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Evaluation of the effectiveness and safety of deferiprone compared to deferasirox in pediatric patients with transfusion-dependent hemoglobinopathies (DEEP-2): a multicenter, randomized, open label, non-inferiority, phase-III trial

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Abstract:	Background Transfusion-dependent hemoglobinopathies (TDHs) require life-long iron chelation

therapy. Randomized clinical trials comparing deferiprone (DFP) and deferasirox (DFX), in pediatric patients are lacking. The study aim was to show the non-inferiority (NI) of DFP versus DFX.
Methods
The DEEP-2 was a phase-III, multicenter, randomized, trial in well treated pediatric patients (1 month-18 years) with TDH and good clinical, receiving deferoxamine (DFO) or DFX except those <2 years. Patients were randomized 1:1 to DFP (75-100 mg/kg/day) or DFX (20-40 mg/kg/day) with dose adjustment for 12 months, stratified by <10 years and ≥10 years and balanced by country. The randomization sequence was generated into the electronic-case report form with blocks of variable size (4-6-8). Blinding was not foreseen here.
The primary efficacy endpoint (PCEE) was based on predefined success criteria for changes in serum ferritin (SF) (all patients) and cardiac MRI T2* in patients >10 years to demonstrate the NI of DFP versus DFX in the per-protocol (PP) and intent-to treat (ITT) populations (EudraCT, 2012-000353-31; ClinicalTrials.gov, NCT NCT01825512).
Findings
Overall 393 patients were randomized between March 17, 2014 and June 16, 2016 (194 DFP and 199 DFX) with a median (IQR) follow-up time of 379 (98) days for DFP and 381 (42) days for DFX. The mean age was 112·6 months (30% <6 years, 5·9% <2 years), 90·3% with thalassemia major. NI was established between DFP and DFX (55·2% vs 54·8% success, difference 0·4%, 95%CI: -11·9,12·6). No significantly difference was shown in serious and drug-related adverse events. Three cases of reversible agranulocytosis and two cases of reversible renal and urinary disorders occurred in DFP and DFX, respectively. Compliance was comparable between both drugs.
Interpretation
In pediatrics patients with TDH, DFP was effective and safety in inducing iron overloading control during 12 months treatment. Considering the needing to have availability of more chelation treatments in pediatrics population, DFP offers a valuable and safe treatment option at this age.
Funding
DEferiprone Evaluation in Pediatrics (DEEP)–FP7-HEALTH-2010 Grant Agreement n. 261483.

Evaluation of the effectiveness and safety of deferiprone compared to deferasirox in pediatric patients with transfusion-dependent hemoglobinopathies (DEEP-2): a multicenter, randomized, open label, non-inferiority, phase-III trial

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SUMMARY

Background

Transfusion-dependent hemoglobinopathies (TDHs) require life-long iron chelation therapy. Randomized clinical trials comparing deferiprone (DFP) and deferasirox (DFX), in pediatric patients are lacking. The study aim was to show the non-inferiority (NI) of DFP versus DFX.

Methods

The DEEP-2 was a phase-III, multicenter, randomized, trial in well treated pediatric patients (1 month-18 years) with TDH and good clinical, receiving deferoxamine (DFO) or DFX except those <2 years. Patients were randomized 1:1 to DFP (75-100 mg/kg/day) or DFX (20-40 mg/kg/day) with dose adjustment for 12 months, stratified by <10 years and ≥10 years and balanced by country. The randomization sequence was generated into the electronic-case report form with blocks of variable size (4-6-8). Blinding was not foreseen here.

The primary efficacy endpoint (PCEE) was based on predefined success criteria for changes in serum ferritin (SF) (all patients) and cardiac MRI T2* in patients >10 years to demonstrate the NI of DFP versus DFX in the per-protocol (PP) and intent-to treat (ITT) populations (EudraCT, 2012-000353-31; ClinicalTrials.gov, NCT NCT01825512).

Findings

Overall 393 patients were randomized between March 17, 2014 and June 16, 2016 (194 DFP and 199 DFX) with a median (IQR) follow-up time of 379 (98) days for DFP and 381 (42) days for DFX. The mean age was 112·6 months (30% <6 years, 5·9% <2 years), 90·3% with thalassemia major. NI was established between DFP and DFX (55·2% vs 54·8% success, difference 0·4%, 95%CI: -11·9,12·6). No significantly difference was shown in serious and drug-related adverse events. Three cases of reversible agranulocytosis and two cases of reversible renal and urinary disorders occurred in DFP and DFX, respectively. Compliance was comparable between both drugs.

Interpretation

In pediatrics patients with TDH, DFP was effective and safety in inducing iron overloading control during 12 months treatment. Considering the needing to have availability of more chelation treatments in pediatrics population, DFP offers a valuable and safe treatment option at this age.

Funding

DEferiprone Evaluation in Pediatrics (DEEP)–FP7-HEALTH-2010 Grant Agreement n. 261483.

INTRODUCTION

Around 7% of the global population carries an abnormal hemoglobin gene and an estimated 300,000-500,000 babies are born each year with clinically significant hemoglobinopathies, mainly beta-thalassemia, alpha-thalassemia and sickle cell disease.[1] A considerable proportion of these patients become transfusion-dependent and, being at risk of iron overload-related morbidity and mortality,[2] require life-long iron chelation therapy with one of the three iron chelators (deferiprone, DFP; deferasirox, DFX; and deferoxamine, DFO) currently available.[3,4]

Efficacy and safety data in varying age subsets of paediatric patients are available.[4] Data on DFP from a recent review[5] and from a search conducted by the authors (last updated November 1, 2016) in Pubmed,[6] clinicaltrials.gov,[7] Eudract[8] and "The European Union electronic Register of Post-Authorisation Studies" (EU PAS Register-ENCEEP)[9] indicate the availability of 23 studies[5] evaluating DFP in paediatric patients (<18 years) and two further studies including subgroup analysis by age.[5] Among these 23 studies, 14 were interventional (8 controlled, 6 non-controlled)[5] and 9 were observational.[5] Therefore, the use of DFP in paediatric patients is still limited. This is mainly attributed to lack of data from a randomized trial evaluating DFP against an 'appropriate' comparator, namely DFX, in very young children. This limitation was recognised by the European Commission, and in compliance with the Paediatric Regulation (Regulation (EC) No 1901/2006),[10] a paediatric work programme was funded: the DEEP project (DEferiprone Evaluation in Pediatrics – FP7-HEALTH-2010 Grant Agreement n. 261483).[11] The aim of DEEP was to conduct studies supporting a pediatric developmental plan, enabling a Pediatric Use Marketing Authorisation (PUMA) submission and approval.

We herein report results from the randomized, (Non-inferiority) NI, phase-III DEEP-2 clinical trial, aimed to investigate the effectiveness and safety of DFP compared with DFX in paediatric patients with TDH.

METHODS

Study design and participants

This was a phase-III, multicenter, randomized, open label, NI trial comparing DFP to DFX in pediatric patients affected by TDH (https://www.clinicaltrialsregister.eu/ctr-search/search?query=n.+2012-000353-31). The following countries participated in the study: Italy, Egypt, Greece, Albania, Cyprus, Tunisia, UK; Appendix 1 (p 2) shows the laboratory tests required to assess eligibility and wash-out period. Any previous chelation treatment was permitted for the study.

Clinical Trial Applications were submitted to each of the seven participating countries to obtain local ethical approval and Competent Authority authorization. The consent was obtained by the legal component persons (parents), according to the local legislation. Moreover, according to the local ethical committees and the age of the patient, an assent was also obtained from the patient. An age-specific booklet was distributed. The Ethics Committee approvals and Competent Authority authorisations were issued between August 2, 2012 and November 27, 2015. Eligible patients had to be between 1 month and 18 years of age, with a confirmed diagnosis of TDH and receiving at least 150 mL/kg/year of packed red blood cells. Patients could be included irrespective of the type of prior iron chelation therapy while patients naïve to iron chelation treatment had to have a SF level ≥800 ng/mL at screening. Female patients of childbearing age were required to use double-barrier contraception.

The number of screened patients was not recorded. Patients were screened and identified by the PIs among the cohort of TDH subjects requiring chronic transfusion therapy that were periodically managed at centers involved in the study.

Patients were excluded if they had: known intolerance or contraindication to either DFP or DFX; were receiving DFX at a dose >40 mg/kg/day or DFP at a dose >100 mg/kg/day at screening; platelet count <100000/μL at the wash-out visit (day -7); absolute neutrophil count <1500/μL at the wash-out visit (day -7); hemoglobin levels <8 g/dL at the wash-out visit (day -7); evidence of alanine aminotransferase [ALT] level >5 × upper limit of normal [ULN]; iron overload from causes other than transfusional haemosiderosis; heart failure or severe arrhythmia or myocardial T2* <10 ms; creatinine level >ULN for age at the wash-out visit (day -7); history of significant medical or psychiatric disorder; received another investigational drug within 30 days prior to consent to study participation; fever or other

signs/symptoms of infection at the wash-out visit (day -7); concomitant use of trivalent cation-dependent medicinal products or a positive test for beta-HCG or lactating female patients.

Randomization and masking

Patients, randomized in a 1:1 ratio to DFP or DFX, were stratified into two groups according to age (<10 years and ≥10 years, considering their different capabilities in undergoing cardiac MRI T2*). Randomization was centralized and balanced by country. The randomization sequence was generated directly into the electronic-case report form (e-CRF) with blocks of variable size (4-6-8) and random seeds to ensure that allocation concealment could not be violated by guessing the allocation sequence at the end of each block. No fixed number of patients per age group was specified and 10% of the patients were required to be <6 years of age. Blinding was not foreseen for this trial because of the different pharmaceutical forms and posology of investigational medicinal products which would have heavily impacted on the study feasibility.

Procedures

DFP (ApoPharma – Toronto, Canada) was administered orally, daily at 75-100 mg/kg/day. It was formulated in a new 80 mg/mL oral solution packaged in 250 mL bottles, using an administration device to ensure accurate measurement of dose volumes. The trial opened to children less than 6 years of age (10% of the total sample size) after dosing was confirmed by the results of the DEEP-1 pharmacokinetics (PK) Study.[12] DFX (Novartis, Basel, Switzerland) was administered as dispersible tablets at 125 mg, 250 mg and 500 mg. DFX daily dosage ranged from 20 to 40 mg/kg/day as recommended in the Summary of Product Charactheristics (SmPC).[13] Dose adjustments were allowed for efficacy (scaling up) or for safety reasons including over-chelation (scaling down). If SF increased by >20% compared with the previous determination, or remained >1500 ng/mL (no increase or any increase <20%) in the absence of a downtrend over a 3-month-period, DFP could be scaled up in steps of 12·5 mg/kg/day (to a maximum daily dose of 100 mg/kg) and DFX in steps of 5 to 10 mg/kg (to a maximum daily dose of 40 mg/kg). DFP or DFX could be adjusted for safety reasons including: creatinine increased by >33% from baseline or decrease in creatinine clearance (only for DFX); urine protein/creatinine ratio ≥0·5 in two consecutive measurements (only for DFX); ALT or aspartate aminotransferase (AST) >10 ULN (for both); severe skin rash (only DFX); SF level ≤500 ng/mL (for both); neutropenia (neutrophil

count <1500/μL and ≥1000/μL in two consecutive measurements) (for both); infection (for both); arthralgia (for both); nausea/abdominal pain/vomiting (for both).

Reasons leading to withdrawals were serious adverse events (SAEs), consent/assent withdrawal, lost to follow-up, and significant protocol violations, moderate neutropenia (neutrophil count <1000/μL but >500/μL) or severe neutropenia/agranulocytosis (neutrophil count <500/μL), or any other event leading to drug suspension for more than 4 weeks. After a 28 day washout period, the treatment period lasted 12 months. Monthly visits were performed.

Patient compliance was estimated from e-CRF data and each patient was evaluated in terms of percentage of compliance. In cases where treatment compliance could not be automatically calculated, a case-by-case evaluation was made on the basis of the difference between the amount of drug that should have been returned and that actually returned. Compliance was defined appropriate if percentage of prescribed therapy taken was ≥80%. Assessments were performed in four phases: 1) run-in including screening from -28 to -7 days and wash-out from -7 to -1 days; 2) baseline (day 0) at randomization and clinical evaluation; 3) treatment, one visit per month for 12 months; and 4) follow-up at month 13 (Appendix 1, p 2). SF levels were analyzed monthly at local and central laboratories. LIC was measured at baseline and 12 months by hepatic R2 MRI (Ferriscan®). Cardiac T2* MRI was measured at baseline, 6 months and 12 months. Children ≥10 years old who did not need sedation had LIC R2-MRI and cardiac T2* assessments. The cardiac T2* protocol included analysis of full-thickness region of interest in the left ventricular septum.[14] LIC R2-MRI was based on protocol described by St Pierre et al.[15] MRI evaluations were centralized at Resonance Health, Perth, Australia. Full blood counts were done weekly for patients in both arms of the study for early detection of neutropenia and agranulocytosis. AEs were collected at every monthly visit in the e-CRF and reported to a pharmacovigilance system. SAEs were reported within 24 hours of the awareness of the event. Assessment of severity for each AE/SAE was performed using the following categories: *mild*, an event that was easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities; *moderate*, an event that was sufficiently discomforting to interfere with normal everyday activities; *severe*, an event that prevented normal everyday activities. Neutropenia <1000/μL and creatinine increase or reduction in creatinine clearance were considered relevant safety concerns and subjected to special monitoring and recording.

Data were entered directly into the e-CRF or indirectly from source data documents. All data collected were reviewed for completeness and accuracy. Any query was solved using an electronic data query system. Any deviations from the protocol, such as failure to obtain patient assent or parent consent, failure of SF tests, or reasons related to the non compliance with study requirements, have been recorded during the trial.

Laboratory samples were processed centrally and all results recorded electronically in the e-CRF. Sites were regularly monitored for patient records, accuracy of entries on e-CRFs, adherence to the protocol and to Good Clinical Practice (GCP), progress of enrolment and monitoring that study medication was being stored, dispensed and accounted for were even performed. The dropout rate was increased from 10% to 20% with a protocol amendment on December 10, 2015, increasing the number of enrolled patients from 344 to 388.

Outcomes

The primary composite efficacy endpoint (PCEE) required both SF and myocardial T2* criteria to be met and was defined according to patient's age, as follows: - in patients < 10 years of age treatment success is defined only in terms of SF level; - in patients ≥ 10 years of age treatment success is defined in terms of both SF level and cardiac MRI T2*. In patients ≥10 years of age who would require sedation for the MRI scan, treatment success is defined only in terms of SF level.

The criteria for definition of treatment success were based on SF levels: if baseline SF level was ≥2500 ng/mL, reduction of 20% or more after 1 year treatment, while if baseline SF level was <2500 ng/mL any decrease or an increase <15% as long as the increase does not result in SF levels ≥2500 ng/mL; Myocardial T2*: if baseline of T2* was <20 ms increase of 10% or more after 1-year treatment, while if baseline of T2* was >20 ms any increase or a decrease <10% after 1-year treatment as long as the decrease does not result in myocardial T2* value <20 ms. Baseline SF and myocardial T2* were considered at randomization visit (Visit 3). The PCEE required both SF and myocardial T2* criteria to be met.

Secondary endpoints: these included changes in SF level, myocardial T2*, and LIC from baseline to end of study, safety, pharmacokinetic, QoL and compliance. Treatment success by LIC was also assessed and defined as LIC <7 mg/g at end of treatment.

Pharmacokinetic and QoL data have not been reported in this paper since they deserve publication in different medical journal. The paper with these data is under publication.

Statistical analysis

As per the EMA Guideline E9[16] the PP population was considered the primary basis for the investigation of the NI hypothesis. The PP includes all subjects that have received the study drugs and for whom the PCEE measures were available at baseline and after 1 year of treatment, without major protocol violations. PP populations include: 1) PP1 patients in which the PCEE was available at baseline and after 1-year of treatment; 2) PP2 patients in which the per-protocol centralized SF level were available at baseline and after 1 year of treatment. This population was more represented in comparison with PP1 because it included patients who did not perform myocardial T2*; 3) PP3 patients in which LIC and myocardial T2* were available at baseline and after 1 year of treatment.

The ITT population included all patients randomized that received at least one dose of study medications.

The primary efficacy end-point analysis was based on the PCEE in the PP1 and in the analysis of ITT population. In the ITT population patients who prematurely discontinue the trial for safety reasons, detailed in the protocol, were considered as treatment failures. In all other suspended patients, according to EMA Guidelines for missing data[17], the last-observation-carried-forward (LOCF) methodology was applied, as imputation method, to SF levels. Myocardial T2* determinations were not included in LOCF analysis because of low number of withdrawn patients having almost one post baseline MRI. A complementary ITT analysis, not including handling of missing data, was also conducted. This analysis considered all patients that prematurely discontinued the trial as treatment failures. The primary and secondary end-points analyses were corrected for country level by Generalized Linear Modeling (GLM). The country level was introduced in the statistical model as factor. By using this procedure, it is possible to test the null hypothesis for the effects of covariates on the means of grouping variables (factors) of a single dependent variable.

NI of the PCEE, in the PP1 population, was based on the 2-sided 95% confidence interval (CI_L, CI_U) of the difference in the success rate between the two arms and was established if the CI_L was greater than -0·125. The choice of this NI margin was based on clinical considerations of the available evidence regarding the effects of DFP and DFX on SF levels and myocardial iron overload.[18-21]

GLM was used for evaluation of SF levels and cardiac T2*. Concerning SF levels, NI was established if the 95% CI of the difference DFX-DFP was less than 400 ng/mL between

baseline and end of study. SF levels were compared between the two groups at each study visit using ANOVA (one-way analysis-of-variance).

Cardiac T2* and LIC data were analyzed using GLM, with cardiac T2* and LIC changes from baseline as dependent variables, the treatment group as predictor variable.

Means were reported with standard deviations (SD). Proportions and differences between proportions were reported with 95% CI. Continuous scale values were compared between the two intervention groups by a paired t-test. A p-value of 0·05 was considered statistically significant. The minimum level of statistical significance was set at 5% (two-sided). Differences in proportions observed on contingency tables were assessed by chi-square analysis. Statistical analyses were performed using SPSS version 21.0 (IBM Clinical Software). All statistical analyses were performed under code at Biostatistics and Data Management Unit, Medi Service, Genoa (Italy) by a biostatistician (G.R.) blinded to the trial interventions. This study is registered on EudraCT, 2012-000353-31 and on ClinicalTrials.gov, NCT NCT01825512.

Sample size of 310 patients, randomized in a 1:1 ratio, was considered appropriate to show NI of DFP versus DFX based on a 95% CI_L NI margin of -0·125 with 80% power and one sided test with type I error of 0·025. However, anticipating a possible 20% dropout rate, an overall enrolment of 388 patients, aged from 1 month to less than 18 years, was planned.

Role of funding source

The sponsor had role in the study design, in the collection, analysis, interpretation of the data and in the writing of the report. MF, AC, BT, DB, GR had access to the raw data. The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit.

RESULTS

Overall 393 patients were randomized at 21 centers in 7 countries (194 to DFP and 199 to DFX) between March 17, 2014 and June 16, 2016 (Appendix 1 p 4). The mean (SD) age at randomization was 112·6 months, while 117 patients (30%, 117/390) were <6 years, including 23 patients (5·9%, 23/390) <2 years. TDH included 352 patients with beta-thalassemia major (90·3%, 352/390), 27 (6·9%, 27/390) with sickle cell disease, 5 (1·3%, 5/390) with sickle cell/beta-thalassemia, and 6 (1·5%, 6/390) with other hemoglobinopathies.

The mean (SD) age at diagnosis was lower in patients receiving DFP than DFX (11·5 [9·68] versus 15·7 [21·37] months, p = 0·01). Mean age at first transfusion, age at first chelation, and the time interval from diagnosis to first transfusion were not statistically different between DFP and DFX (Table 1). Other baseline characteristics of the study population are shown in Appendix 1 (p 5).

Figure 1 illustrates the CONSORT diagram of the study including patients allocated to the two study arms. Forty-two patients were enrolled but not randomized (N=17 did not meet inclusion criteria, N= 5 withdrew consent, N=20 lost to follow-up). The diagram shows the total number of patients evaluated in the PP1 (DFX = 146/199; DFP = 125/194), PP2 (DFX = 166/199, DFP = 137/194), PP3 (DFX = 61/199, DFP = 50/1194) and ITT (DFX = 193/194; DFP = 197/199) populations. Three patients were excluded from the efficacy analysis due to not taking study medication (DFP= 1; DFX=2).

Table 2 shows results of the primary and secondary efficacy endpoints. The PCEE was successfully reached in 55·2% (69/125) and 54·8% (80/146) of the DFP and DFX arms, respectively. The difference between the two percentages (DFP – DFX) was 0·4% (95%CI: -11·9, 12·6), which is consistent with NI for DFP compared to DFX. Baseline (Visit 3) SF levels sub-group analysis in the PP population is shown in Appendix 1 (p 6). The age at diagnosis had no significant effect on baseline SF (p = 0·44) and cardiac T2* values (p = 0·61).

Table 2 shows ITT analysis where LOCF was applied, with imputation of 104 missing data, (26·7 %, 104/390), NI was also obtained between DFP versus DFX (-1·7% (95%CI: -12·1, 8·6)). NI was not shown in the ITT analysis where LOCF was not applied (-9·4%; 95% CI: -19·4, 0·9). NI was also maintained (3·2%; 95%CI: -13·0, 19·1) in the 153 children who were <10 years old. No statistically significant difference was shown between the two treatment groups in 84 (21·5%, 84/390) patients <6 years of age (44 in DFX and 40 in DFP, p = 0·76). NI was not reported because of the low number of patients.

Table 2 shows, in the PP2 population, the mean change in SF between baseline and end of study was -397·6 ng/mL (2468 to 2120 ng/mL) and -398·2 ng/mL (2822 to 2328 ng/mL) in the DFP and DFX arms, respectively (mean difference 0·60, 95%CI: -323·6, 324·8). No statistically significant difference between the two treatment arms (Figure 2; Appendix 1 p 7) was supported by analysis of SF changes from baseline by treatment and by study visit.

North Africa versus Europe post-hoc analysis for change of SF between baseline and end of study was not significantly different (p = 0·53). The percentage of treatment success, based on SF levels, was similar (Appendix 1 p 8).

The PP3 population showed a mean change in cardiac T2* (mean difference -0·6 ms, 95%CI: -4·1, 2·8) and LIC (mean difference 2·1 mg/g, 95%CI: -0·21, 4·5) from baseline to end of study (Table 2) that were not statistically significant different. This included patients >10 years old (n=111). Treatment success by LIC (PP3, n=106, liver MRI was not available for 5 patients) at end of study was similar between both groups (41%, 19/46 DFP vs 48%, 29/60 DFX, p=0·47).

AE evaluation is shown in Appendix 1 (p 9). There were 450 AEs reported in the DFP arm and 416 in DFX, 151 and 71 being drug-related (p <0·001). Among these, 14 AEs in DFP and 21 in DFX were graded as serious, drug-related being 9 for DFP (3 agranulocytosis, 2 hypertransaminasemia, 1 pneumonia, 2 neutropenia, and 1 seizure) and 3 for DFX (1 acute renal failure, 1 gastroenetritis, and 1 hypertransaminasemia).

Table 3 shows AEs by causation. Arthralgia and gastrointestinal disturbance were common in DFP and renal function abnormalities in DFX. Monitoring of neutrophil count revealed values <1500/μL in 12·4%, 24/193 and 13·7%, 27/197 in DFP and DFX, respectively. Twenty-eight cases in 23 DFP-treated patients and 15 cases in 11 DFX-treated patients were reported by physicians to the pharmacovigilance system. Neutropenia had a global incidence rate of 10·4% (20/193) in DFP and 5·6% (11/197) in DFX (p=0·08). Neutropenia, considered drug-related, was reported in 82·1% (23/28) in DFP vs 13·3%(2/15) in DFX.

Mild or moderate neutropenia were reported after 127 (SD 96·1) and 101 (SD 85·7) days from treatment with either DFP or DFX, respectively. Three patients, treated with DFP and not included in the neutropenia analysis, experienced agranulocytosis (neutrophil values <500/μL). Overall 77 patients were withdrawn (51 in DFP and 26 in DFX). A Kaplan-Meier analysis of withdrawal events is shown in Appendix 1 (p 17).

Reasons for withdrawal are shown in Appendix 1 (p 15). More discontinuations, due to non-SAE and not mandated by the protocol, were observed in the DFP arm (11 cases corresponding to arthralgia, joint effusion, nausea, abdominal discomfort, fatigue, joint swelling, epistaxis, upper respiratory tract infections, abdominal pain upper, vomiting, palpitation in DFP versus 1 case of pyrexia in DFX arm).

Compliance was not significantly different (p=0·07) and appropriate in 183 [mean (SD)=92·0% (17·35), median (IQR)=93·3% (13·6)] and 192 [mean (SD)=95·3% (18·56), median (IQR)=96·8%, (11·1)] patients in DFP and DFX, respectively. The mean (SD) of treatment days was 319·7 (116·87) versus 344·8 (93·55) for DFP and DFX and the median (IRQ) follow-up time was 379 (98) days for DFP and 381 (42) days for DFX patients. Overall mean daily doses of DFP and DFX are shown in Appendix 1 (p 16).

DISCUSSION

The study showed that NI was established between DFP and DFX. Changes in SF, cardiac T2*, and LIC from baseline to end of study were comparable. SAEs and drug-related events were not statistically different between the two groups, and comparable to the adult population. Neutropenia occurrence was not significantly different between the two groups. Three reversible cases of agranulocytosis and two cases of reversible renal and urinary disorders were shown in DFP and DFX arm, respectively. Compliance was comparable between both drugs.

DEEP-2 is the largest randomized clinical trial on oral iron chelation in the paediatric population, generating clinically applicable data that were previously lacking, including populations in North Africa such as Egypt and Tunisia where TDH is common.[22]

The design of the study aimed at detecting liver and heart iron overload, to address the controversial results reported on myocardial iron overload in children.[23] SF levels and not LIC were selected as a primary endpoint, since the use of LIC-R2 together with myocardial T2* may lead to higher drop-outs in view of the difficulty in performing pediatric MRI. The study showed that treatment with DFP was not inferior to DFX, in patients who completed 12 months of treatment, for all the parameters evaluated (namely changes in SF, LIC, and myocardial T2*). The NI was also shown when a population <10 years was considered and treatment success was comparable in patients aged <6 years. No additional safety concerns appeared in very young children, suggesting that DFP is safe at the same dosage of adults.

The ITT population where LOCF was applied confirmed these results. NI was not demonstrated in the complementary ITT analysis where LOCF method was not applied. This may be since more patients on DFP discontinued treatment for non-serious AE compared with DFX. This could have a significant ‘bias’ on the efficacy evaluation of the ITT population if all discontinuations are considered treatment failures. In fact, the investigators clinical decision to withdraw a patient seems to have been based on their perception of the

risks associated with a treatment group rather than to strictly adhering to the recommendations of the protocol. This effect (quite expected in similar groups of patients) has been taken into account at the regulatory level leading to the recommendation in the EMA Guidelines on Missing Data (2010)[17] and has been avoided in our analysis where the LOCF methodology was adopted.

Countries representation in this study, even outside of Northern Africa, was similar to that in the phase-III study of DFX making our findings similarly generalizable.[24]

Many patients had a high iron burden at the beginning of the study, partially reflecting inadequate previous chelation history. Chelation dosages were initiated at low levels, focusing mainly on safety, and were adjusted slowly and with stringent criteria throughout the study. Given that chelation efficacy is dose-dependent, these factors prevented a more rapid decline of iron. Dose adjustment with the goal of reaching a maximum tolerable dose may be required in young patients with TDH. Indeed, a multiparametric survey of myocardial and liver iron overload by T2* together with SF level monitoring in 107 pediatric TDH patients in Italy (median age 14·4 years), showed that 21·4% had significant myocardial iron overload, high SF levels (>2000 ng/mL) and LIC (>14 mg/g).[25] This further supports our observation that a TDH cohort with severe iron burden, necessitating chelation treatment optimization, is still present in Western countries.

The first limitation of this study is that liver iron overload was measured as a secondary endpoint and was not included in the composite primary endpoint. Since patients with TDH rarely show myocardial iron overload before the age of ten,[23] the primary endpoint might have been more accurate if it had also included LIC. Second is, the significantly higher discontinuation of DFP versus DFX (Appendix 1 p 15), despite similar occurrence of non-SAEs (Appendix 1 p 15). This reflects varying physician perception on the etiological relationship between AEs and treatment. This is reasonable, considering that research in children should strike the right balance between protecting underage study subjects and advancing the medical field to the benefit of all children, and remains the reason why drug innovations are often limited in children.[26] This, together with the difficulty of having cardiac T2* in children, was the reason why the number of patients in the PP group was fewer than planned. Third, reduction in LIC values was non-significantly higher in DFX compared with DFP. LIC values >7mg/g have the best response to DFX.[27] Therefore, the higher number of patients with LIC >15mg/g in DFX than DFP may explain better response to DFX.[27] Long-

term follow-up is necessary to evaluate the difference in reduction of LIC between DFP and DFX.[28] Finally, because of the low representation of sickle cell disease studies including higher numbers are called for.

In conclusion, our trial supports the use of DFP in pediatric patients with TDH based on data from the largest randomized clinical trial of iron chelation therapy in these patients.

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Authors Contribution: Study conception and design: AC, AM, AK and all other authors. Acquisition and assembly of data: MF, GR, BT, AV. Analysis and interpretation of data: MF, AC, BT, DB, GR. Drafting of the manuscript: AM, AK, AC, MF. Critical revision of the manuscript for important intellectual content: PT, MS, FT, YCT, OD. Statistical analysis: GR. All authors gave final approval of the manuscript for submission.

Data Access and Responsibility: AM and AK had full access to all the data in the study and take responsibility for the accuracy of the data analysis. GR and MF made the analysis and are responsible of the integrity of the data. All authors had final responsibility for the decision to submit for publication.

Conflict of Interest: AM is a member of Novartis and Bluebird bio Advisory Boards, AK reports grants from FP7 - EUROPEAN UNION, during the conduct of the study; grants and personal fees from NOVARTIS ONCOLOGY, personal fees from APOPHARMA, VERTEX, VIFOR, and IONIS, personal fees and non-financial support from CELGENE, outside the submitted work; GR reports grants from CVBF, during the conduct of the study; grants from Medi Service, outside the submitted work; ODP is an employees of Glaxo Smith Kline; RO is a member of Novartis and Bluebird bio Advisory Boards; RO declares Speaker's honoraria and consultant fee for Novartis, and Consultant fee for BlueBird Bio, outside the submitted work; MS reports grants from European Commission, during the conduct of the study, other from ApoPharma Inc. outside the submitted work, and he has a patent WO 2009/129592 Al issued; PT reports personal fees from ApoPharma, out of the submitted paper; YCT and FT are employees of Apotex Inc; MF, GR, AE-B, MB, LS, SC, CC, GCDV, AF, LC, HH, MK, MCP, BT, AV, AZ, DB, AC have no competing financial interests to disclose.

DATA SHARING STATEMENT

1) The data will be made available to others;
2) The indentified participant data will be made available;
3) Related documents will be available (statistical analysis plan and informed consent);
4) These data will be available from data of publication of this paper until 6 months after;
5) The data will be available writing to this e-mail address: md.amaggio@gmail.com;
6) The access criteria to data will be the approval of a proposal with a signed data access agreement.

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Research in Context

Evidence before this study

Patients with TDH require life-long iron chelation therapy, with three iron chelators currently available. Efficacy and safety data for the use of deferoxamine (DFO), deferasirox (DFX) and deferiprone (DFP) in varying age subsets of pediatric patients are available. However, despite the evidence, the use of DFP in pediatric patients, especially very young children, is still limited. This is mainly attributed to lack of data from a randomized trial evaluating DFP against an 'appropriate' comparator, namely DFX, in pediatric patients including very young children. This limitation was recognised by the European Commission, and in compliance with the Pediatric Regulation (Regulation (EC) No 1901/2006), a pediatric work programme was funded: the DEEP project (DEferiprone Evaluation in Pediatrics – FP7-HEALTH-2010 Grant Agreement n. 261483). We searched PubMed, clinicaltrials.gov, Eudract and "The

European Union electronic Register of Post-Authorisation Studies" (EU PAS Register-ENCEEP). (November 1, 2016). DFP efficacy was derived from 14 interventional studies and 2 observational studies. However, these studies showed methodological limitations including low number of patients, duration of the study less than 1 year or undetermined, problems with randomization, different standard methods used for liver and cardiac MRI. To our knowledge the effectiveness of DFP, in pediatric age group, has not been previously studied in a large phase-III, multicenter, randomized, non-inferiority (NI) trial and we sought to investigate this in DEEP-2 study.

Added value of this study

This study fills, for the first time, the gap in data, on the effectiveness and safety of DFP in the pediatric population, through a large randomized clinical trial in 7 countries. Effectiveness and safety were similar between DFP and DFX, although with significantly higher discontinuation of DFP for non-SAE.

Implications of all the available evidence

Using data from a large, 1-year, phase-III, multicenter, randomized, NI trial, our results show that DFP has an effectiveness and safety profile similar to that of DFX in pediatric patients using the same dosage as that of adults. This is provided patients do not discontinue treatment. This may have the implication of a wider evidence-based use of DFP in the paediatric age group. These data have to be confirmed in a long-term follow-up that extends beyond 1 year.

FIGURE LEGENDS

Figure 1. CONSORT flow diagram.

Figure 2. Centralized serum ferritin (ng/mL) change from baseline by treatment arm and study Visit, (PP-2, N = 303).

Figure 1

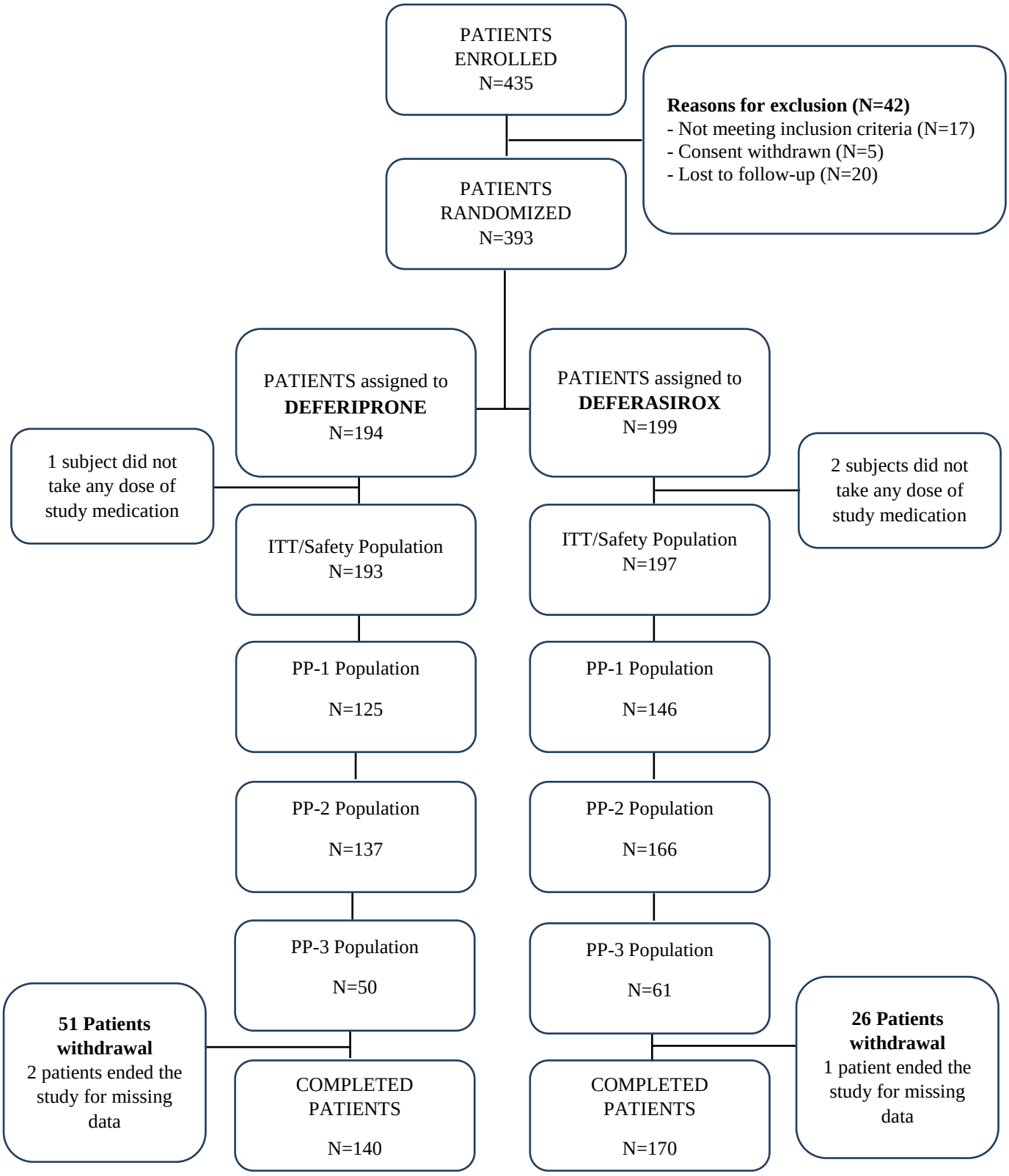
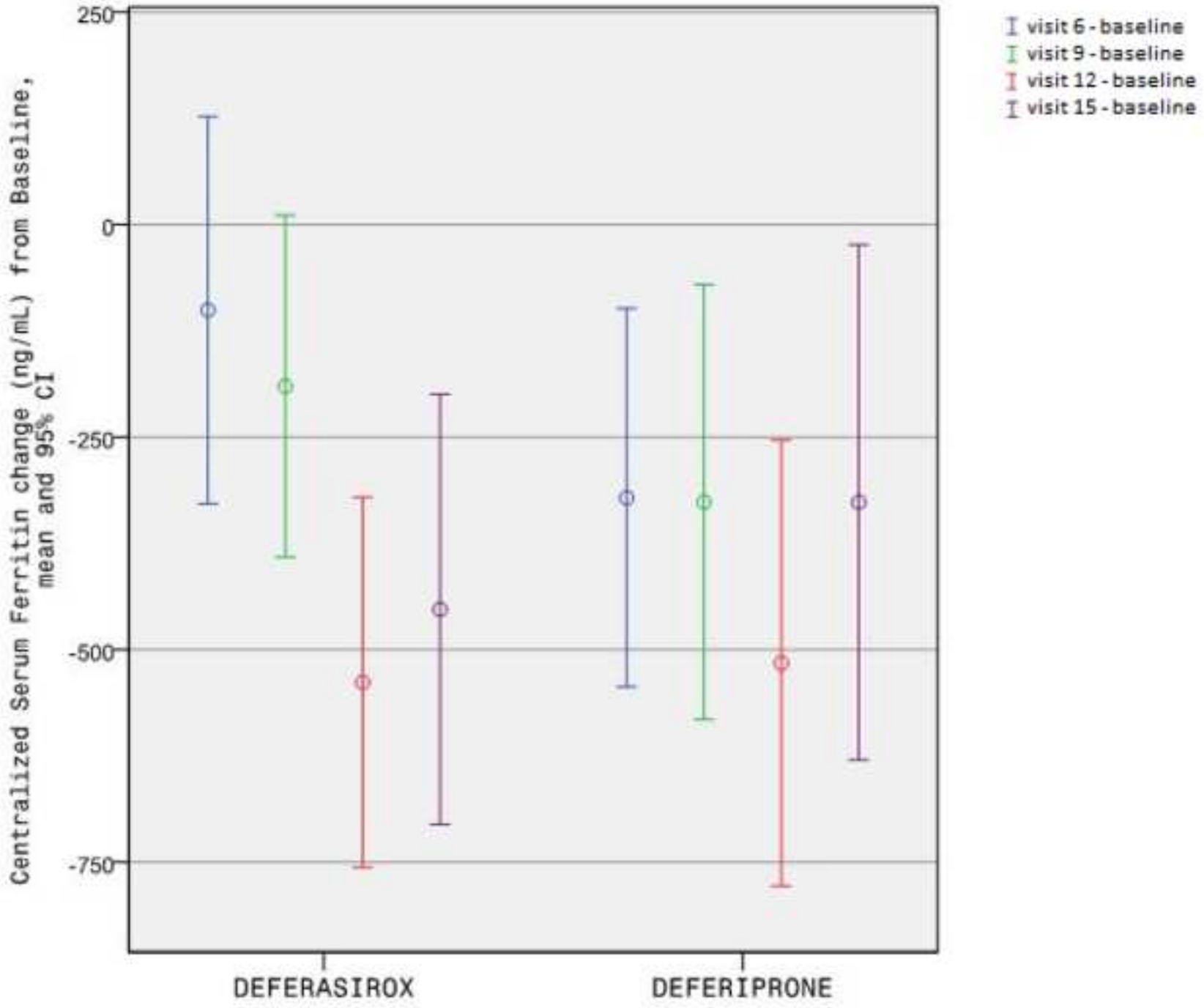

PATIENTS ENROLLED N=435
Reasons for exclusion (N=42)
- Not meeting inclusion criteria (N=17)
- Consent withdrawn (N=5)
- Lost to follow-up (N=20)
PATIENTS RANDOMIZED N=393
PATIENTS assigned to DEFERIPRONE N=194
PATIENTS assigned to DEFERASIROX N=199
1 subject did not take any dose of study medication
2 subjects did not take any dose of study medication
ITT/Safety Population N=193
ITT/Safety Population N=197
PP-1 Population N=125
PP-1 Population N=146
PP-2 Population N=137
PP-2 Population N=166
PP-3 Population N=50
PP-3 Population N=61
51 Patients withdrawal
2 patients ended the study for missing data
26 Patients withdrawal
1 patient ended the study for missing data
COMPLETED PATIENTS N=140
COMPLETED PATIENTS N=170

Figure 2



TABLES

Table 1. Demographic characteristics.

Baseline Demographic	DFP N = 193	DFX N = 197	Total N = 390
Gender, n (%)			
Female	80 (41·5)	93 (47·2)	173 (44·4)
Male	113 (58·5)	104 (52·8)	217 (55·6)
Age (years), n (%)			
<6	59 (30·6)	58 (29·4)	117 (30·0)
≥6 and <10	47 (24·4)	47 (23·9)	94 (24·1)
≥10	87 (45·1)	92 (46·7)	179 (45·9)
Disease, n (%)			
beta-thalassemia major	175 (90·7)	177 (89·8)	352 (90·3)
Sickle cell disease	12 (6·2)	15 (7·6)	27 (6·9)
sickle cell/beta-thalassemia	3 (1·6)	2 (1·0)	5 (1·3)
Other hemoglobinopathy	3 (1·6)	3 (1·5)	6 (1·5)
Naïve to chelation, n (%)			
Yes	27 (14·0)	27 (13·7)	54 (13·8)
No	166 (86·0)	170 (86·3)	336 (86·2)
Age at first chelation (months), mean (SD)	46·8 (36·2)	49·4 (37·1)	48·1 (36·6)
Age at first transfusion (months), mean (SD)	15·6 (21·3)	18·8 (26·9)	17·2 (24·2)

Three patients that did not take any study medicine dose are not included.

Table 2. Primary and secondary efficacy endpoints.

End point	Variable	Population	Result DFP - DFX (95%CI)	p-values
Primary Composite Efficacy DFX = 146; DFP = 125 Total = 271	Treatment success (%)	PP1	55·2 – 54·8 = 0·4 (-11·9, 12·6)	0·79
Primary Composite Efficacy DFX = 197; DFP = 193 Total = 390	Treatment success (%)	ITT [1]	52·2 – 53·9 = -1·7 (-12·1, 8·6)	0·73
Primary Composite Efficacy DFX = 197; DFP = 193 Total = 390	Treatment success (%)	ITT	38·8 – 48·2 = -9·4 (-19·4, 0·9)	0·06
Secondary SF DFX = 166; DFP = 137 Total = 303	SF mean change from baseline (ng/mL)	PP2	-397·6 – -398·2 = 0·6 (-323·6, 324·8)	0·99
Secondary Myocardial T2* DFX = 61; DFP = 50 Total = 111	Myocardial T2* mean change from baseline (ms)	PP3	0·5 – 1·1 = -0·6 (-4·1, 2·8)	0·71
Secondary LIC DFX = 60; DFP = 46 Total = 106[2]	LIC mean change from baseline (mg/g)	PP3	-0·9 – -3·0 = 2·1 (-0·2, 4·5)	0·07

[1]Last observation carried forward (LOCF) analysis.
[2] Liver MRI was not available for 5 patients.
CI, confidence interval; DFP, deferiprone; DFX, deferasirox; SF, serum ferritin; LIC, liver iron concentration.

Table 3. Patients with at least one Adverse Event (ITT population, N = 390).

	DFP N (%)	**DFX N (%)**	**p-value**
Number of Patients in the Safety Population	**193**	**197**	**-**
SAE	13* (6·7)	14** (7·1)	0·88
Neutropenia	18 (9·3)	11 (5·6)	0·15
Arthralgia	23 (11·9)	5 (2·5)	< 0·001
Abnormal renal function	1 (0·5)	14 (7·1)	< 0·001
Abnormal liver function	9 (4·7)	12 (6·1)	0·53
Other laboratory abnormality	19 (9·8)	28 (14·2)	0·18
Gastrointestinal problems	51 (26·4)	20 (10·2)	< 0·001
Other non-serious events	44 (22·8)	72 (36·5)	0·003

Data reported as n (%) of patients.
*Included 6 SARs.
**Included 4 SARs.
SAE, serious adverse event; SAR, serious adverse reaction; DFP, deferiprone; DFX, deferasirox.

Check list Consort

Evaluation of the effectiveness and safety of deferiprone compared to deferasirox in pediatric patients with transfusion-dependent hemoglobinopathies (DEEP-2): a multicenter, randomized, open label, non-inferiority, phase-III trial

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SUMMARY

Background

Transfusion-dependent hemoglobinopathies (TDHs) require life-long iron chelation therapy. Randomized clinical trials comparing deferiprone (DFP) and deferasirox (DFX), in pediatric patients are lacking. The study aim was to show the non-inferiority (NI) of DFP versus DFX.

Methods

The DEEP-2 was a phase-III, multicenter, randomized, trial in well treated pediatric patients (1 month-18 years) with TDH and good clinical, receiving deferoxamine (DFO) or DFX except those <2 years. Patients were randomized 1:1 to DFP (75-100 mg/kg/day) or DFX (20-40 mg/kg/day) with dose adjustment for 12 months, stratified by <10 years and ≥10 years and balanced by country. The randomization sequence was generated into the electronic-case report form with blocks of variable size (4-6-8). Blinding was not foreseen here.

The primary efficacy endpoint (PCEE) was based on predefined success criteria for changes in serum ferritin (SF) (all patients) and cardiac MRI T2* in patients >10 years to demonstrate the NI of DFP versus DFX in the per-protocol (PP) and intent-to treat (ITT) populations (EudraCT, 2012-000353-31; ClinicalTrials.gov, NCT NCT01825512).

Findings

Overall 393 patients were randomized between March 17, 2014 and June 16, 2016 (194 DFP and 199 DFX) with a median (IQR) follow-up time of 379 (98) days for DFP and 381 (42) days for DFX. The mean age was 112·6 months (30% <6 years, 5·9% <2 years), 90·3% with thalassemia major. NI was established between DFP and DFX (55·2% vs 54·8% success, difference 0·4%, 95%CI: -11·9,12·6). No significantly difference was shown in serious and drug-related adverse events. Three cases of reversible agranulocytosis and two cases of reversible renal and urinary disorders occurred in DFP and DFX, respectively. Compliance was comparable between both drugs.

Interpretation

In pediatrics patients with TDH, DFP was effective and safety in inducing iron overloading control during 12 months treatment. Considering the needing to have availability of more chelation treatments in pediatrics population, DFP offers a valuable and safe treatment option at this age.~~has comparable effectiveness and safety to DFX in the whole paediatric TDH~~

~~population, including younger children.~~

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DEferiprone Evaluation in Pediatrics (DEEP)–FP7-HEALTH-2010 Grant Agreement n. 261483.

INTRODUCTION

Around 7% of the global population carries an abnormal hemoglobin gene and an estimated 300,000-500,000 babies are born each year with clinically significant hemoglobinopathies, mainly beta-thalassemia, alpha-thalassemia and sickle cell disease.[1] A considerable proportion of these patients become transfusion-dependent and, being at risk of iron overload-related morbidity and mortality,[2] require life-long iron chelation therapy with one of the three iron chelators (deferiprone, DFP; deferasirox, DFX; and deferoxamine, DFO) currently available.[3,4]

Efficacy and safety data in varying age subsets of paediatric patients are available.[4] Data on DFP from a recent review[5] and from a search conducted by the authors (last updated November 1, 2016) in Pubmed,[6] clinicaltrials.gov,[7] Eudract[8] and "The European Union electronic Register of Post-Authorisation Studies" (EU PAS Register-ENCEEP)[9] indicate the availability of 23 studies[5] evaluating DFP in paediatric patients (<18 years) and two further studies including subgroup analysis by age.[5] Among these 23 studies, 14 were interventional (8 controlled, 6 non-controlled)[5] and 9 were observational.[5] Therefore, the use of DFP in paediatric patients is still limited. This is mainly attributed to lack of data from a randomized trial evaluating DFP against an 'appropriate' comparator, namely DFX, in very young children. This limitation was recognised by the European Commission, and in compliance with the Paediatric Regulation (Regulation (EC) No 1901/2006),[10] a paediatric work programme was funded: the DEEP project (DEferiprone Evaluation in Pediatrics – FP7-HEALTH-2010 Grant Agreement n. 261483).[11] The aim of DEEP was to conduct studies

supporting a pediatric developmental plan, enabling a Pediatric Use Marketing Authorisation (PUMA) submission and approval.

We herein report results from the randomized, (Non-inferiority) NI, phase-III DEEP-2 clinical trial, aimed to investigate the effectiveness and safety of DFP compared with DFX in paediatric patients with TDH.

METHODS

Study design and participants

This was a phase-III, multicenter, randomized, open label, NI trial comparing DFP to DFX in pediatric patients affected by TDH (https://www.clinicaltrialsregister.eu/ctr-search/search?query=n.+2012-000353-31). The following countries participated in the study: Italy, Egypt, Greece, Albania, Cyprus, Tunisia, UK; Appendix 1 (p 2) shows the laboratory tests required to assess eligibility and wash-out period. Any previous chelation treatment was permitted for the study.

Clinical Trial Applications were submitted to each of the seven participating countries to obtain local ethical approval and Competent Authority authorization. The consent was obtained by the legal component persons (parents), according to the local legislation. Moreover, according to the local ethical committees and the age of the patient, an assent was also obtained from the patient. An age-specific booklet was distributed. The Ethics Committee approvals and Competent Authority authorisations were issued between August 2, 2012 and November 27, 2015. Eligible patients had to be between 1 month and 18 years of age, with a confirmed diagnosis of TDH and receiving at least 150 mL/kg/year of packed red blood cells. Patients could be included irrespective of the type of prior iron chelation therapy while patients naïve to iron chelation treatment had to have a SF level ≥800 ng/mL at screening. Female patients of childbearing age were required to use double-barrier contraception.

The number of screened patients was not recorded. Patients were screened and identified by the PIs among the cohort of TDH subjects requiring chronic transfusion therapy that were periodically managed at centers involved in the study.

Patients were excluded if they had: known intolerance or contraindication to either DFP or DFX; were receiving DFX at a dose >40 mg/kg/day or DFP at a dose >100 mg/kg/day at

screening; platelet count <100000/μL at the wash-out visit (day -7); absolute neutrophil count <1500/μL at the wash-out visit (day -7); hemoglobin levels <8 g/dL at the wash-out visit (day -7); evidence of alanine aminotransferase [ALT] level >5 × upper limit of normal [ULN]; iron overload from causes other than transfusional haemosiderosis; heart failure or severe arrhythmia or myocardial T2* <10 ms; creatinine level >ULN for age at the wash-out visit (day -7); history of significant medical or psychiatric disorder; received another investigational drug within 30 days prior to consent to study participation; fever or other signs/symptoms of infection at the wash-out visit (day -7); concomitant use of trivalent cation-dependent medicinal products or a positive test for beta-HCG or lactating female patients.

Randomization and masking

Patients, randomized in a 1:1 ratio to DFP or DFX, were stratified into two groups according to age (<10 years and ≥10 years, considering their different capabilities in undergoing cardiac MRI T2*). Randomization was centralized and balanced by country. The randomization sequence was generated directly into the electronic-case report form (e-CRF) with blocks of variable size (4-6-8) and random seeds to ensure that allocation concealment could not be violated by guessing the allocation sequence at the end of each block. No fixed number of patients per age group was specified and 10% of the patients were required to be <6 years of age. Blinding was not foreseen for this trial because of the different pharmaceutical forms and posology of investigational medicinal products which would have heavily impacted on the study feasibility.

Procedures

DFP (ApoPharma – Toronto, Canada) was administered orally, daily at 75-100 mg/kg/day. It was formulated in a new 80 mg/mL oral solution packaged in 250 mL bottles, using an administration device to ensure accurate measurement of dose volumes. The trial opened to children less than 6 years of age (10% of the total sample size) after dosing was confirmed by the results of the DEEP-1 pharmacokinetics (PK) Study.[12] DFX (Novartis, Basel, Switzerland) was administered as dispersible tablets at 125 mg, 250 mg and 500 mg. DFX daily dosage ranged from 20 to 40 mg/kg/day as recommended in the Summary of Product Charactheristics (SmPC).[13] Dose adjustments were allowed for efficacy (scaling up) or for safety reasons including over-chelation (scaling down). If SF increased by >20% compared with the previous determination, or remained >1500 ng/mL (no increase or any increase

<20%) in the absence of a downtrend over a 3-month-period, DFP could be scaled up in steps of 12·5 mg/kg/day (to a maximum daily dose of 100 mg/kg) and DFX in steps of 5 to 10 mg/kg (to a maximum daily dose of 40 mg/kg). DFP or DFX could be adjusted for safety reasons including: creatinine increased by >33% from baseline or decrease in creatinine clearance (only for DFX); urine protein/creatinine ratio ≥0·5 in two consecutive measurements (only for DFX); ALT or aspartate aminotransferase (AST) >10 ULN (for both); severe skin rash (only DFX); SF level ≤500 ng/mL (for both); neutropenia (neutrophil count <1500/μL and ≥1000/μL in two consecutive measurements) (for both); infection (for both); arthralgia (for both); nausea/abdominal pain/vomiting (for both).

Reasons leading to withdrawals were serious adverse events (SAEs), consent/assent withdrawal, lost to follow-up, and significant protocol violations, moderate neutropenia (neutrophil count <1000/μL but >500/μL) or severe neutropenia/agranulocytosis (neutrophil count <500/μL), or any other event leading to drug suspension for more than 4 weeks. After a 28 day washout period, the treatment period lasted 12 months. Monthly visits were performed.

Patient compliance was estimated from e-CRF data and each patient was evaluated in terms of percentage of compliance. In cases where treatment compliance could not be automatically calculated, a case-by-case evaluation was made on the basis of the difference between the amount of drug that should have been returned and that actually returned. Compliance was defined appropriate if percentage of prescribed therapy taken was ≥80%. Assessments were performed in four phases: 1) run-in including screening from -28 to -7 days and wash-out from -7 to -1 days; 2) baseline (day 0) at randomization and clinical evaluation; 3) treatment, one visit per month for 12 months; and 4) follow-up at month 13 (Appendix 1, p 2). SF levels were analyzed monthly at local and central laboratories. LIC was measured at baseline and 12 months by hepatic R2 MRI (Ferriscan®). Cardiac T2* MRI was measured at baseline, 6 months and 12 months. Children ≥10 years old who did not need sedation had LIC R2-MRI and cardiac T2* assessments. The cardiac T2* protocol included analysis of full-thickness region of interest in the left ventricular septum.[14] LIC R2-MRI was based on protocol described by St Pierre et al.[15] MRI evaluations were centralized at Resonance Health, Perth, Australia. Full blood counts were done weekly for patients in both arms of the study for early detection of neutropenia and agranulocytosis. AEs were collected at every monthly visit in the e-CRF and reported to a pharmacovigilance system. SAEs were reported within 24 hours of the awareness of the event. Assessment of severity for each AE/SAE was performed using

the following categories: *mild*, an event that was easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities; *moderate*, an event that was sufficiently discomforting to interfere with normal everyday activities; *severe*, an event that prevented normal everyday activities. Neutropenia <1000/μL and creatinine increase or reduction in creatinine clearance were considered relevant safety concerns and subjected to special monitoring and recording.

Data were entered directly into the e-CRF or indirectly from source data documents. All data collected were reviewed for completeness and accuracy. Any query was solved using an electronic data query system. Any deviations from the protocol, such as failure to obtain patient assent or parent consent, failure of SF tests, or reasons related to the non compliance with study requirements, have been recorded during the trial.

Laboratory samples were processed centrally and all results recorded electronically in the e-CRF. Sites were regularly monitored for patient records, accuracy of entries on e-CRFs, adherence to the protocol and to Good Clinical Practice (GCP), progress of enrolment and monitoring that study medication was being stored, dispensed and accounted for were even performed. The dropout rate was increased from 10% to 20% with a protocol amendment on December 10, 2015, increasing the number of enrolled patients from 344 to 388.

Outcomes

The primary composite efficacy endpoint (PCEE) required both SF and myocardial T2* criteria to be met and was defined according to patient's age, as follows: - in patients < 10 years of age treatment success is defined only in terms of SF level; - in patients ≥ 10 years of age treatment success is defined in terms of both SF level and cardiac MRI T2*. In patients ≥10 years of age who would require sedation for the MRI scan, treatment success is defined only in terms of SF level.

The criteria for definition of treatment success were based on SF levels: if baseline SF level was ≥2500 ng/mL, reduction of 20% or more after 1 year treatment, while if baseline SF level was <2500 ng/mL any decrease or an increase <15% as long as the increase does not result in SF levels ≥2500 ng/mL; Myocardial T2*: if baseline of T2* was <20 ms increase of 10% or more after 1-year treatment, while if baseline of T2* was >20 ms any increase or a decrease <10% after 1-year treatment as long as the decrease does not result in myocardial T2* value <20 ms. Baseline SF and myocardial T2* were considered at randomization visit (Visit 3). The PCEE required both SF and myocardial T2* criteria to be met.

Secondary endpoints: these included changes in SF level, myocardial T2*, and LIC from baseline to end of study, safety, pharmacokinetic, QoL and compliance. Treatment success by LIC was also assessed and defined as LIC <7 mg/g at end of treatment.

Pharmacokinetic and QoL data have not been reported in this paper since they deserve publication in different medical journal. The paper with these data is under publication.

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Statistical analysis

As per the EMA Guideline E9[16] the PP population was considered the primary basis for the investigation of the NI hypothesis. The PP includes all subjects that have received the study drugs and for whom the PCEE measures were available at baseline and after 1 year of treatment, without major protocol violations. PP populations include: 1) PP1 patients in which the PCEE was available at baseline and after 1-year of treatment; 2) PP2 patients in which the per-protocol centralized SF level were available at baseline and after 1 year of treatment. This population was more represented in comparison with PP1 because it included patients who did not perform myocardial T2*; 3) PP3 patients in which LIC and myocardial T2* were available at baseline and after 1 year of treatment.

The ITT population included all patients randomized that received at least one dose of study medications.

The primary efficacy end-point analysis was based on the PCEE in the PP1 and in the analysis of ITT population. In the ITT population patients who prematurely discontinue the trial for safety reasons, detailed in the protocol, were considered as treatment failures. In all other suspended patients, according to EMA Guidelines for missing data[17], the last-observation-carried-forward (LOCF) methodology was applied, as imputation method, to SF levels. Myocardial T2* determinations were not included in LOCF analysis because of low number of withdrawn patients having almost one post baseline MRI. A complementary ITT analysis, not including handling of missing data, was also conducted. This analysis considered all patients that prematurely discontinued the trial as treatment failures. The

primary and secondary end-points analyses were corrected for country level by Generalized Linear Modeling (GLM). The country level was introduced in the statistical model as factor. By using this procedure, it is possible to test the null hypothesis for the effects of covariates on the means of grouping variables (factors) of a single dependent variable.

NI of the PCEE, in the PP1 population, was based on the 2-sided 95% confidence interval (CI_L, CI_U) of the difference in the success rate between the two arms and was established if the CI_L was greater than -0·125. The choice of this NI margin was based on clinical considerations of the available evidence regarding the effects of DFP and DFX on SF levels and myocardial iron overload.[18-21]

GLM was used for evaluation of SF levels and cardiac T2*. Concerning SF levels, NI was established if the 95% CI of the difference DFX-DFP was less than 400 ng/mL between baseline and end of study. SF levels were compared between the two groups at each study visit using ANOVA (one-way analysis-of-variance).

Cardiac T2* and LIC data were analyzed using GLM, with cardiac T2* and LIC changes from baseline as dependent variables, the treatment group as predictor variable.

Means were reported with standard deviations (SD). Proportions and differences between proportions were reported with 95% CI. Continuous scale values were compared between the two intervention groups by a paired t-test. A p-value of 0·05 was considered statistically significant. The minimum level of statistical significance was set at 5% (two-sided). Differences in proportions observed on contingency tables were assessed by chi-square analysis. Statistical analyses were performed using SPSS version 21.0 (IBM Clinical Software). All statistical analyses were performed under code at Biostatistics and Data Management Unit, Medi Service, Genoa (Italy) by a biostatistician (G.R.) blinded to the trial interventions. This study is registered on EudraCT, 2012-000353-31 and on ClinicalTrials.gov, NCT NCT01825512.

Sample size of 310 patients, randomized in a 1:1 ratio, was considered appropriate to show NI of DFP versus DFX based on a 95% CI_L NI margin of -0·125 with 80% power and one sided test with type I error of 0·025. However, anticipating a possible 20% dropout rate, an overall enrolment of 388 patients, aged from 1 month to less than 18 years, was planned.

Role of funding source

The sponsor had role in the study design, in the collection, analysis, interpretation of the data and in the writing of the report. MF, AC, BT, DB, GR had access to the raw data. The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit.

RESULTS

Overall 393 patients were randomized at 21 centers in 7 countries (194 to DFP and 199 to DFX) between March 17, 2014 and June 16, 2016 (Appendix 1 p 4). The mean (SD) age at randomization was 112·6 months, while 117 patients (30%, 117/390) were <6 years, including 23 patients (5·9%, 23/390) <2 years. TDH included 352 patients with beta-thalassemia major (90·3%, 352/390), 27 (6·9%, 27/390) with sickle cell disease, 5 (1·3%, 5/390) with sickle cell/beta-thalassemia, and 6 (1·5%, 6/390) with other hemoglobinopathies. The mean (SD) age at diagnosis was lower in patients receiving DFP than DFX (11·5 [9·68] versus 15·7 [21·37] months, p = 0·01). Mean age at first transfusion, age at first chelation, and the time interval from diagnosis to first transfusion were not statistically different between DFP and DFX (Table 1). Other baseline characteristics of the study population are shown in Appendix 1 (p 5).

Figure 1 illustrates the CONSORT diagram of the study including patients allocated to the two study arms. Forty-two patients were enrolled but not randomized (N=17 did not meet inclusion criteria, N= 5 withdrew consent, N=20 lost to follow-up). The diagram shows the total number of patients evaluated in the PP1 (DFX = 146/199; DFP = 125/194), PP2 (DFX = 166/199, DFP = 137/194), PP3 (DFX = 61/199, DFP = 50/1194) and ITT (DFX = 193/194; DFP = 197/199) populations. Three patients were excluded from the efficacy analysis due to not taking study medication (DFP= 1; DFX=2).

Table 2 shows results of the primary and secondary efficacy endpoints. The PCEE was successfully reached in 55·2% (69/125) and 54·8% (80/146) of the DFP and DFX arms, respectively. The difference between the two percentages (DFP – DFX) was 0·4% (95%CI: -11·9, 12·6), which is consistent with NI for DFP compared to DFX. Baseline (Visit 3) SF levels sub-group analysis in the PP population is shown in Appendix 1 (p 6). The age at diagnosis had no significant effect on baseline SF (p = 0·44) and cardiac T2* values (p = 0·61).

Table 2 shows ITT analysis where LOCF was applied, with imputation of 104 missing data, (26·7 %, 104/390), NI was also obtained between DFP versus DFX (-1·7% (95%CI: -12·1, 8·6)). NI was not shown in the ITT analysis where LOCF was not applied (-9·4%; 95% CI: -19·4, 0·9). NI was also maintained (3·2%; 95%CI: -13·0, 19·1) in the 153 children who were <10 years old. No statistically significant difference was shown between the two treatment groups in 84 (21·5%, 84/390) patients <6 years of age (44 in DFX and 40 in DFP, p = 0·76). NI was not reported because of the low number of patients.

Table 2 shows, in the PP2 population, the mean change in SF between baseline and end of study was -397·6 ng/mL (2468 to 2120 ng/mL) and -398·2 ng/mL (2822 to 2328 ng/mL) in the DFP and DFX arms, respectively (mean difference 0·60, 95%CI: -323·6, 324·8). No statistically significant difference between the two treatment arms (Figure 2; Appendix 1 p 7) was supported by analysis of SF changes from baseline by treatment and by study visit.

North Africa versus Europe post-hoc analysis for change of SF between baseline and end of study was not significantly different (p = 0·53). The percentage of treatment success, based on SF levels, was similar (Appendix 1 p 8).

The PP3 population showed a mean change in cardiac T2* (mean difference -0·6 ms, 95%CI: -4·1, 2·8) and LIC (mean difference 2·1 mg/g, 95%CI: -0·21, 4·5) from baseline to end of study (Table 2) that were not statistically significant different. This included patients >10 years old (n=111). Treatment success by LIC (PP3, n=106, liver MRI was not available for 5 patients) at end of study was similar between both groups (41%, 19/46 DFP vs 48%, 29/60 DFX, p=0·47).

AE evaluation is shown in Appendix 1 (p 9). There were 450 AEs reported in the DFP arm and 416 in DFX, 151 and 71 being drug-related (p <0·001). Among these, 14 AEs in DFP and 21 in DFX were graded as serious, drug-related being 9 for DFP (3 agranulocytosis, 2 hypertransaminasemia, 1 pneumonia, 2 neutropenia, and 1 seizure) and 3 for DFX (1 acute renal failure, 1 gastroenetritis, and 1 hypertransaminasemia).

Table 3 shows AEs by causation. Arthralgia and gastrointestinal disturbance were common in DFP and renal function abnormalities in DFX. Monitoring of neutrophil count revealed values <1500/μL in 12·4%, 24/193 and 13·7%, 27/197 in DFP and DFX, respectively. Twenty-eight cases in 23 DFP-treated patients and 15 cases in 11 DFX-treated patients were reported by physicians to the pharmacovigilance system. Neutropenia had a global incidence

rate of 10·4% (20/193) in DFP and 5·6% (11/197) in DFX (p=0·08). Neutropenia, considered drug-related, was reported in 82·1% (23/28) in DFP vs 13·3%(2/15) in DFX.

Mild or moderate neutropenia were reported after 127 (SD 96·1) and 101 (SD 85·7) days from treatment with either DFP or DFX, respectively. Three patients, treated with DFP and not included in the neutropenia analysis, experienced agranulocytosis (neutrophil values <500/μL). Overall 77 patients were withdrawn (51 in DFP and 26 in DFX). A Kaplan-Meier analysis of withdrawal events is shown in Appendix 1 (p 17).

Reasons for withdrawal are shown in Appendix 1 (p 15). More discontinuations, due to non-SAE and not mandated by the protocol, were observed in the DFP arm (11 cases corresponding to arthralgia, joint effusion, nausea, abdominal discomfort, fatigue, joint swelling, epistaxis, upper respiratory tract infections, abdominal pain upper, vomiting, palpitation in DFP versus 1 case of pyrexia in DFX arm).

Compliance was not significantly different (p=0·07) and appropriate in 183 [mean (SD)=92·0% (17·35), median (IQR)=93·3% (13·6)] and 192 [mean (SD)=95·3% (18·56), median (IQR)=96·8%, (11·1)] patients in DFP and DFX, respectively. The mean (SD) of treatment days was 319·7 (116·87) versus 344·8 (93·55) for DFP and DFX and the median (IRQ) follow-up time was 379 (98) days for DFP and 381 (42) days for DFX patients. Overall mean daily doses of DFP and DFX are shown in Appendix 1 (p 16).

DISCUSSION

The study showed that NI was established between DFP and DFX. Changes in SF, cardiac T2*, and LIC from baseline to end of study were comparable. SAEs and drug-related events were not statistically different between the two groups, and comparable to the adult population. Neutropenia occurrence was not significantly different between the two groups. Three reversible cases of agranulocytosis and two cases of reversible renal and urinary disorders were shown in DFP and DFX arm, respectively. Compliance was comparable between both drugs.

DEEP-2 is the largest randomized clinical trial on oral iron chelation in the paediatric population, generating clinically applicable data that were previously lacking, including populations in North Africa such as Egypt and Tunisia where TDH is common.[22]

The design of the study aimed at detecting liver and heart iron overload, to address the controversial results reported on myocardial iron overload in children.[23] SF levels and not LIC were selected as a primary endpoint, since the use of LIC-R2 together with myocardial T2* may lead to higher drop-outs in view of the difficulty in performing pediatric MRI. The study showed that treatment with DFP was not inferior to DFX, in patients who completed 12 months of treatment, for all the parameters evaluated (namely changes in SF, LIC, and myocardial T2*). The NI was also shown when a population <10 years was considered and treatment success was comparable in patients aged <6 years. No additional safety concerns appeared in very young children, suggesting that DFP is safe at the same dosage of adults.

The ITT population where LOCF was applied confirmed these results. NI was not demonstrated in the complementary ITT analysis where LOCF method was not applied. This may be since more patients on DFP discontinued treatment for non-serious AE compared with DFX. This could have a significant 'bias' on the efficacy evaluation of the ITT population if all discontinuations are considered treatment failures. In fact, the investigators clinical decision to withdraw a patient seems to have been based on their perception of the risks associated with a treatment group rather than to strictly adhering to the recommendations of the protocol. This effect (quite expected in similar groups of patients) has been taken into account at the regulatory level leading to the recommendation in the EMA Guidelines on Missing Data (2010)[17] and has been avoided in our analysis where the LOCF methodology was adopted.

Countries representation in this study, even outside of Northern Africa, was similar to that in the phase-III study of DFX making our findings similarly generalizable.[24]

Many patients had a high iron burden at the beginning of the study, partially reflecting inadequate previous chelation history. Chelation dosages were initiated at low levels, focusing mainly on safety, and were adjusted slowly and with stringent criteria throughout the study. Given that chelation efficacy is dose-dependent, these factors prevented a more rapid decline of iron. Dose adjustment with the goal of reaching a maximum tolerable dose may be required in young patients with TDH. Indeed, a multiparametric survey of myocardial and liver iron overload by T2* together with SF level monitoring in 107 pediatric TDH patients in Italy (median age 14·4 years), showed that 21·4% had significant myocardial iron overload, high SF levels (>2000 ng/mL) and LIC (>14 mg/g).[25] This further supports our

observation that a TDH cohort with severe iron burden, necessitating chelation treatment optimization, is still present in Western countries.

The first limitation of this study is that liver iron overload was measured as a secondary endpoint and was not included in the composite primary endpoint. Since patients with TDH rarely show myocardial iron overload before the age of ten,[23] the primary endpoint might have been more accurate if it had also included LIC. Second is, the significantly higher discontinuation of DFP versus DFX (Appendix 1 p 15), despite similar occurrence of non-SAEs (Appendix 1 p 15). This reflects varying physician perception on the etiological relationship between AEs and treatment. This is reasonable, considering that research in children should strike the right balance between protecting underage study subjects and advancing the medical field to the benefit of all children, and remains the reason why drug innovations are often limited in children.[26] This, together with the difficulty of having cardiac T2* in children, was the reason why the number of patients in the PP group was fewer than planned. Third, reduction in LIC values was non-significantly higher in DFX compared with DFP. LIC values >7mg/g have the best response to DFX.[27] Therefore, the higher number of patients with LIC >15mg/g in DFX than DFP may explain better response to DFX.[27] Long-term follow-up is necessary to evaluate the difference in reduction of LIC between DFP and DFX.[28] Finally, because of the low representation of sickle cell disease studies including higher numbers are called for.

In conclusion, our trial supports the use of DFP in pediatric patients with TDH based on data from the largest randomized clinical trial of iron chelation therapy in these patients.

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Authors Contribution: Study conception and design: AC, AM, AK and all other authors. Acquisition and assembly of data: MF, GR, BT, AV. Analysis and interpretation of data: MF, AC, BT, DB, GR. Drafting of the manuscript: AM, AK, AC, MF. Critical revision of the manuscript for important intellectual content: PT, MS, FT, YCT, OD. Statistical analysis: GR. All authors gave final approval of the manuscript for submission.

Data Access and Responsibility: AM and AK had full access to all the data in the study and take responsibility for the accuracy of the data analysis. GR and MF made the analysis and are responsible of the integrity of the data. All authors had final responsibility for the decision to submit for publication.

Conflict of Interest: AM is a member of Novartis and Bluebird bio Advisory Boards, AK reports grants from FP7 - EUROPEAN UNION, during the conduct of the study; grants and personal fees from NOVARTIS ONCOLOGY, personal fees from APOPHARMA, VERTEX, VIFOR, and IONIS, personal fees and non-financial support from CELGENE, outside the submitted work; GR reports grants from CVBF, during the conduct of the study; grants from Medi Service, outside the submitted work; ODP is an employees of Glaxo Smith Kline; RO is a member of Novartis and Bluebird bio Advisory Boards; RO declares Speaker's honoraria and consultant fee for Novartis, and Consultant fee for BlueBird Bio, outside the submitted work; MS reports grants from European Commission, during the conduct of the study, other from ApoPharma Inc. outside the submitted work, and he has a patent WO 2009/129592 Al issued; PT reports personal fees from ApoPharma, out of the submitted paper; YCT and FT are employees of Apotex Inc; MF, GR, AE-B, MB, LS, SC, CC, GCDV, AF, LC, HH, MK, MCP, BT, AV, AZ, DB, AC have no competing financial interests to disclose.

DATA SHARING STATEMENT

1) The data will be made available to others;

2) The indentified participant data will be made available;

3) Related documents will be available (statistical analysis plan and informed consent);

4) These data will be available from data of publication of this paper until 6 months after;

5) The data will be available writing to this e-mail address: md.amaggio@gmail.com;

6) The access criteria -to data will be the approval of a proposal with a signed data access agreement.

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Research in Context

Evidence before this study

Patients with TDH require life-long iron chelation therapy, with three iron chelators currently available. Efficacy and safety data for the use of deferoxamine (DFO), deferasirox (DFX) and deferiprone (DFP) in varying age subsets of pediatric patients are available. However, despite the evidence, the use of DFP in pediatric patients, especially very young children, is still limited. This is mainly attributed to lack of data from a randomized trial evaluating DFP against an 'appropriate' comparator, namely DFX, in pediatric patients including very young children. This limitation was recognised by the European Commission, and in compliance with the Pediatric Regulation (Regulation (EC) No 1901/2006), a pediatric work programme was funded: the DEEP project (DEferiprone Evaluation in Pediatrics – FP7-HEALTH-2010 Grant Agreement n. 261483). We searched PubMed, clinicaltrials.gov, Eudract and "The European Union electronic Register of Post-Authorisation Studies" (EU PAS Register-ENCEEP). (November 1, 2016). DFP efficacy was derived from 14 interventional studies and 2 observational studies. However, these studies showed methodological limitations including low number of patients, duration of the study less than 1 year or undetermined, problems with randomization, different standard methods used for liver and cardiac MRI. To our knowledge the effectiveness of DFP, in pediatric age group, has not been previously studied in a large phase-III, multicenter, randomized, non-inferiority (NI) trial and we sought to investigate this in DEEP-2 study.

Added value of this study

This study fills, for the first time, the gap in data, on the effectiveness and safety of DFP in the pediatric population, through a large randomized clinical trial in 7 countries. Effectiveness and safety were similar between DFP and DFX, although with significantly higher discontinuation of DFP for non-SAE.

Implications of all the available evidence

Using data from a large, 1-year, phase-III, multicenter, randomized, NI trial, our results show that DFP has an effectiveness and safety profile similar to that of DFX in pediatric patients using the same dosage as that of adults. This is provided patients do not discontinue treatment. This may have the implication of a wider evidence-based use of DFP in the paediatric age group. These data have to be confirmed in a long-term follow-up that extends

beyond 1 year.

FIGURE LEGENDS

Figure 1. CONSORT flow diagram.

Figure 2. Centralized serum ferritin (ng/mL) change from baseline by treatment arm and study Visit, (PP-2, N = 303).

Number of the Reviewer comments	Editors' comments	Author response and changes made	Page number and paragraph in the tracked paper
1	We appreciate that not all secondary endpoints will be reported in this paper; however, these endpoints should still be described in the outcomes section of the methods section of the main text, with an explanation as to why they are not being reported in this paper. Please add this to the manuscript.	This was done. " Secondary endpoints: these included changes in SF level, myocardial T2*, and LIC from baseline to end of study, safety, pharmacokinetic, QoL and compliance. Treatment success by LIC was also assessed and defined as LIC <7 mg/g at end of treatment. Pharmacokinetic and QoL data have not been reported in this paper since they deserve publication in different medical journal. The paper with these data is under publication."	Outcomes-page 9
2	Thank you for editing the Interpretation section of the Summary; however, we still feel that you are restating your findings in this section. Instead, please change this section to describe what your findings mean clinically and what their implications are. Please see the following previously published article for an example of what should be included in this section: https://nam03.safelinks.protection.outlook.com/?url=https%3A%2F%2Fwww.thelancet.com%2Fjournals%2Flanhae%2Farticle%2FPIIS2352-3026(19)30236-4%2Ffulltext&data=02%7C01%7Cemma.cookson%40lancet.com%7C0d191e7b18c740e0b84e08d7ca649254%7C9274ee3f94254109a27f9fb15c10675d%7C0%7C0%7C637200405611427670&sdata=KVVZxLUb5dZeiNX%2B%2FAZeY%2B8FwRIRvuvxRS2I0UgDKi4%3D&reserved=0	This was done. " In pediatrics patients with TDH, DFP was effective and safety in inducing iron overloading control during 12 months treatment. Considering the needing to have availability of more chelation treatments in pediatrics population, DFP offers a valuable and safe treatment option at this age."	Summary-Interpretation, Page 3