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by Sebastian Botzenhardt, Mariagrazia Felisi, Donato Bonifazi, Giovanni Carlo Del Vecchio, Maria Caterina Putti, Antonis Kattamis, Adriana Ceci, Ian CK Wong, and Antje Neubert Collaborative Groups: the DEEP Consortium)

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Long-term safety of deferiprone treatment in children from the Mediterranean region with beta-thalassaemia major: the DEEP-3 multi-centre observational safety study

Authors

Sebastian Botzenhardt[1], Mariagrazia Felisi[2], Donato Bonifazi[2], Giovanni Carlo Del Vecchio[3], Maria Caterina Putti[4], Antonis Kattamis[5], Adriana Ceci[2,6], Ian CK Wong[7,8], Antje Neubert[1]; on behalf of the DEEP consortium *(collaborative group)*

Affiliations

[1] Department of Paediatrics and Adolescent Medicine, Faculty of Medicine, Friedrich-Alexander University Erlangen-Nu□rnberg (FAU), Erlangen, Germany

[2] Consorzio per Valutazioni Biologiche e Farmacologiche, Pavia, Italy

[3] Azienda Ospedaliera Universitaria Consorziale, Policlinico di Bari, Bari, Italy

[4] Azienda Ospedaliera di Padova, Clinica di Oncoematologia Pediatrica, Padova, Italy

[5] First Department of Pediatrics, National and Kapodistrian University of Athens, Athens, Greece

[6] Gianni Benzi Pharmacological Research Foundation, Bari, Italy

[7] Centre for Medicines Optimisation Research and Education, Research Department of Practice & Policy, University College London School of Pharmacy, London, United Kingdom

[8] Centre for Safe Medication Practice and Research, Department of Pharmacology and Pharmacy, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China

Corresponding author

PD Antje Neubert, PhD
Department of Paediatrics and Adolescent Medicine
Friedrich-Alexander University Erlangen-Nürnberg
Loschgestrasse 15
91054 Erlangen, Germany
Phone: +49 9313 85-41237
Email: antje.neubert@uk-erlangen.de

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Trial registration

ENCePP registry of studies: no. 12034

Early iron chelation therapy is fundamental in the treatment of β-thalassemia major (β-TM) to avoid the adverse effects of chronic transfusional iron overload. Deferiprone (DFP) was the first oral iron chelator, but remains second line because of safety concerns.[1] Superior efficacy in reducing cardiac iron, especially in combination with deferoxamine (DFO), has been shown.[2] Its favourable cost-effectiveness and its off-patent status make it economically interesting both for developed and developing countries. Utilization in children is still limited because studies in pediatric patients are scarce.

The independent consortium *Deferiprone Evaluation in Paediatrics* (DEEP), born within the context of the TEDDY Network and funded by the EU Commission, investigates safety and efficacy of DFP. The DEEP-3 study investigated the long-term safety of DFP therapy alone and in combination with DFO.

DEEP-3 was a multi-center, retro- and prospective, non-interventional cohort study which was conducted in 16 hospitals in six Mediterranean countries between November 2012 and April 2016: Albania (1), Cyprus (1), Egypt (1), Greece (1), Italy (11) and Tunisia (1). Endpoints were the nature and incidence of serious and non-serious adverse drug reactions (ADR) to DFP, and potential risk factors for ADRs and DFP withdrawals.

Inclusion criteria were: diagnosis of β-TM and transfusional iron overload, start of DFP at age one month to less than 18 years, and at least one dose of DFP. Patients were excluded if previous medical records could not be obtained or were incomplete.

Start of observation was the initiation of DFP therapy. Follow-up ended when the patient either completed the study (e.g. turned 18 years of age or end of study) or permanently withdrew from DFP for any reason (e.g. adverse reaction).

The study was approved by all local ethics committees. For prospective data collection, parents or guardians provided written, informed consent.

The investigators were asked to report the following adverse events (AE): hospitalization, fever of unknown origin, thrombocytopenia (platelet count $<150 \times 10^9$/L), neutropenia (neutrophil count 0.5-1.5×10^9/L), agranulocytosis (neutrophil count $<0.5 \times 10^9$/L), increased transaminases, arthropathies, neurological symptoms, hearing impairments, cardiac symptoms, endocrine dysfunctions, renal dysfunctions, weight gain >5% of baseline, gastrointestinal disorders, cutaneous reactions, death, or any other clinically important events.

An independent safety committee evaluated causality, severity and seriousness of each AE. Disagreements were solved by consensus. Only AEs deemed at least possibly related to DFP by the committee and with positive investigator-reported causality were considered to be an ADR to DFP. Additional information on the methods can be found in the Supplementary File.

Two hundred ninety-seven patients were enrolled and observed from March 1994 to October 2015 with 717.4 person-years (PY) of follow-up (Table 1). Median patient age at start of DFP therapy was 8.5 years (range 0.6-17.6 years). One patient died from non-DFP-related vomitus aspiration and severe convulsions. Median follow-up per patient was 1.7 years (IQR 0.8-3.5) and ranged from two days to 15.2 years. Further details are outlined in Supplementary Table S2.

Most patients received DFP monotherapy (68.3%). Simultaneous therapy with DFO was predominate at Cyprus, Greek and Italian sites (Supplementary Table S3).

491 AEs including 158 serious AEs in 183 patients underwent the causality assessment (see full list in Supplementary Table S4). After assessment, 172 AE episodes in 104 patients were considered to be DFP related ADRs (Table 2). The incidence and incidence rate of serious ADRs was 14.8% and 9.5 per 100 PY, respectively. The probability of experiencing a serious ADR was 20.6% after 36 months of treatment (*data not shown*). ADR incidence did not differ significantly between patients using DFP products *Ferriprox* or *Kelfer* (P=0.491).

DFP-related neutropenia was identified in 25 non-splenectomized patients (incidence 8.4%). There was no difference in neutropenia incidence between normal spleen size and splenomegaly (P=0.737).

In three patients, neutropenia was recurrent despite temporary termination and DFP was eventually discontinued.

Agranulocytosis occurred in two female patients in the first 12 months of treatment without previous neutropenia (Figure 1) and recovered upon DFP discontinuation (incidence 0.7%); one patient was on combined therapy and one on monotherapy. Both patients received standard DFP and DFO doses and had splenomegaly with normal neutrophil levels at baseline. A third case of agranulocytosis was demonstrated to be parvovirus B19 related.

Gastrointestinal disorders, e.g. abdominal pain, diarrhea, dyspepsia, or nausea, were observed with incidences ranging between 0.3% and 3.4%. None of these ADRs were serious. Median time to occurrence was three months (IQR 0.0-9.0).

Increased transaminases were seen in 31 patients (incidence 10.4%). The respective alanine transaminase levels were as follows: two ADRs <2x upper limit of normal (ULN), 24 ADRs 2-5x ULN, 14 ADRs 5-10x ULN, and two ADRs >10x ULN. There was no difference between HCV positive and negative patients (P=0.549). Median time to first occurrence was eight months (IQR 4-23).

Arthropathy including arthralgia and swelling, mostly in the knee joints, was the most common ADR (Table 2). In five patients therapy was discontinued due to re-occurrence or severe, disabling symptoms. More than half of the affected patients experienced symptoms in the first 12 months of treatment (Figure 1). North African patients (24.2%) were more often affected than European patients (3.8%) (P<0.001).

Other ADRs identified included mild rash and urticaria, moderate fatigue, mild weight increase, and mild chromaturia. No drug-related worsening of hepatic fibrosis, audiological disorders, visual toxicities or renal impairments were observed. Adverse reactions disappeared upon temporary DFP interruption, dose reduction or even without intervention.

DFP was discontinued due to AEs (52.9%), lack of efficacy (17.0%) and non-compliance (7.8%). Supplementary Tables S5 and S6 provide more details.

In 23.2% of patients an ADR to DFP led to permanent therapy discontinuation, most commonly due to arthropathy, increased transaminases, neutropenia, and gastrointestinal disorders (Table 1). There were significantly more ADR-related discontinuations in children under the age of 10 years than in older children (P=0.025). No specific risk factors for ADRs or DFP withdrawals could be identified (Supplementary Table S7).

DEEP-3 was the largest safety study of DFP in children so far with more than half of our patients below the age of 10 years and more than one-third below six years.

Compared to adults, neutropenia seems to occur more often in children, even healthy ones.[3] However, our agranulocytosis and neutropenia incidences are similar to those reported in children and adolescents on DFP.[4] The association between neutropenia and DFP remains uncertain. Our and other studies showed that patients recovered despite DFP continuation or did not have neutropenia on re-challenge.[5-7] Splenectomy may protect from neutropenia as only non-splenectomized patients were affected from neutropenia and agranulocytosis. Similar observations have been reported before.[1, 8, 9] Ethnic differences may influence the occurrence of agranulocytosis as reported in Chinese children on DFP.[10] Combined therapy was not associated with a higher risk for neutropenia consistent with existing data.[4] European sites reported neutropenia more often than non-European sites (13.5% vs. 4.3%, P=0.004) due to a closer neutrophil monitoring.

For agranulocytosis, we can confirm the following observations: it is most likely seen in the first 12 months of treatment, more often in female patients, and independent from dose and combination with DFO.[3] The rare occurrence did not allow to investigate potential risk factors. In the literature, it is depicted to be idiosyncratic, unpredictable and not dose-dependent.[4]

Arthropathy incidence is in agreement with previous studies.[4] We could not find a correlation between high serum ferritin (SF) and arthropathy, but we found significantly more cases in North African

patients. There may be ethnic differences similar to the very high incidences reported in Indian children.[5, 11, 12] We cannot confirm the influence of different DFP products on the occurrence of arthropathy.[8, 13]

Elevated transaminases were the second most frequently identified ADR to DFP. We could not find an association between the adverse reaction and HCV serostatus or SF levels, respectively.

Gastrointestinal disorders affected 6.4% of the patients which is lower than reported in previous pediatric studies with incidences between 11% and 27.3%.[4] The actual incidence may be higher because we must take underreporting of mild events into account.

DFP discontinuation rate was 23.2% in our pediatric cohort. Other observational studies involving real-world data reported rates of 8.8% and 29.3%.[14, 15] ADR-related DFP discontinuations were more common in children under the age of 10 years. It may be more difficult to re-administer a drug associated with burdensome effects in younger children.

In conclusion, we did not find any unexpected ADRs and incidences are in accordance with available data. Most reactions were mild or moderate and patients recovered shortly upon dose reduction, temporary interruption or withdrawal. Neither patients under the age of 6 years, nor under 10 years, experienced more ADRs than older patients. We did also not find a greater risk for ADRs in patients on combined iron chelation therapy with DFO.

However, agranulocytosis was also present in this pediatric population. Weekly monitoring of neutrophils is warranted. Arthropathy has significant impact on quality of life in children and needs to be closely monitored. A new age-appropriate oral formulation of DFP developed in the DEEP project may improve gastrointestinal tolerability. Further efforts should be made to facilitate the availability of affordable, safe and effective iron chelation for children worldwide.

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Participating sites and principle investigators

Cairo University, Cairo, Egypt (**Amal El-Beshlawy**); National and Kapodistrian University of Athens, Athens, Greece (**Antonis Kattamis**); Qendra Spitalore Universitare Nene Tereza, Tirana, Albania (**Eleni Nastas**); Cyprus Ministry of Health, Nicosia Thalassaemia Center, Nicosia, Cyprus (**Soteroula Christou**); Centre national de Greffe de Moelle Osseuse, Tunis, Tunisia (**Mohamed Bejaoui**); Azienda Ospedaliera di Rilievo Nazionale Antonio Cardarelli, Naples, Italy (**Aldo Filosa**); Azienda Ospedaliera Ospedali Riuniti Villa Sofia-Cervello, Palermo, Italy (**Aurelio Maggio**); Azienda Ospedaliera di Padova, Padua, Italy (**Maria Caterina Putti**); Azienda Ospedaliero-Universitaria Consorziale Policlinico di Bari, Bari, Italy (**Giovanni Carlo Del Vecchio**); ARNAS-Civico G. di Cristina-Benfratelli, Palermo, Italy (**Liana Cuccia**); Azienda Ospedaliera di Cosenza, Consenza, Italy (**Mariagrazia Bisconte**); Ospedale Civile di Lentini, Lentini, Italy (**Francesca Commendatore**); Policlinico di Modena, Modena, Italy (**Giovanni Palazzi**); Azienda Mista Ospedaliera-Universitaria di Sassari, Sassari, Italy (**Carlo Cosmi**); Ospedale Pediatrico Microcitemico "A.Cao", University of Cagliari, Cagliari, Italy (**Raffaella Origa**); Azienda Ospedaliero-Universitaria Meyer, Florence, Italy (**Tommaso Casini**).

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Tables

Table 1: Baseline characteristics

Patients	297 (100.0)
Age (years)	8.5 (4.0-12.2)
< 6 years	112 (37.7)
6-10 years	66 (22.2)
> 10 years	119 (40.1)
at diagnosis of β-TM	0.8 (0.4-1.2)
at start of transfusion therapy	0.7 (0.5-1.2)
at start of chelation therapy	2.8 (2.0-4.5)
Gender	297 (100.0)
Female	145 (48.8)
Male	152 (51.2)
Origin	297 (100.0)
Europe	131 (44.1)
North Africa	154 (51.9)
Rest of Africa	1 (0.3)
North America	6 (2.0)
Latin America	1 (0.3)
Asia	4 (1.4)
β-TM type	84 (100.0)
β^0 / β^0	23 (27.4)
β^0 / β^+	28 (33.3)
β^+ / β^+	30 (35.7)
Hb Lepore / β^0	1 (1.2)
Hb Lepore / β^+	1 (1.2)
δβ / β^+	1 (1.2)
Transfusional iron intake	293 (100.0)
Low (< 0.3 mg/kg/day)	46 (15.7)
Intermediate (0.3-0.5 mg/kg/day)	197 (67.2)
High (> 0.5 mg/kg/day)	50 (17.1)
Annual blood requirement (mL pure RBC/kg/year)	125 (117-156)
Spleen status	289 (100.0)
Normal	51 (17.7)
Splenomegaly	168 (58.1)
Splenectomized	70 (24.2)
Serum ferritin	268 (100.0)
< 2,000 ng/mL (n, %)	143 (53.4)
2,000-4,000 ng/mL (n, %)	93 (34.7)
> 4,000 ng/mL (n, %)	32 (11.9)
HIV seropositive (n = 183)	0 (0.0)
Hepatitis B seropositive (n = 237)	10 (4.2)
Hepatitis C seropositive (n = 242)	41 (16.9)

Values are median (interquartile range) and number of patients, n (%), if not stated otherwise; β-TM: β-thalassemia major; RBC: red blood cells

Table 2: Identified adverse drug reactions (ADR) to deferiprone (DFP)

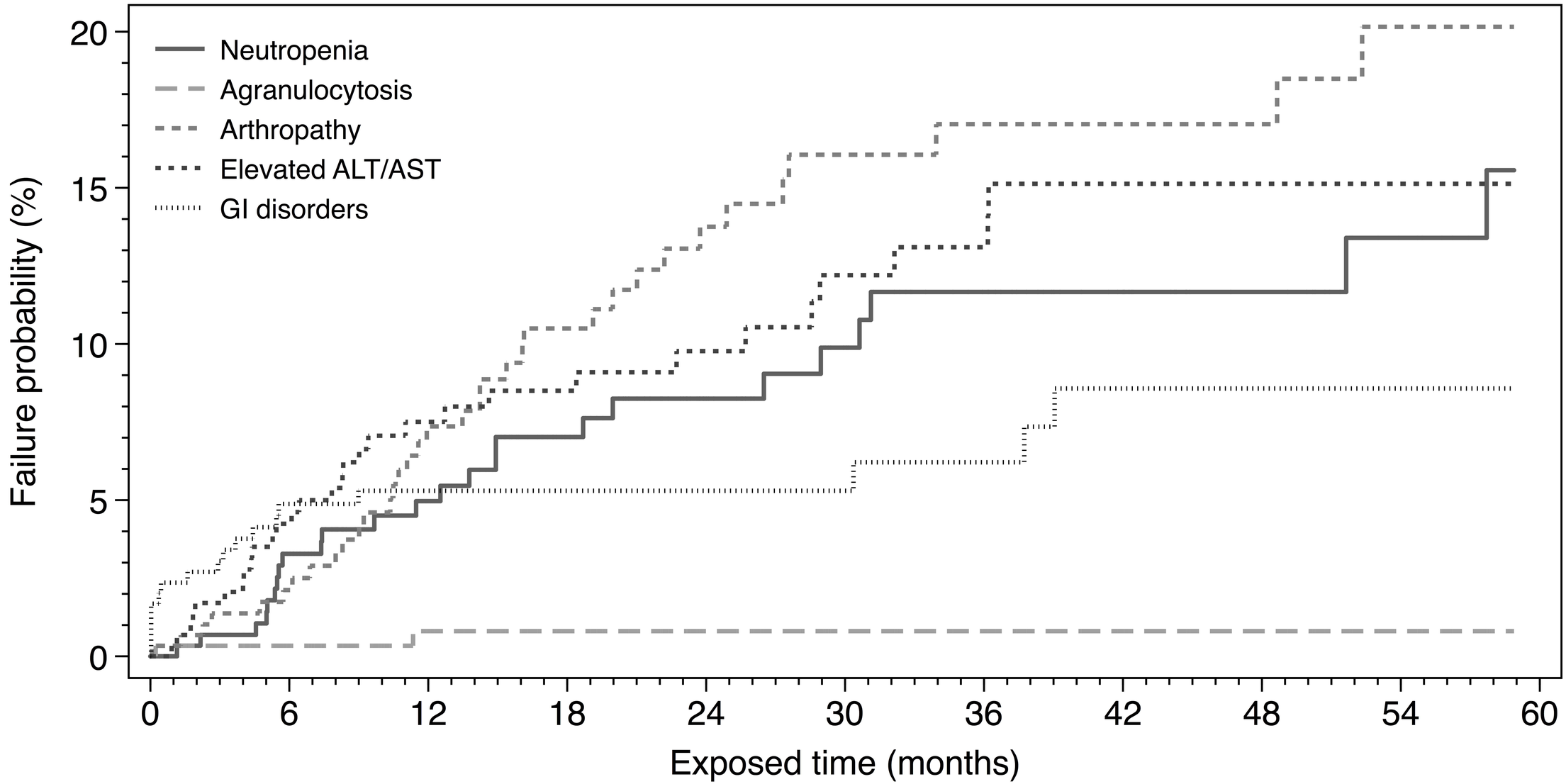
MedDRA SOC / Preferred Term	**ADRs / Patients**	**Severity** mild / moderate / severe	**Seriousness** non-serious / serious	**Incidence**[1] (95% CI)	**Incidence rate**[2] (95% CI)	**DFP discontinuation**[3] (95% CI)
Blood and lymphatic system disorders						
Agranulocytosis	2/2	0/2/0	0/2	0.7 (0.1-2.4)	0.3 (0.0-1.0)	0.7 (0.1-2.4)
Leukopenia	3/2	3/0/0	3/0	0.7 (0.1-2.4)	0.4 (0.1-1.2)	0.3 (0.0-1.9)
Neutropenia	38/25	26/12/0	0/38	8.4 (5.5-12.2)	5.3 (3.7-7.3)	5.1 (2.9-8.2)
Thrombocytopenia	1/1	1/0/0	0/1	0.3 (0.0-1.9)	0.1 (0.0-0.8)	0.3 (0.0-1.9)
Gastrointestinal disorders						
Abdominal pain	9/8	6/3/0	9/0	2.7 (1.2-5.2)	1.3 (0.6-2.4)	1.0 (0.2-2.9)
Diarrhea	1/1	1/0/0	1/0	0.3 (0.0-1.9)	0.1 (0.0-0.8)	none
Dyspepsia	4/4	4/0/0	4/0	1.3 (0.4-3.4)	0.6 (0.2-1.4)	0.3 (0.0-1.9)
Nausea	3/3	1/2/0	3/0	1.0 (0.2-2.9)	0.4 (0.1-1.2)	0.3 (0.0-1.9)
Salivary hypersecretion	1/1	1/0/0	1/0	0.3 (0.0-1.9)	0.1 (0.0-0.8)	none
Vomiting	14/10	3/11/0	14/0	3.4 (1.6-6.1)	2.0 (1.1-3.3)	2.7 (1.2-5.2)
General disorders and administration site conditions						
Fatigue	1/1	0/1/0	1/0	0.3 (0.0-1.9)	0.1 (0.0-0.8)	0.3 (0.0-1.9)
Investigations						
Transaminases increased	42/31	35/7/0	17/25	10.4 (7.2-14.5)	5.9 (4.2-7.9)	6.4 (3.9-9.8)
Weight increased	1/1	1/0/0	1/0	0.3 (0.0-1.9)	0.1 (0.0-0.8)	none
Musculoskeletal and connective tissue disorders						
Arthropathy	43/35	10/30/3	41/2	11.8 (8.3-16.0)	6.0 (4.3-8.1)	8.4 (5.5-12.2)
Bone pain	5/5	2/3/0	5/0	1.7 (0.5-3.9)	0.7 (0.2-1.6)	1.3 (0.4-3.4)
Renal and urinary disorders						
Chromaturia	1/1	1/0/0	1/0	0.3 (0.0-1.9)	0.1 (0.0-0.8)	none
Skin and subcutaneous tissue disorders						
Rash	2/1	2/0/0	2/0	0.3 (0.0-1.9)	0.3 (0.0-1.0)	none
Urticaria	1/1	0/1/0	1/0	0.3 (0.0-1.9)	0.1 (0.0-0.8)	none
TOTAL						
Any ADR	172/104	97/72/3	104/68	35.0 (29.6-40.7)	24.0 (20.5-27.8)	23.2 (18.6-28.5)
Any serious ADR	68/44	48/19/1	0/68	14.8 (11.0-19.4)	9.5 (7.4-12.0)	9.1 (6.1-13.0)

[1] Number of patients with at least one ADR from the corresponding group divided by all exposed patients in percent (95% CI); [2] Number of ADR episodes divided by total observation time per 100 PY (95% CI); [3] Number of patients permanently discontinuing DFP due to the ADR divided by all exposed patients in percent (95% CI). PY: person-years; CI: confidence interval; MedDRA SOC: MedDRA System Organ Class

Figure legend

Figure 1: Kaplan-Meier failure analysis

Values are number of patients at risk at the corresponding time point and in brackets the number of deferiprone-related adverse drug reactions during the respective 6-month period (only first occurrence considered per patient). Censored observation markers and exposure after 60 months have been omitted for the sake of curve simplicity.



Neutropenia	297	(9)	258	(4)	197	(4)	158	(2)	124	(2)	103	(2)	86	(0)	71	(0)	58	(1)	46	(1)	35
Agranulocytosis	297	(1)	262	(1)	202	(0)	162	(0)	129	(0)	108	(0)	91	(0)	75	(0)	61	(0)	49	(0)	38
Arthropathy	297	(6)	257	(12)	195	(6)	152	(5)	121	(3)	100	(1)	83	(0)	70	(0)	57	(2)	47	(0)	37
Elevated ALT/AST	297	(12)	257	(8)	196	(2)	158	(2)	124	(3)	102	(1)	86	(2)	69	(0)	55	(0)	44	(0)	34
GI disorders	297	(14)	253	(1)	197	(0)	160	(0)	127	(0)	106	(1)	88	(2)	70	(0)	56	(0)	46	(0)	36

Supplementary Information & Data

Data collection

Data for this study was collected from medical records at each of the participating sites. This comprised demographic data (e.g. gender, year of birth, origin), disease related data (e.g. genotype, age at diagnosis, transfusion therapy), medical data (e.g. body weight, Tanner stages, hepatitis B/C or HIV status, spleen status), iron chelation details (e.g. actual dose, daily and weekly frequency, reason for discontinuation, drug product), laboratory records, co-morbidities, chronic co-medications, and occurrence of any adverse event potentially related to DFP therapy. Main laboratory parameters included hemoglobin (Hb), white blood count (WBC), neutrophil count (ANC), platelet count (PLT), serum creatinine (SCR), alanine transaminase (ALT), aspartate transaminase (AST), gamma-glutamyl transferase (GGT), and serum ferritin (SF). Investigators were instructed to collect and record data on a three-monthly basis with the latest available laboratory assessments. All data were recorded, stored and handled confidentially in an internet-based and password-protected database with an electronic case report form (eCRF). An independent safety committee evaluated causality, severity and seriousness of each AE using commonly used assessment tools.[1-3]

Statistical methods

The sample size calculation was based on published incidences of agranulocytosis, the most serious known ADR to DFP. Based on the expected agranulocytosis incidence from the SPC of 1.1%, it was anticipated to observe enough patients to detect at least one case of agranulocytosis with a 95% probability.[4] The required minimum sample size needed to detect this was 271 patients.[5] Including a safety margin of 10% (e.g. exclusions due to insufficient data), we planned to enroll at least 298 patients.

Demographic and baseline characteristics are presented using descriptive statistics. Differences in proportions of independent groups were analyzed using Pearson's chi-square test or Fisher's exact test, as appropriate. Kruskal-Wallis equality-of-populations rank test was used for comparisons of continuous variables of independent groups.

ADR incidences and incidence rates were calculated by dividing the number of patients with ADR by the total number of exposed patients, and by dividing the number of ADRs by the total person-time expressed per 100 person-years (PY), respectively. ADR incidences, ADR-related discontinuation rates and ADR incidence rates per person-time are presented with 95% confidence intervals (exact binomial and Poisson, respectively). For a graphical evaluation of the time-to-occurrence of ADRs and therapy discontinuations, Kaplan-Meier failure functions were plotted taking censoring of follow-up into account.

Logistic regression and Cox proportional hazards methods were used to further explore potential risk factors for the dichotomous outcomes *'occurrence of ADRs (yes, no)'* and *'withdrawal from DFP treatment (yes, no)'*, respectively. First, statistically significant predictor variables were identified in univariate models using a manual *stepwise forward* approach. Then, the identified independent variables were combined in multivariate models for the dependent outcome variable. If the resulting odds ratios (OR) and hazard ratios (HR) were still statistically significant, the predictor was considered a potential risk factor for the outcome. The ORs and HRs are reported including 95% confidence interval and *P*-value. We analyzed the following co-variates: gender (*female, male*), age *(≤10 years, >10 years*), mean DFP dose, chelation regimen (*mono, combined*), baseline ferritin, HCV serological status (*yes, no*), spleen status (*non-splenectomized, splenectomized*). Due to the rare incidence of some ADRs, we limited this analysis to arthropathy, neutropenia, increased transaminases and gastrointestinal disorders. As we expected influences from local practice and center effects, we adjusted all multivariate models for country to control for that fact.

Data processing and statistical analyses were performed using Stata Version 13.1 (StataCorp, College Station, Texas, USA). For all statistical tests a type I error (α) of 0.05 was defined and a *P*-value of <0.05 was considered statistically significant.

Table S1: Definitions applied in the DEEP-3 study

Term	Definition
Adverse event (AE)	Any untoward medical occurrence in a patient or clinical trial subject administered a medical product and which does not necessarily have a causal relationship with this treatment.[6]
Adverse drug reactions (ADR)	Response to a medicinal product which is noxious and unintended and which occurs at doses normally used in man for the prophylaxis, diagnosis or therapy of disease or for the restoration, correction or modification of physiological function.[7]
Serious adverse event (SAE)	An AE was considered serious, if one of the following criteria were met: results in death, is life-threatening (at time of occurrence), requires or prolongs existing hospitalization, results in disability or incapacity, or results in congenital abnormality.[8] Additionally, the following important and severe medical events were always considered as serious: acute liver failure, acute renal failure, acute respiratory failure, agranulocytosis (neutrophil count less than $0.5x10^9$/L), anaphylaxis, any malignancy, aplastic anemia, elevated liver enzymes (alanine transaminase (ALT) or aspartate transaminase (AST) more than five times baseline values), liver necrosis, malignant hypertension, neutropenia (neutrophil count less than $1.5x10^9$/L), pulmonary fibrosis, pulmonary hypertension, sclerosing syndromes, seizure (only central neurological seizures), thrombocytopenia (platelet count $<150x10^9$/L), torsades de pointes, toxic epidermal necrolysis, ventricular fibrillation.
Severity	Mild (‘an event that is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities’); Moderate (‘an event that is sufficiently discomforting to interfere with normal everyday activities’); Severe (‘an event that prevents normal everyday activities’).

Table S2: Study characteristics

	Albania	Cyprus	Egypt	Greece	Italy	Tunisia
Study sites	1	1	1	1	11	1
Start of recruitment	Sept 2013	Mar 2015	Apr 2013	Nov 2012	May 2013	Feb 2015
Enrolled subjects	5	13	154	23	101	14
With complete records	5	13	148	23	97	11
Age (median, range, in years)[1]	13.3 (10.3-14.4)	11.1 (5.0-14.1)	4.6 (0.6-17.6)	10.5 (3.3-16.9)	10.5 (1.1-17.4)	7.5 (4.0-12.6)
Follow-up (total person-years)	13.7	30.8	253.6	73.6	326.2	19.3
Retrospective observation	11.3	29.4	230.9	70.3	302.6	19.3
Prospective observation	2.4	1.5	22.7	3.4	23.6	0
Per patient (median, IQR)	3.3 (1.9-3.5)	1.6 (0.7-3.7)	1.2 (0.7-2.1)	2.7 (1.4-4.8)	2.7 (1.4-4.9)	1.1 (0.3-3.4)

[1] *P*-value <0.001

Table S3: Deferiprone chelation therapy characteristics

Chelation regimen	297 (100.0)
Monotherapy (DFP)	203 (68.3)
Combined therapy (DFP/DFO)	59 (19.9)
Switched combined regimen	35 (11.8)
Combined regimen	94 (100.0)
Sequential combination	9 (9.6)
Simultaneous combination	75 (79.8)
Switched combined regimen	10 (10.6)
Follow-up (years)	717.4 (100.0)
On monotherapy (DFP)	497.9 (69.4)
Per subject	1.5 (0.8-3.0)
On combined therapy (DFP/DFO)	219.4 (30.6)
Per subject	1.7 (0.7-3.5)
DFP product used	297 (100.0)
Ferriprox (Apopharma, Canada)	218 (73.4)
Kelfer (Cipla, India)	59 (19.9)
Multiple products	20 (6.7)
Chelator dose (mg/kg/day)	
DFP dose	71.4 (62.5-77.6)
DFO dose (in combined therapy)	38.0 (31.2-42.3)
Co-medications	3 (1-3)
Co-morbidities	0 (0-1)

Values are median (IQR) and number of patients, n (%).

Table S4: All identified adverse events in DEEP-3

MedDRA SOC / Preferred Term	AEs / Patients	Severity mild / moderate / severe	Seriousness non-serious / serious
Blood and lymphatic system disorders			
Agranulocytosis	3/3	0/2/1	0/3
Anemia	1/1	0/0/1	0/1
Hypersplenism	1/1	1/0/0	1/0
Leukocytosis	4/3	3/1/0	4/0
Leukopenia	6/5	5/1/0	6/0
Lymphadenitis	1/1	1/0/0	1/0
Neutropenia	55/38	42/13/0	1/54
Thrombocytopenia	18/18	17/1/0	1/17
Cardiac disorders			
Cardiac ventricular disorder	1/1	1/0/0	1/0
Mitral valve incompetence	1/1	1/0/0	1/0
Palpitations	1/1	1/0/0	1/0
Endocrine disorders			
Hyperglycemia	3/2	2/1/0	3/0
Eye disorders			
Visual impairment	1/1	1/0/0	1/0
Gastrointestinal disorders			
Abdominal distension	1/1	1/0/0	1/0
Abdominal pain	27/19	15/10/2	26/1
Diarrhea	10/9	5/5/0	10/0
Dyspepsia	6/6	5/1/0	6/0
Dysphagia	1/1	1/0/0	1/0
Enteritis	3/2	2/1/0	3/0
Gastrointestinal disorder	1/1	1/0/0	1/0
Mesenteritis	1/1	0/0/1	0/1
Nausea	6/5	1/5/0	6/0
Salivary hypersecretion	1/1	1/0/0	1/0
Vomiting	23/19	11/12/0	22/1
General disorders and administration site conditions			
Asthenia	4/3	2/1/1	4/0
Fatigue	3/3	2/1/0	3/0
Edema peripheral	1/1	1/0/0	1/0
Pyrexia	60/35	36/24/0	57/3
Hepatobiliary disorders			
Hepatocellular injury	1/1	0/1/0	1/0
Immune system disorders			
Allergic transfusion reaction	4/2	4/0/0	4/0
Infections and infestations			
Bacterial infection	1/1	0/1/0	1/0
Bronchitis	1/1	0/1/0	1/0
Conjunctivitis	1/1	1/0/0	1/0
Ear infection	1/1	0/1/0	1/0
Enterobacter bacteremia	1/1	0/0/1	0/1
Gastroenteritis	2/2	1/1/0	1/1
Herpes simplex	2/2	2/0/0	2/0
Measles	1/1	1/0/0	1/0
Mumps	1/1	1/0/0	1/0
Otitis externa	1/1	0/1/0	1/0
Otitis media	3/3	1/2/0	3/0
Paronychia	1/1	1/0/0	1/0
Periodontitis	2/1	2/0/0	1/1
Pharyngitis	6/4	4/2/0	6/0
Pneumonia	6/4	0/5/1	3/3

Table S4: All identified adverse events in DEEP-3 (continued)

MedDRA SOC / Preferred Term	AEs / Patients	Severity mild / moderate / severe	Seriousness non-serious / serious
Infections and infestations			
Respiratory tract infection	6/3	2/4/0	6/0
Sinusitis	1/1	1/0/0	1/0
Tinea versicolor	1/1	1/0/0	1/0
Tonsillitis	6/4	0/6/0	6/0
Urinary tract infection	1/1	1/0/0	1/0
Viral infection	3/3	3/0/0	2/1
Viral upper respiratory tract infection	1/1	1/0/0	1/0
Injury, poisoning and procedural complications			
Chest injury	1/1	0/1/0	0/1
Femur fracture	1/1	0/0/1	0/1
Hemarthrosis	1/1	0/1/0	1/0
Ligament sprain	1/1	0/1/0	1/0
Investigations			
Blood creatinine increased	1/1	1/0/0	1/0
Transaminases increased	84/59	77/7/0	22/62
Urine calcium increased	4/2	4/0/0	4/0
Weight increased	1/1	1/0/0	1/0
Metabolism and nutrition disorders			
Hypocalcaemia	2/2	2/0/0	2/0
Lactose intolerance	1/1	1/0/0	1/0
Musculoskeletal and connective tissue disorders			
Arthropathy	56/43	17/36/3	52/4
Bone pain	6/5	3/3/0	6/0
Muscle spasms	1/1	1/0/0	1/0
Osteochondrosis	1/1	0/1/0	0/1
Pain in extremity	1/1	1/0/0	1/0
Nervous system disorders			
Dizziness	7/4	6/1/0	7/0
Headache	3/3	2/1/0	3/0
Paresthesia	2/2	2/0/0	2/0
Renal and urinary disorders			
Chromaturia	1/1	1/0/0	1/0
Nephrolithiasis	1/1	1/0/0	1/0
Reproductive system and breast disorders			
Amenorrhea	2/1	2/0/0	2/0
Menstrual disorder	1/1	1/0/0	1/0
Respiratory, thoracic and mediastinal disorders			
Aspiration	1/1	0/0/1	0/1
Cough	1/1	1/0/0	1/0
Skin and subcutaneous tissue disorders			
Dermatitis bullous	1/1	1/0/0	1/0
Erythema	1/1	1/0/0	1/0
Eyelid edema	1/1	1/0/0	1/0
Pruritus	1/1	1/0/0	1/0
Rash	5/4	3/2/0	5/0
Skin irritation	1/1	1/0/0	1/0
Skin ulcer	1/1	1/0/0	1/0
Urticaria	1/1	0/1/0	1/0
TOTAL	491/183	319/159/13	333/158

Table S5: Reasons for DFP discontinuation based on therapy duration

	Duration of DFP treatment			
Reasons for discontinuation	**< 1 year** 62 (100.0)	**1-3 years** 55 (100.0)	**> 3 years** 36 (100.0)	**Total** 153 (100.0)
Adverse event	43 (69.4)	27 (49.1)	11 (30.6)	81 (52.9)
Lack of efficacy	2 (3.2)	11 (20.0)	13 (36.1)	26 (17.0)
Other[1]	8 (12.9)	10 (18.2)	4 (11.1)	22 (14.5)
Non-compliance	5 (8.1)	2 (3.6)	5 (13.9)	12 (7.8)
SF normalized	2 (3.2)	4 (7.3)	2 (5.7)	8 (5.2)
Safety concerns	2 (3.2)	1 (1.8)	1 (5.6)	4 (2.6)

Values are number of patients, n (%); [1] Patient preference for DFX (n=11), DFP not available (n=4), participation in a clinical trial (n=4), reimbursement ceased (n=3); SF: serum ferritin.

Table S6: Adverse events resulting in DFP therapy discontinuation

Adverse events	**n** (%)	**DFP related** yes/no
Arthropathy	26 (8.8)	25/1
Transaminases increased	21 (6.7)	19/2
Neutropenia	18 (6.1)	15/3
Gastrointestinal disorders	9 (3.0)	9/0
Thrombocytopenia	4 (1.3)	1/3
Agranulocytosis	3 (1.0)	2/1
Leukopenia	3 (1.0)	1/2
Bone pain	2 (0.7)	2/0
Palpitations	1 (0.3)	0/1
Enterobacter bacteremia	1 (0.3)	0/1
Fatigue	1 (0.3)	1/0
Worsened hepatocellular injury	1 (0.3)	0/1
Vomitus aspiration (fatal)	1 (0.3)	0/1

Values are number of patients affected (n) and incidence (%) in the total cohort (n=297). AEs add up to more than 81 patients, because in 8 patients a combination of AEs led to discontinuation.

Table S7: Potential risk factors for ADR occurrence or DFP therapy discontinuation

	Univariate regression		Multivariate regression	
ADR / Risk factor	**OR (95% CI)[1]**	***P*-value**	**OR (95% CI)[1]**	***P*-value**
Arthropathy				
Regimen (mono vs. combined therapy)	10.5 (1.4-78.3)	0.022	4.8 (0.6-39.0)	0.139
Splenectomized vs. non-splenectomized	2.4 (1.2-5.1)	0.019	2.4 (1.0-5.9)	0.051
Age (≤10 years vs. >10 years)	2.5 (1.1-5.7)	0.031	2.6 (0.9-7.6)	0.084
Mean DFP dose	1.0 (0.9-1.0)	0.041	1.0 (0.9-1.0)	0.161
Neutropenia				
Regimen (combined vs. monotherapy)	2.9 (1.1-7.2)	0.025	1.7 (0.5-5.4)	0.370
Transaminases increased				
-	-	-	-	-
Gastrointestinal disorders				
-	-	-	-	-
Discontinuation / Risk factor	**HR (95% CI)[2]**	***P*-value**	**HR (95% CI)[2]**	***P*-value**
DFP discontinuation				
Age (≤10 years vs. >10 years)	1.6 (1.1-2.2)	0.009	1.1 (0.7-1.7)	0.636
Regimen (mono vs. combined therapy)	1.6 (1.0-2.4)	0.039	1.2 (0.7-2.1)	0.418
Mean DFP dose	1.0 (0.9-1.0)	0.041	1.0 (0.9-1.0)	0.496

[1] Logistic regression model odds ratio and confidence interval for the dependent variable ADR occurrence.
[2] Cox regression model odds ratio and confidence interval adjusted for the time-dependent variable DFP discontinuation. All multivariate models adjusted for country.

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