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

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

[1]*Department of Paediatrics and Adolescent Medicine, Faculty of Medicine, Friedrich-Alexander University Erlangen-Nürnberg (FAU), Erlangen, Germany;* [2]*Consorzio per Valutazioni Biologiche e Farmacologiche, Pavia, Italy;* [3]*Azienda Ospedaliera Universitaria Consorziale, Policlinico di Bari, Italy;* [4]*Azienda Ospedaliera di Padova, Clinica di Oncoematologia Pediatrica, Italy;* [5]*First Department of Pediatrics, National and Kapodistrian University of 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, UK and* [8]*Centre for Safe Medication Practice and Research, Department of Pharmacology and Pharmacy, Li Ka Shing Faculty of Medicine, The University of Hong Kong, China*

Participating sites and principle investigators. Cairo University, Egypt (Amal El-Beshlawy); National and Kapodistrian University of Athens, Greece (Antonis Kattamis); Qendra Spitalore Universitare Nene Tereza, Tirana, Albania (Eleni Nastas); Cyprus Ministry of Health, Nicosia Thalassaemia Center, 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, Italy (Giovanni Carlo Del Vecchio); ARNAS-Civico G. di Cristina-Benfratelli, Palermo, Italy (Liana Cuccia); Azienda Ospedaliera di Cosenza, Italy (Mariagrazia Bisconte); Ospedale Civile di 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, Italy (Raffaella Origa); Azienda Ospedaliero-Universitaria Meyer, Florence, Italy (Tommaso Casini).

Correspondence: antje.neubert@uk-erlangen.de
doi:10.3324/haematol.2017.176065

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.

References

1. World Health Organization (WHO), The Uppsala Monitoring Centre. The use of the WHO-UMC system for standardized case causality assessment. https://who-umc.org/media/2768/standardised-case-causality-assessment.pdf. Last accessed 24 June 2017;
2. Naranjo CA, Busto U, Sellers EM, et al. A method for estimating the probability of adverse drug reactions. Clin Pharmacol Ther. 1981;30(2):239-245.
3. Dormann H, Muth-Selbach U, Krebs S, et al. Incidence and costs of adverse drug reactions during hospitalisation: computerised monitoring versus stimulated spontaneous reporting. Drug Saf. 2000;22(2):161-168.
4. Apotex Europe B.V. Summary of Product Characteristics (SPC) - Ferriprox. http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/000236/human_med_000789.jsp&mid=WC0b01ac058001d124. Last accessed 24 June 2017;
5. Schneiweiss F, Uthoff VA. Sample Size and Postmarketing Surveillance. Therapeutic Innovation & Regulatory Science. 1985;19(1):13-16.
6. European Commission. Directive 2001/20/EC of the European Parliament and of the Council. http://ec.europa.eu/health/files/eudralex/vol-1/dir_2001_20/dir_2001_20_en.pdf. Last accessed 24 June 2017;
7. European Commission. Directive 2001/83/EC of the European Parliament and of the Council. http://ec.europa.eu/health/files/eudralex/vol-1/dir_2001_83_consol_2012/dir_2001_83_cons_2012_en.pdf. Last accessed 24 June 2017;
8. International Council on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). Clinical safety data management: definitions and standards for expedited reporting (E2A). http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E2A/Step4/E2A_Guideline.pdf Last accessed 24 June 2017;