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# Long-term sequential deferiprone–deferoxamine *versus* deferiprone alone for thalassaemia major patients: a randomized clinical trial

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## Summary

A multicentre randomized open-label trial was designed to assess the effectiveness of long-term sequential deferiprone–deferoxamine (DFO–DFP) *versus* DFP alone to treat thalassaemia major (TM). DFP at 75 mg/kg, divided into three oral daily doses, for 4 d/week and DFO by subcutaneous infusion (8–12 h) at 50 mg/kg per day for the remaining 3 d/week was compared with DFP alone at 75 mg/kg, administered 7 d/week during a 5-year follow-up. The main outcome measures were differences between multiple observations of serum ferritin concentrations. Secondary outcomes were survival analysis, adverse events, and costs. Consecutive thalassaemia patients (275) were assessed for eligibility; 213 of these were randomized and underwent intention-to-treat analysis. The decrease of serum ferritin levels during the treatment period was statistically significant higher in sequential DFP–DFO patients compared with DFP-alone patients ($P = 0{\cdot}005$). Kaplan–Meier survival analysis for the two chelation treatments did not show any statistically significant differences (long-rank test, $P = 0{\cdot}3145$). Adverse events and costs were comparable between the groups. The trial results show that sequential DFP–DFO treatment compared with DFP alone significantly decreased serum ferritin concentration during treatment for 5 years without significant differences regarding survival, adverse events, or costs. This trial was registered at http://www.clinicaltrials.gov as # NCT00733811.

**Keywords:** chelation, thalassaemia, clinical trials, red blood cell disorders, iron overload.

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During the last two decades, changes in chelation treatment and transfusion practices have dramatically improved the prognosis of thalassaemia major (TM) patients (Borgna-Pignatti *et al*, 2004; Maggio *et al*, 2007). In most of the 14 clinical trials published between 1990 and 2008, (Olivieri *et al*, 1990; Olivieri & Brittenham, 1997; Aydinok *et al*, 1999, 2007; Maggio *et al*, 2002; Gomber *et al*, 2004; Galanello *et al*, 2005; Ha *et al*, 2006; Kattamis *et al*, 2006; Pennell *et al*, 2006; Abdelrazik, 2007; Tanner *et al*, 2007, 2008; El-Beshlawy *et al*, 2008), deferiprone (DFP) was compared with deferoxamine (DFO). No statistically significant differences were found between these two interventions over a maximum of 18 months treatment duration (Roberts *et al*, 2007). Two randomized trials that compared sequential DFP–DFO treatment *versus* DFO alone reported conflicting results (Galanello *et al*, 2005; Abdelrazik, 2007), but this could have been due to small sample sizes and short treatment duration. Therefore, we conducted a long-term sequential DFP–DFO treatment *versus* DFP alone treatment trial to assess the impact of these chelation treatments on serum ferritin concentrations, survival analysis, adverse events, and costs in TM patients.

## Patients and methods

### *Design*

The trial was designed as a multicentre randomized open-label trial with blinded data management and data analyses, to assess whether either treatment was superior to the other. The trial was performed on behalf of the Italian Society for the Study of Thalassaemia and Haemoglobinopathies (SoSTE) (http://www.soste.org). The investigators initiated, carried out, and controlled the trial, which was conducted without the influence of the non-commercial sponsor (Maggio *et al*, 2004). A sequential rather than simultaneous chelation treatment by DFP plus DFO was selected because the latter was known to be an hyperchelation protocol with more effects on serum ferritin levels and on urinary iron excretion (Giardina & Grady, 2001; Link *et al*, 2001). Therefore, while simultaneous chelation treatment is indicated more in patients with severe iron overload, a sequential protocol is more suitable for the enrolment of patients with moderate body iron burden and these patients were included in our trial for comparison with DFP-alone treatment.

### *Patients*

Consecutive TM patients observed at 25 SoSTE centres in Italy between September 30, 2000, and January 31, 2008 were considered to be eligible for the trial if they had a serum ferritin concentration between 800 and 3000 μg/l and were over 13 years of age. Parents gave informed consent for patients between 13 and 18 years of age. The data were collected at the coordinating centre (A.O.V. Cervello, U.O.C. di Ematologia II, Palermo, Italy).

Figure 1 shows the trial profile. Before inclusion in the trial, all patients were treated with either subcutaneous (SC) administration of DFO 50 mg/kg per day, 8–12 h for 5 d a week or with DFP 75 mg/kg per day orally for 7 d a week. There were no statistically significant differences in the number of the patients receiving DFO or DFP, before randomization between the two groups. To achieve an acceptable treatment wash-out, chelation therapy was withdrawn for 1 week before randomization, after verifying inclusion and exclusion criteria. The diagnosis of TM was based on accepted clinical and molecular criteria (Modell & Berdoukas, 1984; Maggio *et al*, 1993).

The exclusion criteria were (i) known intolerance to one of the trial treatments, (ii) platelet count $<100 \times 10^9$/l or leucocyte count $<3{\cdot}0 \times 10^9$/l, (iii) severe liver damage as indicated by Child-Plugh C grade classification, and (iv) overt heart failure. Eligibility and exclusion criteria were checked at each participating centre, where the patients were also seen throughout the entire follow-up period.

### *Randomization*

The randomization sequence was based on a computer-randomized list in permuted blocks of 10 with a 1:1 ratio, generated at the Institute Giulio Alfredo Maccacaro, University of Milan, Italy. To ensure allocation concealment, treatment was assigned by telephone contact from the coordinating centre. The sequence was concealed until interventions were assigned. Randomization was performed per each consecutive patient after verification of the exclusion criteria. Treatment was started within the following 24 h.

### *Interventions*

Trial interventions were sequential treatment including DFP (Apotex; Toronto, ON, Canada) at 75 mg/kg, divided into three oral daily doses, for 4 d/week and DFO (Biofutura Pharma, Pomezia, Italy) by SC infusion (8–12 h) at 50 mg/kg per day for the remaining 3 d/week *versus* DFP alone, at the same dosage (75 mg/kg divided into three oral daily doses), administered 7 d a week. A double-blinded design was not considered to be possible because of the SC administration of DFO.

The planned duration of treatment was 5 years. However, because of the beneficial effects, in terms of serum ferritin levels reduction in the sequential DFP–DFO group, observed after the interim analysis performed at January 31, 2008 the trial was stopped before the planned 5 years of treatment were completed for all patients. Therefore, the mean duration of treatment was $2{\cdot}5 \pm 2{\cdot}2$ and $2{\cdot}9 \pm 2{\cdot}1$ years for DFP and sequential DFP–DFO group respectively.

During this period, treatment was only interrupted at the discretion of the investigators for intercurrent illness or adverse events (AEs). Dose modifications were allowed for safety reasons. Criteria for dosage reduction during sequential treatment to DFP 50 mg/kg per day and DFO 30 mg/kg per day were (i) decrease in serum ferritin levels at a final value of

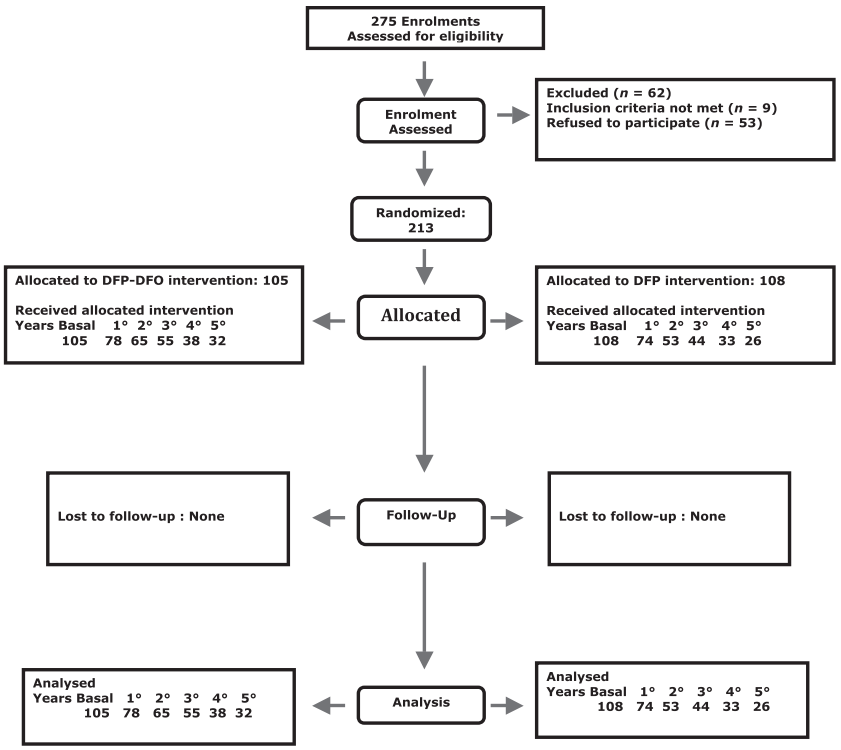


**Fig 1.** Trial profile of sequential deferiprone–deferoxamine *versus* deferiprone-alone intervention groups during the trial.

<700 μg/l, confirmed by two determinations and (ii) decrease in serum zinc levels of more than 12·5 μmol/l. Further criteria for dosage reduction during DFP-alone intervention to DFP 50 mg/kg per day were arthralgia and nausea not controlled by symptomatic therapy. The treatment was stopped and alternative therapy was started if an increase in ferritin levels of more than 1000 μg/l, confirmed at least by two determinations, was detected during the study period. This was reported as treatment failure. All outcome assessments were done under code by physicians blinded to the trial treatment. Drug administration was recorded on the case report form. Assessments for safety and efficacy were performed at monthly planned follow-up visits. The coordinating investigator decided whether each patient could continue on the allocated treatment (sequential DFP–DFO or DFP alone) or should change to DFO alone until the trial analysis was completed at the end of the 5-year treatment period. Compliance was assessed by counting the pills in each returned bag of DFP and by assessing the number of infusions of DFO registered on the electronic pump (Crono$^{TM}$, Gene S.r.l., Italy). Standard transfusion therapy was aimed at maintaining the haemoglobin blood concentration at or above 95 g/l.

### *Primary and secondary outcomes*

The primary outcome measure of treatment efficacy was the difference between multiple observations of serum ferritin concentrations during the 5-year treatment. A correlation between liver iron concentration and serum ferritin levels has previously been shown in cohort of thalassaemia major patients treated with deferiprone (Olivieri *et al*, 1995). Secondary outcome measures were survival analysis, adverse events, and costs.

In addition, a multislice-multiecho T2* magnetic resonance imaging (MRI) scan, available since June 2004, was used in a subgroup of the patients to evaluate variations in the iron content of the heart and liver during the trial (Pepe *et al*, 2006). The mean duration from entry into the trial to the time of the T2* MRI basal assessment ± standard deviation (T0) of heart and liver was 17 ± 18 months in the DFP–DFO treated group and 18 ± 17 months in the DFP treated group ($P = 0{\cdot}73$). The final evaluation (T1) was performed after a further 16 ± 5 and 14 ± 6 months ($P = 0{\cdot}94$) respectively.

### *Follow-up and data collection*

A 5-year follow-up was planned. Patients visited the clinic monthly and, at each visit, clinical and biochemical data, as well as a complete blood cell count, were registered in predefined data collection forms. The data were regularly forwarded to the coordinating centre, where a complete database for all included patients was established.

### *Sample size estimation*

Sample size calculation for two-group repeated-measure experiments was performed as reported by Rochon (1991),

who included tables for derivation of the minimum sample size (for our study, the numbers reported in the tables was between 40 and 100). A minimum number of patients required in each treatment group was calculated assuming equal allocation, under the hypothesis of equality between the two treatment groups at every point during the course of the trial for the autoregressive correlation structure, for a two-sided test at $\alpha = 0{\cdot}05$, $\beta = 0{\cdot}80$, $\Delta = 0{\cdot}41$ (standardized effect), $\rho = 0{\cdot}60$, and number of follow-up measurements $T = 5$.

### *Cost*

The costs of chelation treatments were calculated for the overall period of observation based on cost for 1-day treatment per each person multiplied by the person-years of treatment. Periods of reduced dosage were also considered for the cost analysis.

### *Ethics*

The study protocol was in accordance with the Declaration of Helsinki (http://www.wma.net/e/policy/b3.html) and was approved by the local ethics committee for human investigations (September 13, 2000). The patients (or the parents of minors) gave their written informed consent to participate in the trial. The trial was registered at http://www.clinicltrials.gov, Identifier NCT00733811.

### *Statistical methods*

All of the statistical analyses were performed under code at the Institute Giulio Alfredo Maccacaro, University of Milan, by a biostatistician (A.M.) blinded to the trial interventions.

Means are reported with standard deviations (SD). Proportions and differences between proportions are reported with 95% confidence intervals (CI). The statistical analysis was based on the 'intention-to-treat' principle. Continuous scale values were compared between the two intervention groups by a paired *t*-test. Differences in proportions observed on contingency tables were assessed by chi-square analysis. Serum ferritin levels consisted of repeated observations over time with the same patient.

To model longitudinal data, we used the generalized estimating equation (GEE) model (Hedeker & Gibbons, 2006). This approach has been implemented in the 'xtgee' procedure of STATA 9.2 software (StataCorp, College Station, TX, USA). The Kaplan–Meier method was used to estimate survival analysis among the randomized patients. The survival curves were compared per treatment group using the log-rank test and hazard ratio. Survival analysis was performed from September 30, 2000 to January 31, 2008. All statistical analyses were performed using STATA 9.2 (StataCorp).

## Results

### *Participant recruitment and flow*

Consecutive TM patients ($n = 275$) were observed at the 25 SoSTE centres from September 30, 2000 to January 31, 2008 (Fig 1). Nine patients did not meet inclusion criteria and 53 patients declined to participate (Fig 1). The remaining 213 patients were included; 105 and 108 respectively, were randomly allocated to DFP–DFO sequential treatment or DFP alone (Fig 1). None of the patients were lost to follow-up.

### *Baseline data*

Haematological and clinical findings at enrolment are shown in Table I. No differences were observed at baseline between the two randomized groups (Table I). In particular, the main findings of body iron overloading, expressed as serum ferritin at baseline, liver iron concentration (LIC), total number of blood transfusions, and mean ferritin levels in the year before randomization, were similar in the two groups (Table I).

### *Serum ferritin concentrations*

Table II shows baseline and follow-up changes of the mean serum ferritin values during the 5-year treatment. Figure 2 shows the estimated profiles of serum ferritin levels in the two groups after modelling the longitudinal data by the GEE model (Hedeker & Gibbons, 2006). The regression coefficient of treatment-year showed that there was a significant linear reduction over time of $-115{\cdot}3$ μg/l per year of serum ferritin levels in sequential DFP–DFO patients compared with DFP-alone patients ($P = 0{\cdot}005$) (Table III and Fig 2). There was no correlation between variations in serum ferritin levels and protein C values during the trial follow-up (data not shown).

### *Survival analysis during the trial*

The survival probability curves of the two treatment groups are reported in Fig 3. The intention-to treat analysis did not show any statistically significant difference between the two groups. One patient died during DFP–DFO treatment. This patient had experienced an episode of heart failure 1 year before entry into the trial (Table IV, Patient 1). No deaths occurred during DFP treatment.

### *Late deaths after stopping trial medication*

Five late deaths occurred 11–60 months after definitive withdrawal from the trial medication (Table IV, Patients 2–6). Sequential DFP–DFO treatment had been administered to one of these patients, who withdrew from the study due to liver cancer (Table IV, Patient 2). DFP alone had been administered to the other four patients (Table IV, Patients

**Table I.** Baseline comparison in the 213 patients included in the trial.

| Findings | Sequential DFP–DFO group $n$ = 105 | DFP group $n$ = 108 |
|---|---|---|
| Age, years | 23 ± 8·0 | 23 ± 7·8 |
| Females (%) | 55 (50·9) | 66 (61·1) |
| Hb, g/l* | 99 ± 10 | 98 ± 10 |
| ALT, I/U per liter* | 46 ± 61·6 | 48 ± 43·0 |
| γGT, I/U per liter* | 25 ± 21·7 | 21 ± 17·0 |
| Prothrombin activity, %* | 82 ± 23·5 | 88 ± 30·4 |
| Iron, μmol/l* | 35·6 ± 7·7 | 35·6 ± 8·4 |
| Transferrin, g/l* | 1·56 ± 0·29 | 1·60 ± 0·40 |
| Zinc, μmol/l | 12·7 ± 5·2 | 13·7 ± 5·5 |
| Liver iron concentration, mg/g per dry weight*† | 4·6 ± 2·8 | 4·0 ± 2·3 |
| Total blood transfusion, ml/kg per year | 140 ± 42 | 145 ± 44 |
| Mean Hb pre-Tx, g/l | 97 ± 5·0 | 97 ± 5·0 |
| Mean ferritin,‡ μg/l | 1727 ± 669 | 1868 ± 845 |
| Mean age at DFO starting, years | 5·1 ± 5·2 | 4·3 ± 4·1 |
| Splenectomy (%) | 17 (14·0) | 15 (12·7) |
| Cirrhosis (%) | 4 (3·3) | 6 (5·2) |
| Arrhythmia (%) | 6 (5·0) | 2 (1·7) |
| HCV-RNA positive (%) | 31 (38·3) | 37 (47·4) |

Tx, transfusion.

*Normal values: Hb, males 140–180 g/l, females 120–160 g/l; alanine transaminase (ALT), 2–26 I/U per liter; γ-glutamyltransferase (γGT) 0–30 I/U per liter; Prothrombin activity, 70–110%; Iron, males and females (mean ± 1 SD), 21·8 ± 5·5 μmol/l; Transferrin, 2·5 g/l; Zinc, males and females (mean ± 1 SD), 9·8–18·3 μmol/l; Liver iron concentration, mg/g per dry weight† = males 0·2–2, females 0·2–1·6.

†Liver iron concentration was determined in 39 with DFP-alone *versus* 37 with sequential DFP–DFO- treated patients by liver biopsy and atomic absorption spectrometry (AAS) technique. All patients performed liver biopsy during the year before randomization. The indication for liver biopsy was evaluation for histology and liver iron concentration aimed at antiviral treatment.

‡Value obtained by the mean of the three determinations performed in each patient during the year before randomization.

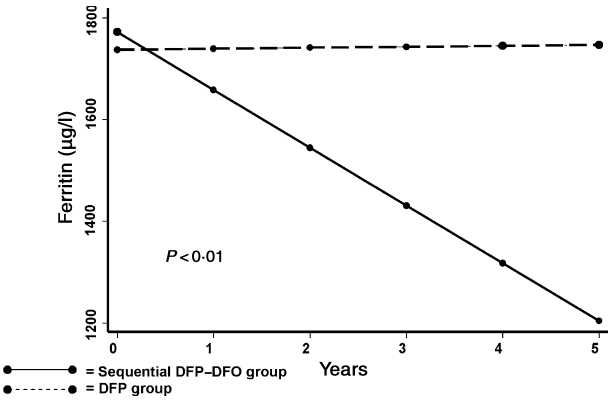


**Fig 2.** Estimated profiles from the GEE-fitted model.

**Table III.** GEE model to evaluate changes in mean serum ferritin levels in sequential DFP–DFO *versus* DFP-alone intervention groups over the course of the trial.

| | Coefficient | 95% CI | $P$ value* |
|---|---|---|---|
| Intercept | 1737·3 | 1541·4 to 1933·1 | 0·0001 |
| Treatment | 34·2 | −238·2 to 306·6 | 0·805 |
| Year | 1·8 | −57·1 to 60·7 | 0·952 |
| Treatment-years | −115·3 | −195·4 to −35·3 | 0·005 |

*$P$ value from null hypothesis coefficient = 0 by Wald's test.

3–6). Causes of withdrawal from DFP are shown in Table IV (Patients 3–6). The main causes of death were heart failure (50%). Other causes were pancreatic cancer, liver cancer, and stroke.

### *Adverse events and treatment failures*

Table V reports the main side effects in the two groups. The overall period of observation was 307·98 person-years for sequential DFP–DFO patients compared with 274·52 person-years for DFP-alone patients. Only 21 (35%) subjects in the

**Table II.** Variations in serum ferritin levels (μg/l) along with their difference during 5 year-treatment*.

| | Sequential DFP–DFO group | | | DFP group | | |
|---|---|---|---|---|---|---|
| Year | $n$ | Mean ± SD | Difference† ± SD | $n$ | Mean ± SD | Difference† ± SD |
| 0 | 105 | 1787 ± 735‡ | – | 108 | 1890 ± 816‡ | – |
| 1 | 78 | 1400 ± 770 | −417 ± 589 | 74 | 1633 ± 841 | −132 ± 724 |
| 2 | 65 | 1480 ± 867 | −362 ± 803 | 53 | 1742 ± 923 | −12·6 ± 732 |
| 3 | 55 | 1351 ± 754 | −403 ± 697 | 44 | 1734 ± 1037 | 5·4 ± 870 |
| 4 | 38 | 1408 ± 869 | −395 ± 940 | 33 | 1856 ± 1315 | 184 ± 1036 |
| 5 | 32 | 1369 ± 816 | −396 ± 894 | 26 | 1588 ± 1217 | −115 ± 1009 |

*These data are repeated measurements so it is not possible to perform single t-test but it is necessary to use generalized estimating equation (GEE) model (Hedeker & Gibbons, 2006) (see Table III and Fig 2).

†Value obtained by the difference of serum ferritin level at each treatment-year less serum ferritin level at baseline.

‡Value obtained by the mean of the determinations performed in the patients after a 1-week treatment wash-out period before randomization.

DFP-alone and 12 (24%) in the sequential DFP–DFO group withdrew definitely from the trial (Table V). The mean time for definitive withdrawal was 152 ± 103 (days) in DFP-alone *versus* 112 ± 76 (days) in the sequential DFP–DFO group respectively. There was no statistically significant difference in temporary and definitive discontinuation of treatment between the groups ($\chi^2 = 3{\cdot}35$; $P = 0{\cdot}07$) during the overall period of observation. Dosage decrease was necessary in 25 (49·0%) of sequential DFP–DFO patients *versus* 26 (56·5%) of DFP-alone patients ($\chi^2 = 0{\cdot}54$; $P = 0{\cdot}4$).

There was no difference in treatment failures ($n = 2$ in sequential DFP–DFO *versus* $n = 8$ in DFP-alone treated group) between the two groups ($\chi^2 = 2{\cdot}9$; $P = 0{\cdot}088$). No patients with agranulocytosis were reported in the sequential DFP–DFO-treated patients, compared with three patients with agranulocytosis in the DFP-alone group ($\chi^2 = 2{\cdot}958$; $P = 0{\cdot}085$) (Table V). Moreover, no significant change was shown in zinc levels from baseline to the end of the study between the two groups (data not shown).

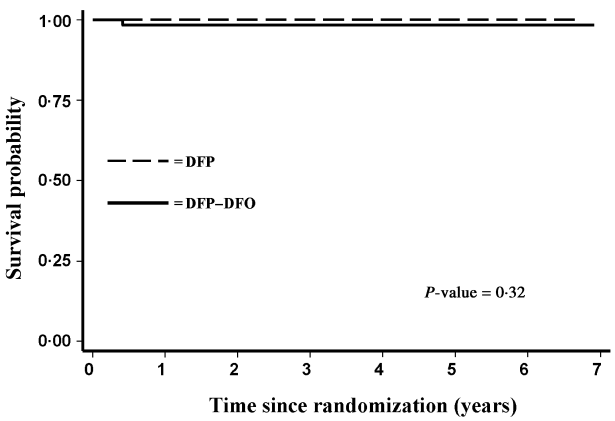


**Fig 3.** Kaplan–Meier survival probability curves in the two treatment groups (DFP–DFO continuous line; DFP dashed line), log-rank test = 0·32.

### *Compliance and costs*

In the sequential DFP–DFO group, compliance was 92·7% (SD ± 15·2%; range 37–100%) with DFP treatment and 70·6% (SD ± 24·1%; range 25–100%) with DFO treatment. Compliance with DFP was 93·6% (SD ± 9·7%; range 56–100%) in the DFP-alone patients. The calculated cost was 6402 ± 4571 Euros per person-year (range 45–15 102) for sequential DFP–DFO *versus* 5634 ± 4824 Euro per person-year (range 138–14 961) for DFP-alone treatment.

### *T2* magnetic resonance imaging*

Data on heart and liver T2* MRI scans, performed on a subgroup of sequential DFP–DFO patients ($n = 34$) *versus* DFP-alone patients ($n = 20$) at T0 and T1, are shown in Table VI. They were not selected and had the same characteristics as the other patients in term of inclusion criteria, including all the patients that were studied since June 2004, when T2* MRI was available, to the date at which trial was stopped. No statistically significant differences in the T2* signals of the heart and liver were found between the two groups (Table VI).

## Discussion

These findings show that sequential DFP–DFO treatment compared with DFP treatment alone significantly decreased serum ferritin concentration over 5 years of treatment without significant differences in survival, adverse events, and costs.

The first success of sequential DFP–DFO treatment was reported by Aydinok *et al* (1999) who found that there was a significant reduction in liver iron concentrations in seven

**Table IV.** Demographics and clinical information for the six patients who died during the trial.

| Patient | Age at start of trial, years* | Age at first transfusion (months) | Age at start of first chelation (years) | Chelation treatment during trial | Cause of definitive withdrawal from trial | Months from withdrawal of trial medication and death | Treatment before death | Cause of death |
|---|---|---|---|---|---|---|---|---|
| 1 | 26 | 3 | 3 | DFP–DFO | Death | 0 | DFP–DFO | Arrhythmia |
| 2 | 41 | 10 | 12† | DFP–DFO | Liver cancer | 11 | DFO | Liver cancer |
| 3 | 41 | 6 | NA | DFP | Pancreatic cancer | 12 | DFO | Pancreatic cancer |
| 4 | 29 | 36 | 7 | DFP | Failure of treatment‡ | 18 | DFO | Heart failure |
| 5 | 46 | 12 | 14† | DFP | Gastrointestinal problems | 34 | DFO | Stroke |
| 6 | 18 | 5 | 1 | DFP | Gastrointestinal problems | 60 | DFO | Myocarditis |

NA, not available.

*Age of patient on the day of study entry.

†This relatively advanced age at the start of the first chelation could have been due to the availability of DFO in Italy only between the end of 1960 and the start of 1970.

‡Treatment failure was defined as an increase in ferritin levels of more than 1000 μg/l with respect to the previous values, confirmed by two separate determinations.

**Table V.** Adverse events in thalassaemia major patients during sequential deferiprone–deferoxamine *versus* deferiprone intervention during the trial.

| | Sequential DFP–DFO group | | DFP group | |
|---|---|---|---|---|
| Adverse event* | *n* | % | *n* | % |
| Agranulocytosis | – | – | 3 | 3·4 |
| Neutropenia | 15 | 23·1 | 11 | 12·5 |
| Arthralgia | 5 | 7·7 | 6 | 6·8 |
| Gastrointestinal problems | 7 | 10·8 | 16 | 18·2 |
| ↑ALT† | 22 | 33·8 | 23 | 26·1 |
| Total‡ | 49 | – | 59 | – |

Dashes indicate that there were no cases.
*Excluding agranulocytosis, the main causes of withdrawal were considered to be adverse events with an incidence rate of >4%.
†Alanine transaminase level increased greater than twofold.
‡One patient in sequential DFP–DFO and one in DFP-alone withdrew from trial for liver and pancreatic cancer respectively.

thalassaemic children, as revealed by liver biopsy after 6 months of treatment (Aydinok *et al*, 1999). Galanello *et al* (2005) compared sequential DFO (2 d/week) and DFP (5 d/week) *versus* DFO alone (5–7 d/week) in a randomized trial, and did not find any statistically significant differences between the two treatment schedules. Recently, Abdelrazik (2007) reported that sequential DFP–DFO treatment was more effective than DFO alone in terms of serum ferritin reduction ($P < 0{\cdot}001$), improvement in the myocardial function as assessed by ejection fraction ($P \leq 0{\cdot}002$) or fractional shortening ($P \leq 0{\cdot}01$), and mean urinary iron excretion ($P \leq 0{\cdot}003$) during a 1-year randomized trial (Abdelrazik, 2007). The differences in the results of this trial compared with those previously published by Galanello *et al* (2005) could be due to the higher number of patients enrolled, the difference in the number of DFO infusion days (3 d in this trial *versus* 2 d in Galanello *et al*, 2005), and the different inclusion criteria in terms of age (>13 years in this trial *versus* >10 years in Galanello *et al*, 2005), and ferritin levels (between 800 and 3000 μg/l in this trial *versus* 1000–4000 μg/l in Galanello *et al*, 2005). However, it is unlikely that this difference could be due to the use of DFP, rather than DFO as the comparator group, because 14 randomized clinical trials comparing DFP with DFO have not found any differences between the two interventions (Olivieri *et al*, 1990; Olivieri & Brittenham, 1997; Aydinok *et al*, 1999, 2007; Maggio *et al*, 2002; Gomber *et al*, 2004; Galanello *et al*, 2005; Ha *et al*, 2006; Kattamis *et al*, 2006; Pennell *et al*, 2006; Abdelrazik, 2007; Tanner *et al*, 2007, 2008; El-Beshlawy *et al*, 2008).

The greater effectiveness of associated DFP–DFO treatment, in which the administration of these chelators occurs on the same days, has been explained as the result of a transfer of chelated iron from DFP to DFO; i.e., the 'shuttle' hypothesis (Giardina & Grady, 2001; Link *et al*, 2001). However, during sequential DFP–DFO therapy, the administration of chelators is not simultaneous. Therefore, a new hypothesis is required to explain the greater reduction in serum ferritin levels by sequential DFP–DFO treatment (Giardina & Grady, 2001; Link *et al*, 2001). Probably, sequential mobilization of iron stores from different compartments could have an additive effect on overall iron excretion from the body. However, further studies are necessary to clarify this issue.

Previous reports, including natural history, retrospective and prospective non-randomized clinical trials, have suggested that mortality, due mainly to cardiac damage, was reduced or completely absent in patients treated with DFP alone (Piga *et al*, 2003; Borgna-Pignatti *et al*, 2006; Ceci *et al*, 2006), or with DFP and DFO associated chelation treatment (Telfer *et al*, 2006). The results of this trial also suggest that sequential treatment does not show differences in survival compared with DFP alone (Fig 3). During this trial, no deaths were observed in the DFP-alone group, while during sequential DFP–DFO treatment, one sudden death due to arrhythmia was registered (Fig 3 and Table IV, Patient 1). This patient experienced ventricular fibrillation and had been diagnosed with heart failure 1 year before entry into the trial. We know that the correlation between arrhythmia and heart iron burden in TM patients is controversial (Cogliandro *et al*, 2008). DFO treatment appears to account for the late deaths, the majority of which were due to heart failure (Table IV). Increases in mortality in patients treated with DFO, mainly due to heart damage, were also previously reported in retrospective and

**Table VI.** Variations in heart and liver T2* signals.

| | Time 0 | | | Time 1 | | |
|---|---|---|---|---|---|---|
| Tissue | Sequential DFP–DFO* | DFP | *P* | Sequential DFP–DFO | DFP | *P* |
| Heart T2*, ms | | | | | | |
| Global left ventricle | 20·1 ± 11·9 | 25 ± 11·3 | 0·14 | 21·8 ± 12·6 | 26 ± 11·8 | 0·26 |
| Septum | 23·5 ± 12·4 | 28·5 ± 13·3 | 0·25 | 24·2 ± 14·6 | 30 ± 13·6 | 0·21 |
| Liver T2*, ms | 4 ± 2·9 | 4 ± 5·9 | 0·97 | 4·4 ± 3·4 | 3·5 ± 4·3 | 0·5 |

T2* signals were determined at T0 and T1 in 34 and 20 patients in the DFP–DFO or DFP intervention groups respectively, during the trial (see text for T0 and T1 times).

prospective non-randomized clinical trials (Piga *et al*, 2003; Borgna-Pignatti *et al*, 2006; Ceci *et al*, 2006; Telfer *et al*, 2006).

The overall period of observation of trials using sequential DFP–DFO treatment is 370·48 person-years, including our present results (Aydinok *et al*, 1999; Galanello *et al*, 2005; Abdelrazik, 2007). The incidence of definitive withdrawal from the study in the sequential DFP–DFO-group (22.4%) was not different from that previously reported in other trials (Galanello *et al*, 2005; Abdelrazik, 2007).

No patients with agranulocytosis were reported during this period of observation. An incidence of agranulocytosis ranging from 0·4 to 0·6/100 patient-years, as was reported for DFP-alone treatment (Cohen *et al*, 2000; Ceci *et al*, 2002), could conceivably have been detected by our present trial. Therefore, these findings suggest that, during sequential treatment, the risk of agranulocytosis may be lower than with DFP-alone treatment. The cause of agranulocytosis is unknown. Potential causes of DFP-induced neutropenia include an immunological mechanism (Al-Refaie *et al*, 1993), or a maturation arrest at the level of granulocyte-monocyte colony-forming units (Al-Refaie *et al*, 1994; Vlachaki *et al*, 2007). Moreover, the higher concentration of DFP in the haematopoietic cells could be due to the higher lipophilic nature of the drug. It is likely that the reduction in the incidence of agranulocytosis during sequential treatment is due to the administration of DFP for only 4 d/week. This could have decreased the bone marrow's exposure to the drug. This hypothesis could be tested by setting up a trial with the use of DFP alone for 5–6 d/week.

Compliance was higher for DFP treatment in both arms of the trial than for DFO treatment. These findings are comparable with those reported in a total of 18 studies by Delea *et al*, (2007), suggesting that compliance with DFP is generally better (range: 79–96%) than with DFO (range: 59–78%). The cost of sequential treatment was not significantly different from that of DFP alone.

Several clinical trials have suggested a significant improvement in the T2* heart signal and the left ventricular ejection fraction (LVEF) when DFP was used alone or in association with DFO, *versus* DFO alone (Origa *et al*, 2005; Kattamis *et al*, 2006; Tanner *et al*, 2007, 2008). However, to our knowledge, changes in liver and heart T2* signals due to sequential treatment have not been reported in the literature. These findings suggest that there is no statistically significant difference between the two interventions (Table VI). This could be due to similar changes in liver and heart T2* signals due to the use of DFP in both groups, the small numbers of evaluated patients, and delays in getting liver and heart baseline T2* signal (T0 time) because of multislice–multiecho T2* MRI availability only since June 2004.

In conclusion, the trial results show that sequential DFP–DFO treatment, compared with DFP-alone treatment, significantly decreases serum ferritin concentrations during treatment for 5 years without significant differences in adverse events, costs and survival. However, longer studies are needed to define if lower ferritin levels will translate into survival benefit over decades of follow-up given that chelation is, after all, a lifelong therapeutic endeavour.

## Conflict of interest disclosure

No relevant conflicts of interest to declare. Role of funding source and ethics committee approval: the investigators initiated, carried out, and controlled the trial, which was conducted without the influence of the non-commercial sponsor. It was approved by the local ethics committee for human investigations (September 13, 2000). The patients (or the parents of minors) gave their written informed consent to participate in the trial. The trial was registered at http://www.clinicaltrials.gov, Identifier NCT00733811.

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## Author contributions

AM: coordination, design, collection and elaboration data, writing and reviewing paper; AV, GD, AM: statistical analysis; CG: reviewing paper and linguistic assistance; AP: T2* Cardiac and Liver Magnetic Resonance measurements; MC, LC, FG, AF, MAR, CM, VC, CA, CG, SC, PV, RM, PC, MR, DGD, AQ, LP, CF, GR, TL, AC, AM, FC, AC, RG, GG, MG, PR: collecting data.

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