Randomized phase II trial of deferasirox (Exjade®, ICL670), a once-daily, orally-administered iron chelator, in comparison to deferoxamine in thalassemia patients with transfusional iron overload

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Background and Objectives. Iron accumulation is an inevitable consequence of chronic blood transfusions and results in serious complications in the absence of chelation treatment to remove excess iron. Deferoxamine (Desferal®, DFO) reduces morbidity and mortality although the administration schedule of slow, parenteral infusions several days each week limits compliance and negatively affects long-term outcome. Deferasirox (Exjade®, ICL670) is an oral chelator with high iron-binding potency and selectivity. In a phase II study, the tolerability and efficacy of deferasirox were compared with those of DFO in 71 adults with transfusional hemosiderosis.

Design and Methods. Patients were randomized to receive once-daily deferasirox (10 or 20 mg/kg; n=24 in both groups) or DFO (40 mg/kg, 5 days/week; n=23) for 48 weeks.

Results. Both treatments were well tolerated and no patient discontinued deferasirox due to drug-related adverse events. The reported frequency of transient, mild to moderate gastrointestinal disturbances was higher in the deferasirox group than in the DFO group, but these disturbances settled spontaneously without dose interruption in all patients. Decreases in liver iron concentration (LIC) were comparable in the deferasirox 20 mg/kg/day and DFO groups; baseline values of 8.5 and 7.9 mg Fe/g dw fell to 6.6 and 5.9 mg Fe/g dw, respectively, by week 48. Deferasirox showed a plasma elimination half-life of 8-16 hours, supporting its once-daily administration.

Interpretation and Conclusions. Deferasirox at daily doses of 10 or 20 mg/kg was well tolerated and, at 20 mg/kg, showed similar efficacy to DFO 40 mg/kg in terms of decreases in LIC.

Key words: iron chelation, deferasirox, thalassemia, well tolerated, efficacy, deferoxamine.

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Patients with chronic anemias such as thalassemia, sickle cell disease, congenital rare anemias and myelodysplastic syndromes require regular blood transfusions in order to improve both quality of life and survival. Humans are unable to eliminate the iron released from the breakdown of transfused red blood cells and the excess iron is deposited as hemosiderin and ferritin in the liver, spleen, endocrine organs and myocardium. The accumulation of toxic quantities of iron causes tissue damage and leads to complications such as heart failure, diabetes, hypothyroidism and liver failure. Morbidity and mortality in regularly-transfused thalassemia patients are due primarily to the effects of iron overload rather than to the underlying disease, with over half of all deaths attributable to cardiac complications.[1] Iron chelators mobilize tissue iron by forming soluble, stable complexes that are then excreted in the feces and/or urine. More than 40 years of clinical experience with deferoxamine (Desferal®, DFO) in iron-overloaded patients has established that chelation therapy, when given at an adequate dose, reduces iron-related complications and thereby improves quality of life and overall survival.[2,3] The poor oral bioavailability and short plasma half-life of DFO necessitates parenteral administration and prolonged infusions. The standard regimen to remove excess iron accumulated through regular transfusion is a subcutaneous (sc) infusion over 8-12 hours, on 3 to 7 days each week. This inconvenient schedule has a negative impact on compliance and eventually on long-term outcome, with some deaths being directly attributable to poor compliance with therapy.[3-5] The need for an effective, well-tolerated, oral iron chelator has been recognized for many years but, despite much effort, few candidate molecules were suitable for development. The oral chelator deferiprone is licensed for use only in β-thalassemia patients when DFO treatment is either inadequate or contraindicated. Its restricted indication is due partly to the incidence of agranulocytosis (1%), which necessitates weekly neutrophil counts, and partly to the limited clinical data available regarding its efficacy.[6] Comparisons of deferiprone and DFO are largely based on retrospective analyses and additional well-designed prospective trials are needed to clarify the comparative effects of these agents.[7-12] Combined treatment with DFO and deferiprone has shown additional efficacy in patients who failed to

respond satisfactorily to DFO or deferiprone treatment alone.[13-17]

Deferasirox (Exjade®, ICL670) is an N-substituted bis-hydroxyphenyl-triazole[18] that was selected from more than 700 compounds as part of a rational drug development program. It represents a new class of tridentate iron chelators with a high specificity for iron.[19] Selective and efficient mobilization of tissue iron has been demonstrated in animal models, with greater efficiency than with DFO.[20] In common with representatives from other classes of iron chelators, renal tubular nephropathy and lens opacities were noted in animal toxicology studies.[19] Phase I clinical evaluation of deferasirox has confirmed that the compound is well tolerated at single oral doses of up to 80 mg/kg.[21,22] Iron excretion occurs almost entirely in the feces and is dose-dependent, averaging 0.13, 0.34 and 0.56 mg/kg/day at deferasirox doses of 10, 20 and 40 mg/kg/day, respectively.[22] The plasma half-life of 11-19 hours supported the once-daily oral dosing regimen which has been used in subsequent clinical trials.[21,22]

This phase II study was carried out primarily to determine the safety and tolerability of deferasirox at doses of 10 and 20 mg/kg/day in comparison with a standard dose of DFO 40 mg/kg in patients with transfusional hemosiderosis. In addition, the 48-week treatment period provided the first opportunity for the collection and analysis of long-term pharmacokinetic data, which are of particular importance for a life-long medication. Changes in liver iron concentration (LIC) and serum ferritin were also assessed as measures of chelation efficacy.

Design and Methods

Quality control, quality assurance and monitoring

This trial was conducted in accordance with Good Clinical Practices, as outlined in the International Conference on Harmonization guidelines. Ethics Committee approval was obtained at each participating institution and written informed consent was obtained from all patients prior to participation in any study procedures. A Study Monitoring Committee comprising the four principal investigators (*AP, RG, GLF, MDC*) supervised the trial conduct, and made decisions regarding dose adjustment for individual patients. An independent Program Safety Board confidentially reviewed clinical and laboratory data on several occasions during this trial in order to ensure patient safety.

Patients

The study recruited 71 β-thalassemia patients aged ≥18 years with transfusional hemosiderosis from four centers in Italy. Patients should have received a mean daily dose of DFO ≥30 mg/kg 5 days/week (a hypothetical weekly dose of ≥150 mg/kg) for ≥4 weeks prior to entering the screening period and should have been regularly transfused. Patients were also required to have had at least two evaluations of serum ferritin values of 2000-8000 ng/mL documented during the preceding 12 months, or a superconducting quantum interference device (SQUID) LIC measurement of 5-15 mg Fe/g dw (performed in the previous year). For admission to the washout period, during
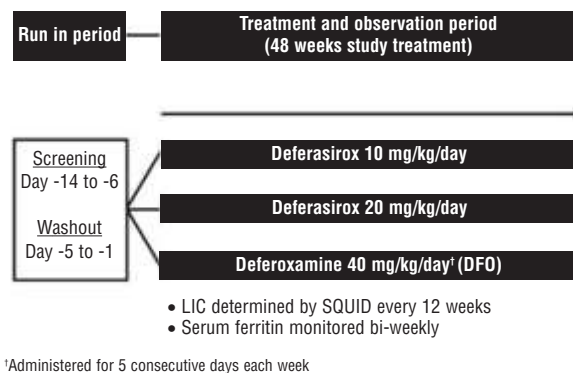

- LIC determined by SQUID every 12 weeks
- Serum ferritin monitored bi-weekly

†Administered for 5 consecutive days each week

Figure 1. Study design.

which DFO was discontinued (Figure 1), the LIC was required to be 5-15 mg Fe/g dw. Average post-transfusion hemoglobin levels were required to be 10.5-13.5 g/dL during the 12 months prior to enrollment, including one measurement during washout. Patients with an aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >250 U/L or a creatinine clearance <80 mL/minute were excluded, as were patients with hypertension and those with any degree of atrio-ventricular (A-V) block, clinically relevant QT interval prolongation, or those requiring treatment with digoxin or any drug that could induce prolongation of A-V conduction. Patients with a diagnosis of cataract or a previous history of clinically relevant ocular toxicity related to iron chelation were also excluded, as this was the first long-term deferasirox study and prospective data on these events were collected.

Study design

This was a multicenter, randomized, open-label study of 48 weeks' duration (Figure 1). The primary objective was to determine the safety and tolerability of deferasirox at daily doses of 10 and 20 mg/kg in comparison with a standard dose of DFO 40 mg/kg in patients with transfusional hemosiderosis. Secondary objectives included evaluation of the effects of deferasirox on LIC, serum ferritin, serum iron, transferrin and transferrin saturation. Other key aims were to explore an effective dose titration strategy that would allow deferasirox to be administered at doses that depleted or maintained iron load according to individual requirements and to evaluate the pharmacokinetic profile of deferasirox. The open-label design was considered appropriate in view of the differences in the treatment regimens and the fact that any potential bias would be counteracted by the objective nature of the efficacy parameters employed. During the 14-day run-in period, eligible patients had their usual DFO therapy adjusted to 40 mg/kg given on 5 consecutive days each week. Baseline LIC values were determined towards the end of the screening period. On day -5, patients were admitted to the study site to receive a blood transfusion to achieve a target hemoglobin level of ≥13 g/dL prior to commencing study treatment. After a DFO washout period of 5 days, patients were randomized in a 1:1:1 ratio to receive either once-daily deferasirox (10 or 20 mg/kg) or DFO (40 mg/kg

on 5 consecutive days per week). Randomization was performed using a validated system that generates an automated random assignment of numbers to treatment groups. Daily doses of deferasirox were prepared using 250 mg tablets which were divisible into four parts. The correct number of tablets was dispersed in a glass of non-carbonated mineral water, stirred and ingested 30 minutes before breakfast. DFO doses were prepared as a 10% solution using commercially available vials of 500 or 2000 mg dry powder. Subcutaneous infusion was performed using a Microject Crono® pump over 8-12 hours for 5 consecutive days each week. The study protocol allowed for dose adjustment within the range of 5-40 mg/kg/day in the deferasirox groups and 20-50 mg/kg in the DFO group. Depending on the response of each patient, assessed primarily using the change in LIC at three consecutive determinations, dose increases or decreases were made by ±5 or ±10 mg/kg in the deferasirox groups and by ±10 mg/kg in the DFO group. Dose reductions were performed if the decrease in LIC was extrapolated to fall below 2 mg Fe/g dw within the next 12 weeks and dose increases were prescribed if an increasing trend in LIC was noted. Dose adjustments were decided on a case-by-case basis in joint consultation between the Study Monitoring Committee and the sponsor. Patients continued to receive regular transfusions throughout the study. At the end of the study, patients, including those who were originally randomized to DFO, were offered the option of continuing therapy with deferasirox during a non-comparative extension phase. The results of this phase of the study will be reported separately.

Safety assessments

Laboratory tests, including evaluation of blood indices, liver and renal function, serum electrolytes, copper and zinc, were performed at baseline and at 2-weekly intervals throughout the study. All laboratory parameters were measured at a central laboratory (EXACTA Clinical Trials Services, Verona, Italy). Second void urine samples were collected for measurement of N-acetyl-β-glucosaminidase and an aliquot of urine was alkalinized for measurement of β-2 microglobulin. An ophthalmology examination, including a slit lamp examination of the lens and retinal fundoscopy, was performed every 2 weeks. Audiometry, ECG and liver ultrasonography were carried out every 3 months. Adverse events were recorded at each study visit and the severity of each adverse event was graded as mild, moderate or severe. A serious adverse event was defined as a medically significant event that was either fatal or life-threatening, required surgical intervention, prolonged hospitalization or resulted in persistent disability. All adverse events and serious adverse events were assessed by the investigator for a possible relationship to the study drug. Adverse events were ranked according to incidence in the deferasirox 20 mg/kg/day treatment group.

Efficacy assessments

The efficacy of iron chelation was evaluated by determining changes in LIC, as this was considered to be the most robust indicator of total body iron.[23] To avoid the need for invasive liver biopsy, LIC was measured non-invasively by biomagnetic susceptometry using a low crit-

ical temperature (low-TC) superconducting quantum interference device (SQUID) biomagnetometer, which allows the measurement of the paramagnetic susceptibility of the iron stored in the liver as hemosiderin and ferritin. In patients with iron overload, the results of biomagnetic susceptometry measurements of hepatic non-heme iron have been reported to be strongly correlated with those obtained by conventional analysis of liver biopsy.[24] The reliability of this methodology is supported by early studies that showed a good correlation between biomagnetic liver susceptometry and liver biopsy determinations of LIC.[25-27] All biomagnetic liver susceptometry evaluations were performed at the Ospedale Regina Margherita, University of Turin, Italy. LIC was determined at screening and then every 12 weeks during treatment and at the end of the study. LIC values derived from magnetic susceptometry as milligrams of iron per gram of liver tissue (wet weight) were multiplied by a factor of 3.33 to provide values per gram of liver tissue dry weight (mg Fe/g dw; the conventional units used for LIC).[18] Responders to chelation were defined as patients exhibiting a >10% fall in LIC (mg Fe/g dw) in the end-of-study biomagnetic susceptometry assessment in comparison to baseline.[28]

During the study, markers of iron metabolism (serum ferritin, serum iron, serum transferrin and transferrin saturation) were analyzed by a central laboratory (EXACTA Clinical Trials Services, Verona, Italy). The transferrin saturation was calculated from the serum iron and the transferrin concentrations. Urinary iron excretion was determined in 24-hour urine collections in ten patients taking deferasirox (five in each dose group) who also underwent blood sampling for pharmacokinetic analyses. Urinary iron excretion was measured using atomic absorption spectrometry.

Pharmacokinetic evaluation

The pharmacokinetic profile of deferasirox was assessed in a subpopulation of ten patients receiving deferasirox (five from each dose group). Blood samples were taken for this purpose at eight time-points during the study, one on day 1 after the first dose and seven during the steady state, and included collection intervals from pre-dose up to 24 hours after dose administration. Trough blood samples (taken 24 hours after the previous dose and immediately prior to the next dose) were taken for all patients, initially after 2 weeks of treatment, then every 4 weeks during the first 3 months of the study and subsequently every 12 weeks. The specific determination of the deferasirox and its iron complex Fe-[deferasirox]$_2$ in plasma was performed using high performance liquid chromatography with UV detection. The lower limits of quantification were 1.34 μmol/L for deferasirox and 0.314 μmol/L for Fe-[deferasirox]$_2$.[29] Standard non-compartmental pharmacokinetic parameters (area under the curve, C_{max}, t_{max} and half-life) were derived from the plasma concentrations of deferasirox and Fe-[deferasirox]$_2$.

Results

Patients' baseline characteristics

There was a preponderance of females in each treatment group (Table 1) and overall there were only 26 males

among the 71 patients in the study. Apart from two individuals with β-thalassemia intermedia, both of whom received deferasirox, all patients had β-thalassemia major. The treatment groups were reasonably comparable with respect to medical history although no patient in the deferasirox 10 mg/kg/day group had either hypothyroidism or a history of hepatitis B infection. Approximately 50% of patients had undergone splenectomy.

Dosing of study drugs

The average daily dose of the study drug over the 48 weeks of treatment was close to the randomized dose in each of the treatment groups (Table 2). By the end of the study, a total of 35 dose adjustments had been performed in 31 of the 69 patients who had had at least one LIC assessment by biomagnetic liver susceptometry. Dose increases were implemented on average after 31 weeks of treatment (range 22-43 weeks), as dictated by the requirement of having LIC values from at least three consecutive biomagnetic liver susceptometry evaluations before dose adjustments were made. Among the treatment groups, dose increases were performed most frequently in patients randomized to deferasirox 10 mg/kg/day and least often in patients receiving DFO. No patient received a deferasirox dose greater than 30 mg/kg/day during the study period. Although one-third of the study population had dose interruptions due to adverse events, with the majority of these interruption occurring in the deferasirox 20 mg/kg/day group, all but three patients resumed therapy at the original dose. Infections were the most frequent cause of dose interruption during the study but no between-group differences were apparent in the frequency of these episodes. The duration of patient exposure to the study drugs is summarized in Table 2; no relevant differences between the treatment groups were observed.

Safety and tolerability

There were no deaths during the study. Four patients (two each in the deferasirox 20 mg/kg/day and DFO groups) were withdrawn prematurely from the study, three due to adverse events and one due to an unsatisfactory therapeutic effect. In one patient receiving DFO, the adverse events leading to discontinuation (arthralgia, headache and fever) were suspected by the investigator to be study drug-related. The other adverse events resulting in withdrawal from the study comprised arrhythmia and cardiac failure in a patient receiving DFO, and trauma due to a road traffic accident in a patient on deferasirox 20 mg/kg/day; neither was considered study drug-related. The patient withdrawn due to unsatisfactory therapeutic effect was randomized to deferasirox 20 mg/kg/day but the dose was reduced to 10 mg/kg/day on day 85 when a fall in LIC from 5.2 to 2.6 mg Fe/g dw was detected. On day 250, the LIC had increased again to 4.5 mg Fe/g dw. The patient was also noted to have QTc prolongation. Since this was an exclusion criterion for study participation, and given the limited clinical experience with deferasirox available at that time, dose re-escalation to 20 mg/kg/day was considered inappropriate and the study drug was therefore discontinued. Adverse events irrespective of presumed drug relationship occurring in more than

Table 1. Patients' baseline characteristics.

	Deferasirox 10 mg/kg/day (n=24)	Deferasirox 20 mg/kg/day (n=24)	DFO 40 mg/kg (n=23)
Mean age in years (range)	23.7 (17-33)	25.6 (19-50)	22.7 (18-29)
Male/female	6/18	10/14	10/13
Mean height in cm (± SD)	155.6 (8.5)	157.0 (9.1)	160.0 (9.3)
Mean weight in kg (± SD)	52.4 (7.5)	50.7 (9.3)	54.3 (9.0)
Underlying disease			
β-thalassemia major	23	23	23
β-thalassemia intermedia*	1	1	0
Medical history			
Splenectomy	8	12	14
Hypogonadism (male or female)	10	12	10
Hypothyroidism (acquired)	0	5	6
Hepatitis B	0	3	2
Hepatitis C[†]	7	6	6
Cardiac disorder	4	5	5

Transfusion-dependent; [†]Anti-HCV-positive or HCV RNA-positive.

Table 2. Dosing details.

	Deferasirox 10 mg/kg/day (n=24)	Deferasirox 20 mg/kg/day (n=24)	DFO 40 mg/kg* (n=23)
Mean daily dose, mg/kg ± SD (range)	11.7±2.12 (8.5-14.7)	19.0±4.10 (10.4-25.6)	28.6±1.06 (26.6-31.6)
Number of patients with dose adjustments	13	14	4
Number of dose adjustments	13	16	6
Number of patients with dose interruptions due to an adverse event	5	11	5
Dose at week 48[†] (mg/kg/day)			
Discontinued	0	2	2
10	11	5	–
20	13	11	–
30	0	6	2
40			18
50			1
Mean duration of exposure in days (min-max)[‡]	345 (332-369)	332 (21-402)	318[§] (13-392)

DFO dose administered on 5 consecutive days per week; [†]Although adjustments of ± 5 mg/kg were made, all adjustments were further altered such that final doses were all in steps of 10 mg/kg only; [‡]The availability of a LIC result by biomagnetic susceptometry marked the end of study and hence of the exposure period to the study drug. For patients for whom there was a delay in performing biomagnetic susceptometry, the duration of exposure to study drug was slightly longer than 48 weeks; [§]Days of participation in the study, as DFO was administered for only 5 days per week.

four patients in any treatment group are listed in Table 3. In general, the adverse events reported were typical of incidental medical problems that are encountered regularly in young adults. With the exception of nausea and vomiting, no marked differences were seen between the groups in the frequency, nature or severity of adverse events. However, differences became more apparent when adverse events with a suspected relationship to study drugs were considered. In patients randomized to receive deferasirox at doses of 10 and 20 mg/kg/day, nausea was seen in one and six patients and vomiting in none

and three patients, respectively, whereas no patients randomized to DFO experienced nausea and vomiting with a suspected relationship to DFO. However, the gastrointestinal adverse events were mild to moderate in severity and resolved spontaneously, generally within a few days and without dose interruption. One patient had a skin rash on two occasions with a suspected relationship to study drug. The first episode was a generalized rash, lasting from days 9-13, which settled with concomitant therapy. The second episode affected only the hands, lasted from days 25-57, and settled spontaneously. The study drug was not interrupted during either episode. There were no episodes of arthralgia in patients randomized to deferasirox. Serious adverse events, none of which was assessed as related to study drug, were reported in 12 of the 71 patients with a similar distribution among the treatment groups.

Isolated serum creatinine values above the upper limit of normal were detected in six patients (in three, one and two patients receiving deferasirox 10 mg/kg/day, deferasirox 20 mg/kg/day and DFO, respectively) during the study. However, no patient had consecutive measurements of serum creatinine above the upper limit of normal. Elevations of urinary β-2 microglobulin were detected in all treatment groups but were more frequent in patients receiving deferasirox 20 mg/kg/day. The elevations were transient and low-grade (<10-fold above the upper limit of normal) and tended to normalize despite continuation of the study drug. In three patients receiving deferasirox, treatment was temporarily interrupted and elevated values normalized within 7 to 10 days. In the two patients treated with deferasirox 20 mg/kg/day who experienced the highest elevations of β-2 microglobulin, the dose of study drug was reduced to 10 mg/kg/day as a precautionary measure, and this was followed by prompt normalization of the β-2 microglobulin levels. There were no consistent changes in levels of urinary N-acetyl-β-glucosaminidase. There were no episodes of neutropenia, agranulocytosis or thrombocytopenia in any of the treatment groups. Most patients had normal AST levels at baseline, though a relevant proportion (32%) had increased ALT, presumably reflecting liver damage due to chronic viral hepatitis and/or iron overload. No patient developed consistent or progressive elevations in transaminase levels. Serum copper and zinc levels fluctuated considerably during the study but no patient developed progressive decreases in these trace elements. No retinal findings, lens abnormalities or hearing losses were detected during regular ophthalmological and auditory testing.

Efficacy

All patients received regular transfusions during the study and the average daily volume of blood transfused was similar across treatment groups, as was the estimated total daily iron intake in mg/kg. Thus, the mean volume of blood given was 0.34 mL RBC/kg/day (range 0.20-0.48) and the mean daily iron intake was 0.37 mg/kg (range 0.22-0.52). Biomagnetic susceptometry assessments performed at the end of study showed average decreases in LIC of similar magnitude in the deferasirox 20 mg/kg/day and DFO groups (-2.1 and -2.0 mg Fe/g dw, respectively), when compared to the values obtained at baseline (Figure

Table 3. Summary of adverse events reported in four or more patients in any treatment group, irrespective of presumed drug relationship.

Adverse event, n (%)	Deferasirox 10 mg/kg/day (n=24)	Deferasirox 20 mg/kg/day (n=24)	DFO 40 mg/kg (n=23)
Any adverse event	24 (100.0)	23 (95.8)	21 (91.3)
Back pain	8 (33.3)	10 (41.7)	8 (34.8)
Cough	5 (20.8)	10 (41.7)	4 (17.4)
Pyrexia	7 (29.2)	10 (41.7)	6 (26.1)
Abdominal pain	14 (58.3)	9 (37.5)	8 (34.8)
Rhinitis	7 (29.2)	9 (37.5)	6 (26.1)
Nausea	2 (8.3)	8 (33.3)	2 (8.7)
Vomiting	–	8 (33.3)	2 (8.7)
Asthenia	3 (12.5)	7 (29.2)	4 (17.4)
Headache	9 (37.5)	7 (29.2)	4 (17.4)
Pharyngitis	10 (41.7)	7 (29.2)	8 (34.8)
Diarrhea	7 (29.2)	6 (25.0)	6 (26.1)
Pharyngolaryngeal pain	5 (20.8)	6 (25.0)	6 (26.1)
Influenza	1 (4.2)	5 (20.8)	5 (21.7)
Allergic conjunctivitis	–	4 (16.7)	–
Dyspepsia	1 (4.2)	4 (16.7)	2 (8.7)
Influenza-like illness	7 (29.2)	3 (12.5)	4 (17.4)
Arthralgia	4 (16.7)	2 (8.3)	3 (13.0)
Vertigo	5 (20.8)	2 (8.3)	3 (13.0)
Urinary tract infection	4 (16.7)	1 (4.2)	1 (4.3)
Bronchitis	5 (20.8)	–	1 (4.3)

2). The number of responders, defined as patients exhibiting a >10% fall in LIC by the end of study, was equal in these two groups (Table 4). In contrast, deferasirox 10 mg/kg/day resulted in only a minimal fall in LIC of -0.4 mg Fe/g dw.

Over the study duration, mean serum ferritin levels remained stable in the deferasirox 20 mg/kg/day and DFO groups whereas there was a tendency for ferritin values to increase modestly over time in patients randomized to deferasirox 10 mg/kg/day (Figure 3). There were also no consistent changes in the levels of other markers of iron homeostasis (serum iron, transferrin and transferrin saturation).

Pharmacokinetics of deferasirox

The mean steady-state pharmacokinetic profiles of each deferasirox dose are presented in Figure 4A. The median time to reach the maximal concentrations (t_{max}, ss) of deferasirox ranged from 1 to 2 hours in both the 10 and 20 mg/kg/day dose groups. Table 5 summarizes the main pharmacokinetic parameters of deferasirox after administration of the first dose and on three selected days during the steady-state. The area under the curve (AUC) at steady state was dose-dependent and the elimination half-life for 10 and 20 mg/kg/day doses ranged from 7 to 16 hours. Trough concentrations of deferasirox and deferasirox-iron complex were almost always detectable in plasma over the entire dosing interval and were dose-related (Figure 4B). The exposure to the complex was about 5-20 times less than the exposure to deferasirox. The concentrations of deferasirox and deferasirox-iron complex increased in plasma from the first dose to steady state, but showed no further accumulation after multiple dosing during the 48-week treatment period. Consistent with deferasirox's
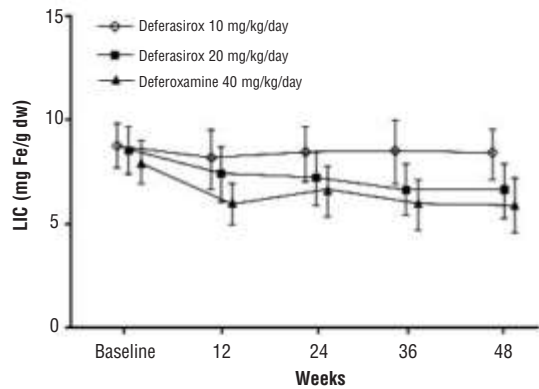
Figure 2. Liver iron concentration at baseline and after 12, 24, 36 and 48 weeks of therapy with deferasirox or DFO (mean data±SD).
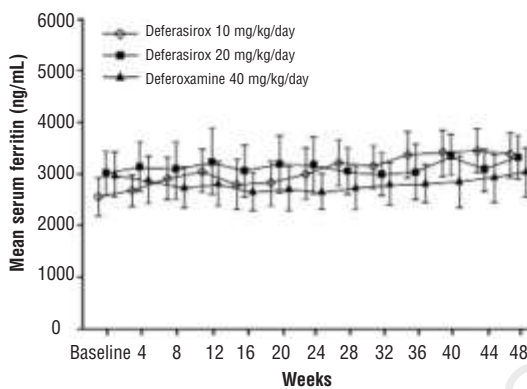

Figure 3. Serum ferritin levels over the course of the study (mean data±SD).

Table 4. Percentage change in liver iron concentration between baseline and final visit.

Percentage change in liver iron concentration	Deferasirox 10 mg/kg/day (n=24) n (%)	Deferasirox 20 mg/kg/day (n=22) n (%)	DFO 40 mg/kg (n=21) n (%)
No change (±10%)	6 (25.0)	4 (18.2)	4 (19.0)
Fall (>10%)	11 (45.8)	16 (72.7)	16 (76.2)
Increase (>10%)	7 (29.2)	2 (9.1)	1 (4.8)

Liver iron concentration was measured non-invasively using a low-TC SQUID biomagnetometer.
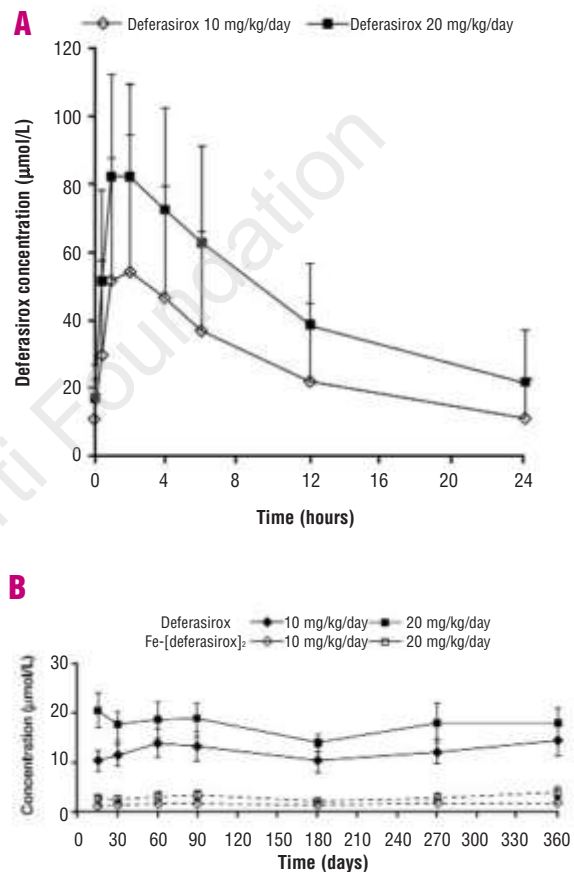

Figure 4. A. Mean steady-state plasma concentrations (+SD) of deferasirox following a dose of 10 or 20 mg/kg/day. **B.** Mean trough concentrations (±SD) of deferasirox and iron complex Fe-[deferasirox]₂.

established mode of iron removal predominantly in the bile, urinary iron excretion levels were very low. The upper limits of urinary iron detected were 58±67 µmoL/24h (mean ± SD, data from four patients) and 40±29 µmoL/24h (data from five patients) in the deferasirox 10 and 20 mg/kg/day groups, respectively.

Discussion

The primary objective of this study was to compare the safety and tolerability of daily oral doses of deferasirox (10 or 20 mg/kg) with a standard dose of DFO (40 mg/kg) over 48 weeks. During the safety evaluation, there was particular focus on toxicities that have been reported previously in the context of iron chelation therapy or that were seen during the preclinical toxicology evaluation of deferasirox. These included assessments of bone marrow, liver and kidney function, trace metal homeostasis, and screening for the development of lens opacities and high-frequency sensorineural hearing loss. Apart from differences related to the respective modes of administration, deferasirox was as well tolerated as DFO and no patient

discontinued deferasirox due to drug-related adverse events. Transient, mild-to-moderate nausea, vomiting and abdominal pain were reported at a higher frequency in patients taking deferasirox, mainly at 20 mg/kg/day, but settled spontaneously without dose interruption in all patients. Other adverse events were as expected for this patient population, with no differences between the treatment groups. No cases of arthropathy, neutropenia, agranulocytosis or falling zinc levels were seen in patients receiving deferasirox. There were no deaths during the study and none of the reported serious adverse events

Table 5. Main pharmacokinetic parameters of deferasirox in plasma after single and multiple deferasirox dose administration at selected visits.

Day	Dose (mg/kg/day) (n. of subjects)	Deferasirox C_{max} ($\mu mol/L$) mean±SD	AUC_{0-24h} ($h.\mu mol/L$) mean±SD	Half-life (h) mean±SD
1	10 (n=5)	41±17	354±186	8.9[†]±2.6
	20 (n=5)	68±23	664±265	7.2[†]±1.8
15	10 (n=4)	64±45	662±492	9.0*±4.4
	20 (n=5)	107±22	1279±208	8.6[†]±2.9
180	10 (n=6)	55±44	486±440	10.9[†]±6.3
	20 (n=4)	99±32	1262±358	9.8[†]±1.4
360	10 (n=1)	103	1210	11.6
	20 (n=8)	95±34	1001±627	9.3[‡]±2.3
	30 (n=1)	161	1811	7.4

Because a precise estimation of terminal elimination half-life was not possible, this parameter was not determined in 2*, 3[†], 4[‡] subjects.

were suspected to be drug-related. No patient developed progressive increases in serum creatinine or consecutive measurements above the upper limit of normal during either the 48-week study or during the ongoing extension study in which the patients have received deferasirox at doses of 20-30 mg/kg/day for periods of up to 3 years (*Novartis, data on file*). Increases in urinary β-2 microglobulin were seen in all treatment groups but are of doubtful clinical relevance. The findings may relate to disturbances in renal tubular function in patients with thalassemia, which have been reported by several groups and which were attributed to the toxic effects of iron overload.[30-32]

Efficacy was assessed by measuring LIC, which is widely accepted to be a reliable indicator of total body iron.[23] The LIC was measured every 3 months using non-invasive biomagnetic susceptometry, since liver biopsy, even though it allows for a direct estimate of iron load, cannot be performed more frequently than once a year. Potential variability in LIC measurements was limited by performing all assessments at a single center. Although there was wide interpatient variability in LIC results in all treatment groups, there was a clear central tendency for LIC reduction in patients receiving deferasirox 20 mg/kg/day and for LIC maintenance over 48 weeks in patients randomized to 10 mg/kg/day. At the deferasirox dose of 20 mg/kg/day, the reduction in LIC was similar to that seen with DFO 40 mg/kg. In general, the data suggest that daily doses of deferasirox in the range of 20-30 mg/kg are required to deplete iron stores in the majority of regularly transfused thalassemia patients, whereas doses of 10 mg/kg/day might be suitable for the maintenance of stable iron levels. This conclusion is partly at variance with the results of a carefully conducted iron-balance study, which showed that the daily iron excretion measured 0.119 mg/kg in a small number of patients treated for 12 days at a daily dose of deferasirox 10 mg/kg.[22] Since the average daily iron intake from blood transfusions in thalassemia patients is almost always greater than 0.3 mg/kg, the results of this earlier study suggest that a dose of deferasirox 10 mg/kg/day might be insufficient to maintain iron balance. Although dose modifications were allowed during the study, these generally occurred after 24-36 weeks of ther-

apy due to the requirement of having LIC values from at least three consecutive biomagnetic liver susceptometry evaluations before making such modifications. The average daily dose of study drug was, therefore, close to the initial dose, being 11.7 and 19 mg/kg in patients randomized to deferasirox 10 and 20 mg/kg, respectively. As they were dictated by a rise in LIC, dose increases occurred in all treatment groups but were more frequent in patients treated with deferasirox 10 mg/kg/day. Dose decreases due to excessive falls in LIC occurred in two patients treated with deferasirox 20 mg/kg/day but in none treated with deferasirox 10 mg/kg/day or DFO.

Surprisingly, mean serum ferritin levels remained more or less constant throughout and did not mirror the clear trends in LIC that were observed across the treatment groups. The absence of concordance between LIC and serum ferritin in this study probably reflects the wide variability in ferritin levels at baseline, both within and between patients, coupled with the relatively small sample size. In contrast, a recently reported large phase III trial of deferasirox found robust parallel trends between LIC and serum ferritin levels.[33] In the relatively small study reported here, no consistent trends were noted in the levels of serum ferritin or other markers of iron overload including serum iron, transferrin and transferrin saturation. Emerging techniques for the non-invasive measurement of cardiac iron have added a new dimension to the analysis of efficacy of iron chelators. Such techniques were in their infancy at the time this preliminary study was designed, and measurement of cardiac iron load was not included. Current trials of deferasirox include analysis of cardiac iron load using T2* cardiovascular magnetic resonance.[34-36] The results of these studies should cast light on the ability of deferasirox to remove excess iron from the heart, and provide valuable additional evidence on the clinical effects of deferasirox.

This study allowed the first evaluation of the pharmacokinetics of deferasirox after long-term administration. Importantly, trough plasma sampling indicated that the levels of deferasirox remained stable throughout the treatment period. Both deferasirox and Fe-[deferasirox]₂ persisted at detectable levels in the blood over the entire dosing interval. Deferasirox was absorbed with a median t_{max} of about 12 hours post dose. The elimination half-life in plasma of deferasirox, which was around 7 to 16 hours and generally longer for the iron complex, allows for a convenient, once-daily administration schedule. In contrast, because of its short elimination half-life, deferiprone must be administered three times a day and strict compliance with this demanding schedule is unlikely. As reported in previous studies,[21,22] urinary iron excretion was negligible indicating that this parameter is unsuitable for monitoring the efficacy of chelation therapy with deferasirox, contrasting with the utility of this approach for monitoring treatment with DFO. In conclusion, this phase II study over 48 weeks comparing two doses of deferasirox with a standard dose of DFO in β-thalassemia patients demonstrated that deferasirox 20 mg/kg given orally, once daily is as well-tolerated and efficacious in reducing LIC as DFO 40 mg/kg given by sc infusions for 5 days each week. The pharmacokinetic profile of deferasirox supports a convenient, once-daily, oral administration schedule, which offers

the potential to improve compliance in comparison with that achieved with other available chelators. These preliminary results suggest that deferasirox is a well-tolerated, oral iron chelator with similar efficacy to DFO and, if confirmed in a larger number of patients, would represent a significant advance in the treatment of iron overload.

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