white cell count and hemoglobin were examined as both were significantly higher in the deferoxamine group. These differences appear to have resulted from the difference in the number of splenectomized patients in the two therapy arms (34% in the deferoxamine group vs 14% in the deferiprone group). The splenectomized patients had significantly higher corrected white cell count than non-splenectomized patients ($p < 0.0001$). Data review showed 4 splenectomized patients in the deferoxamine group had baseline corrected white cell counts $> 20 \times 10^9/L$. The larger number of splenectomized patients in the deferoxamine group could also explain the higher hemoglobin in the deferoxamine group because of decreased RBC consumption to maintain the same hemoglobin level despite similar mean transfusional input between the two groups (151.6 ± 43.4 mL/kg/y in the deferiprone group vs 144.3 ± 44.4 mL/kg/y in the deferoxamine group; $p = 0.52$). Therefore we conducted a multivariate analysis of the effect on the myocardial T2* of baseline differences in log serum ferritin and splenectomy status. This showed that the baseline ferritin does not have a significant impact on the improvement in T2* ($p = 0.66$), but splenectomy was significantly predictive ($p = 0.0018$). Thus splenectomized patients exhibited greater T2* improvement than non-splenectomized patients. The anti-log of the estimated effect size ($e^{0.1850} = 1.20$), which is equal to the ratio of geometric mean between splenectomized and non-splenectomized patients, indicates that the splenectomized patients were 20% better in improving T2* than non-splenectomized patients. After controlling for the baseline ferritin values and the splenectomy status, the difference in T2* favoring deferiprone remained significant, and its significance was substantially greater ($p = 0.0024$). Since the deferoxamine group had more splenectomized patients than the deferiprone group, an even greater difference in mean change from baseline in T2* in favor of deferiprone would have been expected had there been an equal number of splenectomized patients in the two treatment groups.

Cardiac function and volumes

At 12 months, there was a significant difference favoring deferiprone for reduction in end-systolic volume (-6.4 ± 6.8 vs -0.6 ± 7.9 mL; $p=0.0043$) and a borderline difference in reduction end-diastolic volume (-7.8 ± 13 vs -1.2 ± 13 mL; $p=0.060$). The EF rose with deferiprone by absolute units of $2.0 \pm 2.7\%$ at 6 months ($p<0.001$), and $3.1 \pm 3.6\%$ at 12 months ($p<0.001$). The EF rose with deferoxamine by $0.52 \pm 3.5\%$ at 6 months ($p=0.42$), and $0.32 \pm 3.4\%$ at 12 months ($p=0.66$). The difference in the change between groups was borderline at 6 months ($p=0.074$), and significant at 12 months ($p=0.0034$, figure 3). The percent change in T2* and EF with deferiprone was significantly correlated ($r^2 = 0.21$, $p= 0.012$), although this was relatively weak, and not useful to predict the EF change from the change in T2*. There was no correlation with deferoxamine ($r^2= 0.036$, $p= 0.49$).

Figure 3. The change in left ventricular (LV) ejection fraction at 12 months was significantly greater in the patients taking deferiprone rather than deferoxamine. In this analysis are included 29 deferiprone patients and 31 deferoxamine patients (1 deferoxamine patient was excluded because T2* and ejection fraction were not measured at 6 and 12 months, and the 4 drop-outs were included using last observation carried over). Standard error bars are shown.

Figure 3



Other iron measures

One patient in the deferiprone group did not have a LIC at baseline and there were 3 patients (1 deferiprone and 2 deferoxamine) who did not have a LIC value at 12 months. The last observation carried over technique could not be used for those 3 patients and they were excluded from the analysis at 12 months. Therefore LIC was assessed in 27 and 30 patients for the deferiprone and deferoxamine groups, respectively. LIC fell with deferiprone by $0.93 \pm 2.9 \text{ mg/g dw}$ at 12 months (-10.1% , $p=0.11$), compared with a fall with deferoxamine by $1.54 \pm 2.5 \text{ mg/g dw}$ at 12 months (-24.4% , $p=0.002$). The difference between groups at 12 months was not significant ($p=0.40$). The change in ferritin at 6 months with deferiprone and deferoxamine was $151 \pm 713 \text{ ug/L}$ and $-314 \pm 921 \text{ ug/L}$ ($p=0.033$ between groups); and at 12 months $-181 \pm 826 \text{ ug/L}$ and $-466 \pm 739 \text{ ug/L}$ (-10.1% and -16.7% change from baseline values respectively, $p=0.16$ between groups). There was no difference in trend of serum ferritin over time between groups ($p=0.41$).

Vital signs

Weight gain was greater with deferiprone at 6 months (1.6 ± 1.8 vs -0.22 ± 2.7 kg; $p=0.0035$), and at 12 months (2.2 ± 3.0 vs 0.1 ± 2.7 kg; $p=0.0043$). There were no significant differences between groups in other vital signs.

Other laboratory measures

The difference between the deferiprone and deferoxamine groups in change of ALT level at 12 months was not significant (22.9 ± 48.6 vs 4.7 ± 38.2 U/L; $p=0.11$). This includes the 2 patients who dropped out for increased serum enzyme levels. There was no significant difference in trend of ALT over time between groups ($p=0.32$). The difference in percentage of patients with ALT greater than twice the upper limit was not significant at baseline or at 12 months.

There was no significant difference between groups in the change of zinc level at 12 months (-0.80 ± 2.8 vs 0.23 ± 2.3 umol/L; $p=0.12$). There was no significant difference between groups in the change in creatinine at 12 months (3.24 ± 10.5 vs 0.06 ± 12.7 umol/L; $p=0.29$). There were no significant differences between groups in other laboratory data including ANC.

Adverse events

The most frequent adverse events with deferiprone were gastrointestinal symptoms (nausea, vomiting, or abdominal pain), which occurred in 19 (66%) patients. These usually occurred in the first weeks of treatment, were mild to moderate, and usually resolved within a median of 3 days (range 1-17 days) without discontinuation or decreasing the dose of deferiprone. Deferoxamine adverse events included reactions at the infusion site, which occurred in 12

(38%) patients. Joint problems, including pain and/or swelling, occurred in 8 (28%) deferiprone-treated patients and in 6 (19%) deferoxamine-treated patients ($p=0.30$). Nine (31%) deferiprone-treated patients reported increased appetite ($p<0.001$). One episode of neutropenia (1.01×10^9 neutrophils/L) occurred in one deferiprone-treated patient. The event resolved within 3 days (2.69×10^9 neutrophils/L) without discontinuation or decreasing the dose of deferiprone. There were no episodes of agranulocytosis.

DISCUSSION

Heart disease, which results from myocardial iron deposition associated with life-long blood transfusions and increased gut iron uptake, is the commonest cause of death in beta-thalassemia major. Recently it has become possible to target therapy for myocardial siderosis using myocardial T2* allowing comparison of the myocardial efficacy of iron chelators. Using myocardial T2*, continuous intravenous deferoxamine has been shown to reduce myocardial siderosis in acute heart failure,¹¹ although iron clearance was considerably slower from heart than liver. In the current trial, all screened patients had no cardiac symptoms and were receiving chronic deferoxamine monotherapy, and those who were subsequently randomized either continued deferoxamine at an increased dose (+4 mg/kg/day, $p= 0.001$) following entry into the trial with standardization of treatment, or were switched to deferiprone monotherapy. We found a significant reduction in our primary trial endpoint of myocardial siderosis in the deferoxamine group (T2* increased by 13% at 12 months, $p<0.001$) which probably resulted from the increased dose of deferoxamine, and/or improved compliance. Despite this improvement in the control group, the deferiprone group showed superior efficacy in reducing myocardial siderosis (T2* increased by 27% at 12 months, $p<0.001$; $p=0.023$ vs deferoxamine group at 12 months). These data accord with a previous

retrospective study showing lower myocardial iron with deferiprone.²¹ Although the clinical efficacy of raising myocardial T2* for asymptomatic patients remains to be determined, it would appear that reduced levels of myocardial iron are a favorable response.

The deferiprone group also exhibited significantly improvement in secondary trial endpoints compared with deferoxamine, with a reduced end-systolic volume and increased EF. In this trial, we excluded patients with an EF <56% by CMR, which is the lower limit for non-anemic subjects.²⁸ We believed this was sufficient evidence that significant LV systolic dysfunction was present and that best current medical treatment should be offered outside the trial setting. However, “normal” cardiac volumes and function in thalassemia are not well defined. Compared with normal non-anemic subjects, thalassemia patients’ hearts have larger end-diastolic volumes and increased cardiac output,^{7,29,30} which allows for increased perfusion for tissue oxygenation requirements. In addition, the ejection fraction in thalassemia major patients with no iron loading (T2* >20ms), is higher than in controls with a lower limit of up to 63%.^{5,7,30} Thus, because the EF in thalassemia without myocardial siderosis is higher than in normals, we believe that our trial included patients with sub-clinical LV dysfunction. The increase in EF seen in this trial accords with this view, and suggests that deferiprone was effective in relieving this dysfunction. Reduced EF is linked with adverse survival not only in thalassemia,³¹ but also in coronary artery disease,³² and heart failure,³³ and improved prognosis occurs with treatments that improve EF.^{34,35} Deferiprone also reduced end-systolic volume, another prognostic parameter in large,³⁶ and normal sized hearts.³⁷ Heart failure treatments known to improve prognosis reduce the end-systolic volume,³⁸ although this has not been shown directly in thalassemia patients. These prospective randomized data are also consistent with a previous study showing significantly superior EF with deferiprone.²¹

It therefore appears that deferiprone could reduce the risk of progression to iron-related cardiomyopathy by removing more cardiac iron than subcutaneous deferoxamine, and the LV function and volume improvements in patients with sub-clinical LV dysfunction could yield prognostic benefit. Lower levels of myocardial siderosis would allow an increased reserve for any future periods of iron loading, and the potential for greater resistance to catastrophic heart failure that can be seen in thalassemia patients with intercurrent infection. These interpretations accord with longitudinal studies of EF in thalassemia,³¹ and previous retrospective survival data using deferiprone or deferoxamine.³⁹ With >4 years of follow-up, cardiac dysfunction was newly diagnosed in 4% of the deferiprone-treated patients and 20% of the deferoxamine-treated patients (p=0.007). This was accompanied by no deaths with deferiprone but 3 cardiac-related deaths with deferoxamine.

These findings suggest differences in the in-vivo myocardial efficacy of these drugs. Deferoxamine is an important chelator,^{40,41,42} but is a large positively charged molecule, relatively lipophobic, and one which undergoes conformation change on binding of iron which may lead to intracellular trapping.⁴³ These properties potentially limit its ability to chelate intracellular iron, unless there is an active excretion pathway, as postulated for deferoxamine in hepatocytes.^{43,44} By contrast, deferiprone is a small, neutral molecule with greater lipophilicity, and therefore has significantly greater potential to chelate intracellular iron.^{45,46} This suggests that deferiprone may have preferential access to intracellular iron in tissues such as the myocardium, and in-vitro and animal data support this hypothesis.⁴⁷ Because of these variations in action, combination therapy with these chelators may be attractive for which there is supportive in-vitro,⁴⁷ and clinical evidence.⁴⁸ Combination therapy is being subjected to a further randomized controlled trial for cardiac efficacy.⁴⁹

Three previous randomized trials have compared the myocardial effects of chelators. Maggio compared deferiprone (75mg/kg/day) with deferoxamine (50mg/kg/day for 5 days/week).⁵⁰ No difference was found between groups in serum ferritin or T2 weighted myocardial signal intensity ratio (SIR). Peng compared deferiprone (75mg/kg/day) with deferoxamine (50mg/kg/day for ≥ 5 days/week).⁵¹ Deferiprone resulted in a significantly larger improvement in SIR and EF. Galia compared deferiprone (75mg/kg/day) with deferoxamine (50mg/kg/day for 6 days/week).⁵² No significant differences between groups were demonstrated in myocardial SIR or EF. Our current trial differs from these reports in several ways which might explain the efficacy of deferiprone which we found: first, the current trial used deferiprone at 100mg/kg/day; second, compliance was high in the current study, but was not quantified in the previous reports and may have been lower; third, the prior studies used heart SIR to measure myocardial siderosis, which is not an absolute measure and is sensitive to noise, limiting both its reproducibility and interpretation; fourth, the current trial is multicenter and not single center. Our results are consistent with another randomized controlled trial from Olivieri, which has only been published in abstract form; deferiprone (75mg/kg/day) resulted in a significant increase in T2 relaxation time, which was not matched by deferoxamine (50mg/kg/day).⁵³

The patients in the current study were well-matched at baseline for the primary endpoint of myocardial T2*, and they were also well-matched for the secondary endpoint of liver iron. However, the accuracy of SQUID may vary between machines and direct comparison of absolute liver iron levels with studies relating our single center SQUID results to those from other centers may not be straightforward. Despite these issues, it is worth noting that at baseline our patients had a median LIC of 4.30mg/g dw, with only 8% having an LIC >15mg/g dw. Therefore the patients in the current study would appear to be reasonably well-

chelated by liver criteria. In the current study, the 1.5 mg/g dw reduction in LIC over 12 months with deferoxamine was significant, whereas the 0.94 mg/g dw reduction with deferiprone did not achieve significance, although the between groups comparison was not significant. This is the first randomized controlled study to compare changes in LIC at the highest dose of deferiprone currently approved in Europe (100mg/kg/day). Deferiprone appeared to prevent liver iron accumulation, even if the mean decrease appeared marginally less effective than deferoxamine. The patients in our study showed a difference at baseline for the secondary endpoint of serum ferritin. The explanation for this difference is the presence of 2 extreme outliers in the deferoxamine group with very high ferritin levels. After log transformation to achieve a normal distribution, no difference between groups at baseline was present. This lack of difference accords with the lack of baseline statistical difference in the direct measures of liver or myocardial iron. After controlling for the baseline serum ferritin status (>2500 ug/L or ≤ 2500 ug/L) in a covariate analysis, the difference in myocardial T2* at month 12 between the treatment groups remained significant ($p=0.032$), favoring the deferiprone treatment group, and there was no difference in trend of serum ferritin over time between treatment groups.

Compliance with deferiprone at 100mg/kg/day was excellent with no new drug-related complications compared with previous lower dose trials. Contrary to other trials where deferiprone was interrupted at the first sign of neutropenia ($<1.5 \times 10^9/L$), we allowed continuation of deferiprone if the neutrophil count remained above $1.0 \times 10^9/L$. One patient experienced one episode of neutropenia, which resolved without discontinuing deferiprone. No agranulocytosis was seen. Two deferiprone treated patients discontinued the study due to increased liver enzymes, which returned to baseline levels upon interruption of therapy. One deferoxamine-patient developed arrhythmias and a reduction in EF, though still in the normal

range, and an increase in the LV and atrial diameters and was withdrawn from the study for initiation of intensive chelation therapy. Two other deferoxamine-patients discontinued the study for personal reasons.

Limitations

The results of this trial are relevant to patients with no symptoms of heart failure, and who are older than 18 years. Evidence that these findings apply to symptomatic patients, or children awaits trials in these specific populations.

Conclusions

At the doses used in this prospective trial, deferiprone was superior to deferoxamine over 1 year in improving myocardial siderosis in asymptomatic patients with beta-thalassemia major, and this confirms previous retrospective data. This suggests that deferiprone has superior access to myocardial iron stores compared with deferoxamine, which accords with in-vitro data. The combination of detection of myocardial siderosis by T2* CMR and the superior effect of deferiprone in removal of cardiac iron allows a rational paradigm of early management of cardiac siderosis in patients maintained on long term deferoxamine, and this should translate into improved survival.

Acknowledgements

We thank Drs David Lee and Yu-Chung Tsang for expert statistical advice on this study, and the following doctors for their important contributions: Gildo Matta, Carlo Dessi, Annalisa Agus, Simona Piras, GB Melis, P Bina - Cagliari; Filomena Longo, Giulio Lupo - Torino; Christina Fragodimitri, Fotis Karabatsos, Antonia Hatziliami, Jacqueline Youssef, Ekaterini Dokou, Helen Berdoussi, Helen Architektonidou, Dimitris Tsoussis – Aghia Sophia

Children's Hospital; David Firmin, Lisa Anderson - Royal Brompton Hospital. We also thank Professor John Porter for his constructive criticism of the manuscript.

REFERENCES

- ¹ 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 (8653): 27-30.
- ² Olivieri NF, Nathan DG, MacMillan JH, Wayne AS, Liu PP, McGee A, Martin M, Koren G, Cohen AR. Survival in medically treated patients with homozygous beta-thalassemia. *N Engl J Med* 1994; 331: 574-8.
- ³ 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: 227-33.
- ⁴ 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: 1187-93.
- ⁵ Anderson LJ, Holden S, Davies B, Prescott E, Charrier C, Bunce NH, Firmin DN, Porter JB, Wonke B, Walker JM, Pennell DJ. Cardiovascular T2* (T2 star) magnetic resonance for the early diagnosis of myocardial iron overload. *Eur Heart J* 2001; 22: 2171-9.
- ⁶ Cohen AR, Galanello R, Pennell DJ, Cunningham MJ, Vichinsky E. Thalassemia. *Hematology (Am Soc Hematol Educ Program)*. 2004; 14-34.
- ⁷ Wood JC, Tyszka JM, Carson S, Nelson MD, Coates TD. Myocardial iron loading in transfusion-dependent thalassemia and sickle cell disease. *Blood* 2004; 103: 1934-6.
- ⁸ Westwood MA, Wonke B, Maceira AM, Prescott E, Walker JM, Porter JB, Pennell DJ. Left ventricular diastolic function compared with T2* cardiovascular magnetic resonance for early detection of myocardial iron overload in thalassemia major. *J Magn Reson Imaging*. 2005; 22: 229-33.
- ⁹ Westwood MA, Anderson LJ, Tanner MA, Pennell DJ. The relationship between myocardial iron deposition and left ventricular dysfunction in thalassemia using cardiovascular magnetic resonance (abstr). *J Cardiovasc Magn Reson* 2005; 7: 46-7.

- ¹⁰ Tanner MA, Porter JB, Westwood MA, Nair SV, anderson LJ, Walker JM, Pennell DJ. Myocardial T2* in patients with cardiac failure secondary to iron overload (abstr). *Blood* 2005; 106 (suppl): 3838a.
- ¹¹ Anderson LJ, Westwood MA, Holden S, Davis B, Prescott E, Wonke B, Porter JB, Walker JM, Pennell DJ. Myocardial iron clearance during reversal of siderotic cardiomyopathy with intravenous desferrioxamine: A prospective study using T2* cardiovascular magnetic resonance. *Br J Haematol* 2004; 127: 348-55.
- ¹² Kirk P, Pennell DJ. Myocardial T2* is not affected by the structural effects of myocardial fibrosis, ageing or impaired left ventricular function (abstr). *Blood* 2005; 106 (suppl): 1675a.
- ¹³ Westwood M, Anderson LJ, Firmin DN, Gatehouse PD, Charrier CC, Wonke B, Pennell DJ. A single breath-hold multiecho T2* cardiovascular magnetic resonance technique for diagnosis of myocardial iron overload. *J Magn Reson Imaging* 2003; 18: 33-9.
- ¹⁴ Westwood M, Anderson LJ, Firmin DN, Gatehouse PD, Lorenz CH, Wonke B, Pennell DJ. Interscanner reproducibility of cardiovascular magnetic resonance in the early diagnosis of myocardial iron overload. *J Magn Reson Imaging* 2003; 18: 616-20.
- ¹⁵ 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: 531-8.
- ¹⁶ Wood JC, Enriquez CM, Powell A, Ghugre NR, Nelson MD, Polzin J, Coates TD. Single echo versus multiple echo cardiac T2* estimates for cardiac iron estimation in iron overload (abstr). *J Cardiovasc Magn Reson* 2005; 7: 97.
- ¹⁷ Kirk P, Anderson LJ, Tanner MA, Galanello R, Matta G, Tan RS, Ng I, Lai A, Chen K, Firmin DN, Pennell DJ. Intersite validation of a single breath-hold T2* MR technique for heart and liver iron measurement (abstr). *Blood* 2005; 106 (suppl): 3830a.
- ¹⁸ Wood JC, Enriquez C, Ghugre N, Tyzka JM, Carson S, Nelson MD, Coates TD. MRI R2 and R2* mapping accurately estimates hepatic iron concentration in transfusion-dependent thalassemia and sickle cell disease patients. *Blood* 2005; 106: 1460-5.
- ¹⁹ Wood JC, Otto-Duessel M, Aguilar M, Nick H, Nelson MD, Coates TD, Pollack H, Moats R. Cardiac iron determines cardiac T2*, T2, and T1 in the gerbil model of iron cardiomyopathy. *Circulation* 2005 26; 112: 535-43.

- ²⁰ Pennell DJ, Anderson LJ, Kirk P, Forni G, Ellis G, Walker JM, Porter JB. Calibration of myocardial T2* values in post-mortem hearts (abstr). *Blood* 2005; 106 (suppl): 3837 a.
- ²¹ Anderson LJ, Wonke B, Prescott E, Holden S, Walker JM, Pennell DJ. Comparison of effects of oral deferiprone and subcutaneous desferrioxamine on myocardial iron levels and ventricular function in beta thalassemia. *Lancet* 2002; 360: 516-20.
- ²² Ghugre NR, Enriquez CM, Coates TD, Nelson MD, Wood JC. Improved R2* measurements in myocardial iron overload (abstr). *J Cardiovasc Magn Reson* 2005; 7: 32-33.
- ²³ Pennell DJ. Cardiovascular Magnetic Resonance. In: Braunwald's Heart Disease: A textbook of cardiovascular medicine. Eds: Zipes DP, Libby P, Bonow RO, Braunwald E. Elsevier Saunders, USA. 2005; 335-53.
- ²⁴ 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.
- ²⁵ Brittenham GM, Farrell DE, Harris JW, Feldman ES, Danish EH, Muir WA, Tripp JH, Bellon EM. Magnetic-susceptibility measurement of human iron stores. *N Engl J Med* 1982; 307: 1671-5.
- ²⁶ Fung EB, Longo F, Fischer R, Engelhardt R, Pakbaz Z, Optiz H, Fagaly RL, Nick H, Nielsen P, Vichinsky E, Piga A, Harmatz P. Comparison of liver iron concentration measured by the SQUID biosusceptometers at Hamburg, Torino and Oakland in patients with thalassemia and sickle cell disease (abstr). *Blood* 2005; 106 (suppl): 3721a.
- ²⁷ Porter JB. Practical management of iron overload. *Br J Haematol* 2001; 115: 239-52.
- ²⁸ Lorenz CH, Walker ES, Morgan VL, Klein SS, Graham TP Jr. Normal human right and left ventricular mass, systolic function, and gender differences by cine magnetic resonance imaging. *J Cardiovasc Magn Reson*. 1999; 1: :7-21.
- ²⁹ Aessopos A, Farmakis D, Hatziliami A, Fragodimitri C, Karabatsos F, Joussef J, Mitilineou E, Diamanti-Kandaraki E, Meletis J, Karagiorga M. Cardiac status in well-treated patients with thalassemia major. *Eur J Haematol* 2004; 73: 359-66.
- ³⁰ Westwood MA, Anderson LJ, Maceira AM, Prescott E, Porter JB, Wonke B, Walker JM, Pennell DJ. Normalized left ventricular volumes and function in thalassemia major patients with normal myocardial iron (abstr). *Blood* 2005; 106 (suppl): 2707a.

- ³¹ Davis BA, O'Sullivan C, Jarritt PH, Porter JB. Value of sequential monitoring of left ventricular ejection fraction in the management of thalassemia major. *Blood* 2004; 104: 263-9.
- ³² The Multicenter Postinfarction Research Group. Risk stratification and survival after myocardial infarction. *N Engl J Med* 1983; 309: 331-6.
- ³³ Parameshwar J, Keegan J, Sparrow J, Sutton GC, Poole-Wilson PA. Predictors of prognosis in severe chronic heart failure. *Am Heart J* 1992; 123: 421-6.
- ³⁴ The CONSENSUS study group. Effects of enalapril on mortality in severe congestive heart failure. Results of the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS). *N Engl J Med* 1987; 316: 1429-35.
- ³⁵ Packer M, Bristow MR, Cohn JN, Colucci WS, Fowler MB, Gilbert EM, Shusterman NH. The effect of carvedilol on morbidity and mortality in patients with chronic heart failure. U.S. Carvedilol Heart Failure Study Group. *N Engl J Med* 1996; 334: 1349-55.
- ³⁶ White HD, Norris RM, Brown MA, Brandt PW, Whitlock RM, Wild CJ. Left ventricular end-systolic volume as the major determinant of survival after recovery from myocardial infarction. *Circulation* 1987; 76: 44-51.
- ³⁷ Vasan RS, Larson MG, Benjamin EJ, Evans JC, Levy D. Left ventricular dilatation and the risk of congestive heart failure in people without myocardial infarction. *N Engl J Med* 1997; 336: 1350-5.
- ³⁸ Konstam MA, Rousseau MF, Kronenberg MW, Udelson JE, Melin J, Stewart D, Dolan N, Edens TR, Ahn S, Kinan D, et al. Effects of the angiotensin converting enzyme inhibitor enalapril on the long-term progression of left ventricular dysfunction in patients with heart failure. SOLVD Investigators. *Circulation* 1992; 86: 431-8.
- ³⁹ Piga A, Gaglioti C, Fogliacco E, Tricta F. Comparative effects of deferiprone and deferoxamine on survival and cardiac disease in patients with thalassemia major: a retrospective analysis. *Haematologica* 2003; 88: 489-96.
- ⁴⁰ Wolfe L, Olivieri N, Sallan D, Colan S, Rose V, Propper R, Freedman MH, Nathan DG. Prevention of cardiac disease by subcutaneous deferoxamine in patients with thalassemia major. *N Engl J Med* 1985; 312: 1600-3.
- ⁴¹ 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: 567-73.

- ⁴² Giardina PJ, Ehlers KH, Engle MA, Grady RW, Hilgartner MW. The effect of subcutaneous deferoxamine on the cardiac profile of thalassemia major: a five-year study. *Ann N Y Acad Sci* 1985; 445: 282-92.
- ⁴³ Porter JB. Development of primary hepatocyte cultures for the preclinical evaluation of iron chelators. MD Thesis, University of Cambridge, 1999.
- ⁴⁴ Kushner JP, Porter JP, Olivieri NF. Secondary iron overload. *Hematology (Am Soc Hematol Educ Program)*. 2001; 47-61.
- ⁴⁵ de Franceschi L, Shalev O, Piga A, Collell M, Olivieri O, Corrocher R, Hebbel RP, Brugnara C. Deferiprone therapy in homozygous human beta-thalassemia removes erythrocyte membrane free iron and reduces KCl cotransport activity. *J Lab Clin Med* 1999; 133: 64-9.
- ⁴⁶ Shalev O, Repka T, Goldfarb A, Grinberg L, Abrahamov A, Olivieri NF, Rachmilewitz EA, Hebbel RP. Deferiprone (L1) chelates pathologic iron deposits from membranes of intact thalassemic and sickle red blood cells both in vitro and in vivo. *Blood* 1995; 86: 2008-13.
- ⁴⁷ Link G, Konijn AM, Breuer W, Cabantchik ZI, Hershko C. Exploring the "iron shuttle" hypothesis in chelation therapy: effects of combined deferoxamine and deferiprone treatment in hypertransfused rats with labeled iron stores and in iron-loaded rat heart cells in culture. *J Lab Clin Med* 2001; 138:130-8.
- ⁴⁸ Mourad FH, Hoffbrand AV, Sheikh-Taha M, Koussa S, Khoriaty AI, Taher A. Comparison between desferrioxamine and combined therapy with desferrioxamine and deferiprone in iron overloaded thalassaemia patients. *Br J Haematol* 2003; 121: 187-9.
- ⁴⁹ Tanner MA, Westwood MA, Galanello R, Pennell DJ. Baseline findings of a CMR driven randomized controlled trial of iron chelation therapy in thalassemia major (abstr). *J Cardiovasc Magn Reson* 2005; 7: 31-2.
- ⁵⁰ Maggio A, D'Amico G, Morabito A, Capra M, Ciaccio C, Cianciulli P, Di Gregorio F, Garozzo G, Malizia R, Magnano C, Mangiagli A, Quarta G, Rizzo M, D'Ascola DG, Rizzo A, Midiri M. Deferiprone versus deferoxamine in patients with thalassemia major: a randomized clinical trial. *Blood Cells Mol Dis* 2002; 28: 196-208.
- ⁵¹ Peng CT, Chow KC, Chen JH, Chiang YP, Lin TY, Tsai CH. Safety monitoring of cardiac and hepatic systems in beta-thalassemia patients with chelating treatment in Taiwan. *Eur J Haematol* 2003; 70: 392-7.
- ⁵² Galia M, Midiri M, Bartolotta V, Morabito A, Rizzo M, Mangiagli A, Malizia R, Borsellino Z, Capra M, D'Ascola DG, Magnano C, Gerardi C, Rigano P, Maggio A; Multicenter Trial Group of the Society for the Study of Thalassemia and Haemoglobinopathies. Potential myocardial iron content evaluation by magnetic resonance

imaging in thalassemia major patients treated with Deferoxamine or Deferiprone during a randomized multicenter prospective clinical study. *Hemoglobin* 2003; 27: 63-76.

⁵³ Olivieri NF, Brittenham GM, Armstrong SA, Basran RK, Daneman R, Daneman N, Iwanchko RM, Talbot AL, Koren G. First prospective randomized trial of the iron chelators deferiprone (L1) and deferoxamine (abstr). *Blood* 1995; 86 (suppl 1): 249a.