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Safety, immunogenicity and efficacy of subcutaneous biosimilar epoetin-α (HX575) in non-dialysis patients with renal anemia: a multi-center, randomized, double-blind study

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Key words
anemia – chronic kidney disease – HX575 – pre-dialysis – subcutaneous

Abstract. Background: HX575 is a biosimilar version of epoetin-α that is approved for the treatment of anemia associated with chronic kidney disease (CKD) using the intravenous route of administration. Here we report data from a study of anemic pre-dialysis patients to assess the safety, immunogenicity and efficacy of subcutaneous (s.c.) administration of HX575 vs. Erypo®/Eprex® (Ortho Biotech, Neuss, Germany). Methods: This was a randomized, double-blind study in adult patients (n = 337) with Stage III – V CKD and a hemoglobin (Hb) level of 7.5 – 11.0 g/dl. Eligible patients were randomized to 52 weeks of treatment with HX575 or Erypo®/Eprex® at a starting dose of 25 IU/kg body weight 3 times weekly or 75 IU/kg body weight once weekly during Weeks 1 – 5. This could be adjusted after 5 weeks to maintain Hb levels between 10 and 12 g/dl. The primary objective was to assess the safety and immunogenicity of HX575 compared with Erypo®/Eprex®. Efficacy endpoints were mean absolute change in Hb from baseline to end of Week 13 and mean weekly epoetin dosage in Weeks 11 – 13. Results: HX575 was equivalent to Erypo®/Eprex® in terms of maintaining Hb levels and epoetin dose requirements. Two patients in the HX575 group developed neutralizing antibodies (NAbs) to erythropoietin, which resulted in the study being terminated prematurely. Aside from these two events, reported adverse events were as expected for patients with Stage III – V CKD and similar in both treatment groups. Conclusions: This study demonstrated the efficacy and therapeutic equivalence of s.c. HX575 compared with the reference epoetin-α, but 2 patients developed NAbs during treatment with s.c. HX575 in this study. Results of a thorough root-cause analysis reported elsewhere indicate that increased tungsten exposure in pre-filled syringes precipitated immunogenic reactions.

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Introduction

Over the past two decades, erythropoiesis-stimulating agents (ESAs) have become the mainstay of treatment for anemia associated with chronic kidney disease (CKD). HX575 (Binocrit®, Sandoz, Holzkirchen, Germany) is a biosimilar version of epoetin-α that is approved by the European Medicines Agency (EMA) for the treatment of anemia associated with CKD in adult and pediatric patients using the intravenous (i.v.) route of administration [1]. HX575 is also approved for: (1) the treatment of anemia and reduction of transfusion requirements in adult patients receiving chemotherapy for solid tumors, malignant lymphoma or multiple myeloma using the subcutaneous (s.c.) route of administration; (2) to increase the yield of autologous blood from patients in a predonation program (i.v. administration); and (3) to reduce exposure to allogeneic blood transfusions in adult non-iron-deficient patients prior to major elective orthopedic surgery (s.c. administration).

HX575 was not tested for s.c. use in patients with CKD because, at the time the regulatory Phase III studies were performed, the license for s.c. use of the reference product

(Erypo®/Eprex®) had been withdrawn following an increase in the incidence of pure red cell aplasia (PRCA) with this product. Comparison of s.c. administration of HX575 with its reference product in patients with anemia associated with CKD became possible once the licence for s.c. use of Erypo®/Eprex® was re-instated. Here we report data from a randomized, double-blind, multicenter study of anemic pre-dialysis patients to assess the safety, immunogenicity and efficacy of s.c. administration of HX575 vs. Erypo®/Eprex®.

Methods

This was a randomized, controlled, double-blind trial involving patients enrolled at 89 centers across Austria, Bulgaria, the Czech Republic, France, Germany, India, Poland, Romania, Russia and Slovakia. Relevant Ethics Committees/Institutional Review Boards approved the protocol and the trial was conducted in accordance with the Declaration of Helsinki, ICH Good Clinical Practice and any applicable local regulations. All patients provided written informed consent.

Patients

Patients aged ≥ 18 years were eligible for inclusion if they had known CKD of ≥ 4 weeks duration with a diagnosis of CKD Stage III – V, anemia (hemoglobin (Hb) level of ≥ 7.5 and < 11.0 g/dl on at least two visits during the screening period), were naïve to ESA treatment or with an ESA treatment-free period of ≥ 3 months before enrolment, and had adequate iron status (serum ferritin ≥ 100 mg/l or transferrin saturation ≥ 20%). Exclusion criteria included chronic dialysis within the prior 6 months; non-renal anemia; acute deterioration of renal function or blood transfusion during screening; suspicion of, or known, PRCA; any hematological disorder; thrombocytopenia or leucopenia; evidence of uncontrolled diabetes, uncontrolled hypertension, uncontrolled hyperparathyroidism or severe hepatic dysfunction; congestive heart failure and/or angina; myocardial infarction or stroke in the previous 6 months; acute or chronic infection; previous gastrointestinal bleeding (within 6 months) or hemolysis; evidence of active malignancy within the previous 5 years (except non-melanoma skin cancer); therapy with immunosuppressants (other than corticosteroids for chronic disease) within 3 months of screening; or known allergy to test products or hypersensitivity to mammalian-derived products.

Study design

Patient eligibility was assessed during the 2-week screening period, after which eligible patients were randomized to 52 weeks of treatment with HX575 or Erypo®/Eprex®. Block randomization was performed stratified by center. All randomized patients received a starting dose of 25 IU/kg body weight 3 times weekly or 75 IU/kg body weight once weekly during Weeks 1 – 5 (with no randomization or stratification with respect to the starting dose). This could be adjusted after 5 weeks to maintain Hb levels between 10 and 12 g/dl.

Blood samples for determination of erythropoietin antibodies were taken at screening and Weeks 8, 16, 24, 40 and 52, and analyzed using a validated radioimmunoprecipitation (RIP) assay (MDS Pharma Services Switzerland AG, Fehraltorf, Switzerland). If the RIP assay was positive, a second aliquot was analyzed using a validated neutralizing antibody (NAb) assay (Hexal AG, Oberhaching, Germany). This assay determines the ability of erythropoietin antibodies found in patient sera to inhibit the erythropoietin-dependent growth of UT-7 cells.

During the 6-month follow-up period, all RIP-positive samples underwent confirmatory testing in an independent laboratory (Professor Nicole Casadevall, Department of Hematology, Hôpital Saint Antoine, Paris, France).

Objectives

The primary objective was to assess the safety and immunogenicity of HX575 compared with Erypo®/Eprex® during long-term s.c. administration. The study was originally intended as a safety study with no formal sample size calculation and no efficacy variables planned. However, after premature termination of the study two post-hoc efficacy endpoints were introduced before un-

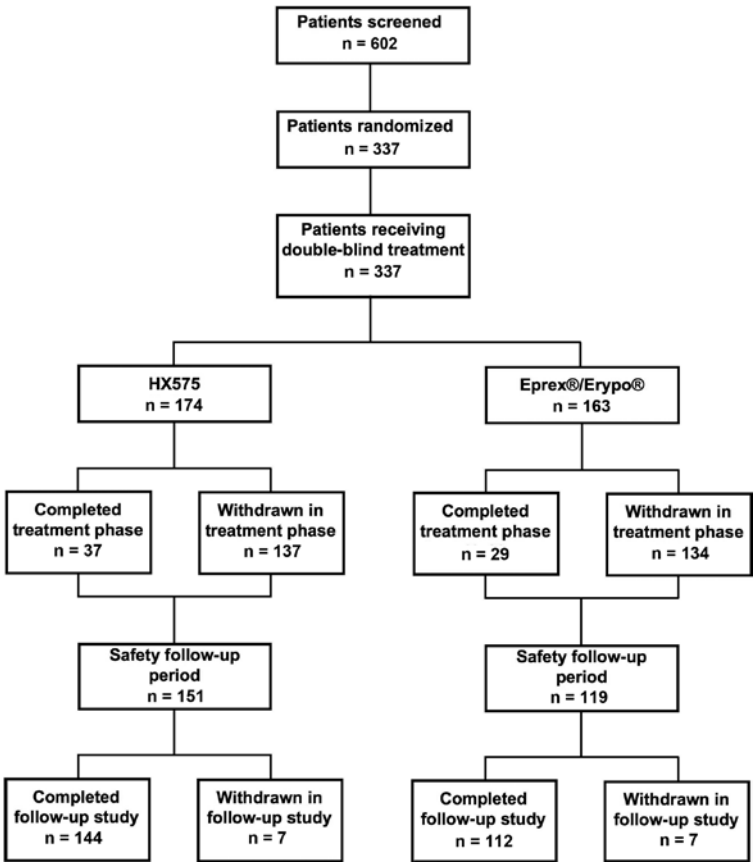

Figure 1. Disposition of patients.

Table 1. Baseline demographic and clinical characteristics (safety population).

	HX575 (n = 174)	Erypo®/Eprex® (n = 163)
Mean age (range), years	64.1 (19 – 88)	64.9 (20 – 90)
Mean weight (range), kg	75.5 (37.5 – 174.0)	73.5 (40.3 – 150.0)
Sex, n (%)		
Male	77 (44.3)	65 (39.9)
Female	97 (55.7)	98 (60.1)
Ethnic group, n (%)		
Caucasian	167 (96.0)	155 (95.1)
Other	7 (4.0)	8 (4.9)
Primary cause of CKD, n (%)		
Diabetes	49 (28.2)	62 (38.0)
Hypertension	36 (20.7)	28 (17.2)
Interstitial nephritis	17 (9.8)	22 (13.5)
Chronic glomerulonephritis	33 (19.0)	26 (16.0)
Polycystic kidney disease	7 (4.0)	7 (4.3)
Urologic	9 (5.2)	6 (3.7)
Other	16 (9.2)	10 (6.1)
Unknown	7 (4.0)	2 (1.2)
Mean baseline Hb (range), g/dl	9.7 (6.8 – 11.6)	9.9 (6.8 – 12.2)

CKD = chronic kidney disease; Hb = hemoglobin.

blinding and data analysis. These were mean absolute change in Hb from baseline to end of Week 13 and mean weekly epoetin dosage in Weeks 11 – 13. Safety analysis was based on the safety population (all patients who received at least one dose of study medication). Efficacy analysis was based on the per-protocol population, which included all patients in the safety set who completed at least 13 weeks of treatment with no major protocol violations.

Results

A total of 602 patients was screened, of whom 337 were randomized and received at least one dose of study treatment (HX575, n = 174; Erypo®/Eprex®, n = 163). 66 patients (19.6%) completed the treatment period before the study was halted. The 6-month safety follow-up included 270 patients (HX575, n = 151; Erypo®/Eprex®, n = 119). Disposition of patients is shown in Figure 1.

Demographic and clinical baseline characteristics were similar between treatment groups (Table 1). Mean ± standard deviation (SD) treatment duration was similar in both groups (31.9 ± 12.5 weeks in the HX575 group vs. 30.2 ± 13.6 weeks in the Erypo®/Eprex® group). Overall patient exposure was 114 patient-years in the HX575 group and 102 patient-years in the Erypo®/Eprex® group.

Efficacy

The mean ± SD increase in Hb from baseline to Week 13 was 2.2 ± 0.9 g/dl in the HX575 group and 2.2 ± 1.0 g/dl in the Erypo®/Eprex® group, with an estimated difference of –0.01 g/dl. Since the 2-sided 95% confidence interval (CI) for the difference (–0.21 – 0.19) was entirely within the predefined equivalence region of –1.0 – 1.0 g/dl, HX575 and Erypo®/Eprex® were considered as equivalent. Figure 2 shows the mean Hb level over time in both treatment groups; the Hb curves overlap in both the correction and maintenance phase of the study.

Mean weekly epoetin dose in Weeks 11 – 13 was 51.7 ± 41.9 IU/kg in the HX575 group and 51.6 ± 43.5 IU/kg in the Erypo®/Eprex® group. The estimated difference was 0.02 IU/kg, with the 2-sided 95% CI of –9.94 – 9.97 entirely within the pre-defined equivalence region (–45 – 45 IU/kg). Mean

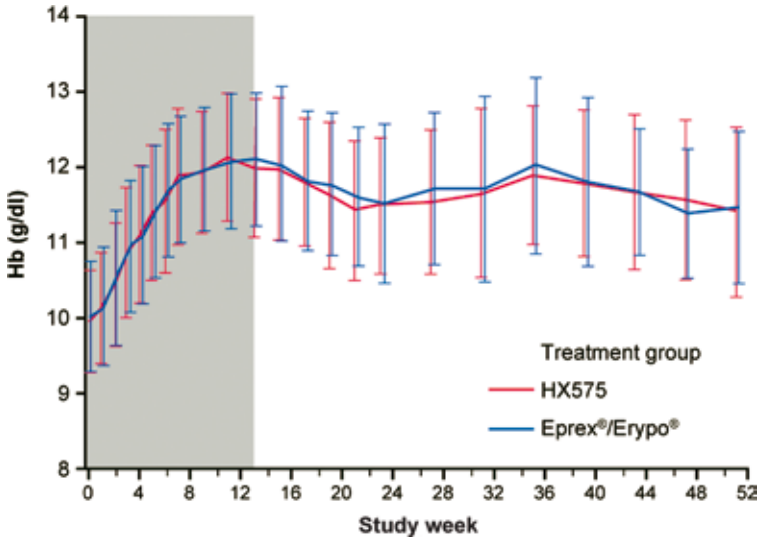

Figure 2. Time course (mean ± SD) of Hb levels (per protocol set).

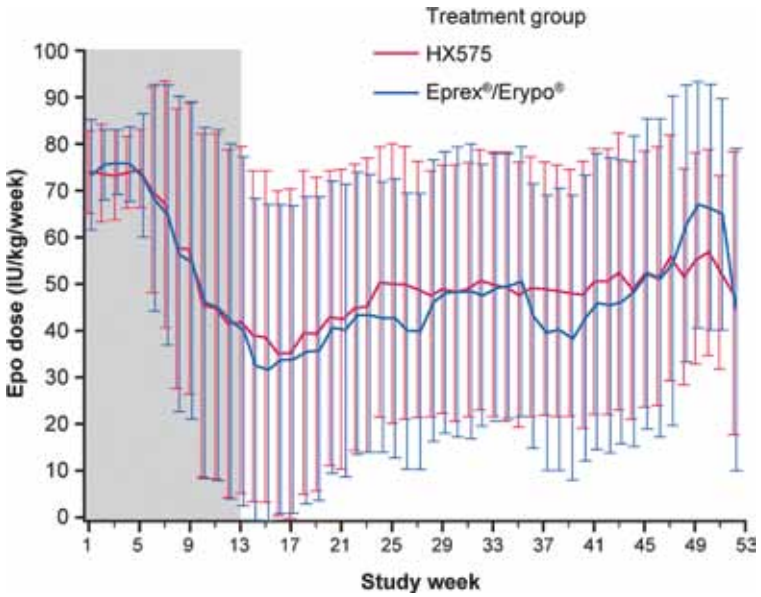

Figure 3. Time course (mean ± SD) of weekly epoetin doses (per protocol set).

epoetin dose over time in both treatment groups is shown in Figure 3; again, overlapping curves are demonstrated for both the correction and maintenance phase of the study.

Premature study discontinuation and safety follow-up

On June 9, 2009 (5 months after the last patient was enrolled), treatment with the investigational and comparator drugs was prematurely halted by the sponsor, with concurrence and the recommendation of the Data Safety Monitoring Board (DSMB), after 2 patients had tested positive for erythropoietin-NAbs. The study continued for safety follow-up, with all randomized patients who had received at least one dose of study medication followed-up for a period of 6 months, during which monthly visits were performed. All patients who had not yet completed the study had to stop treatment with study medication and were switched to standard medical care and ESA treatment available in their respective centers. An extensive investigation was initiated to determine the root-cause of the NAbs.

Incidence of anti-epoetin antibody formation

Three patients showed positive antibody results from the sensitive screening RIP assay and had been randomized to the HX575 group before the results became available. The corresponding NAb tests were negative (the "false-positive" rate of samples positive in the RIP assay but not confirmed in the NAb assay is known to be ~5%). All 3 patients were withdrawn from the study when the positive RIP results became known. No information on pre-treatment with any other ESA was available.

During the treatment period of the study, 5 patients (2.9%) in the HX575 group and 2 patients (1.2%) in the Erypo®/Eprex® group had positive RIP results. Two of the 5 patients in the HX575 group with a positive RIP result had a positive and confirming NAb result. These 2 cases are discussed in more detail below. NAb assays were negative for the other patients with a positive RIP result, suggesting once again "false-positive" RIP assays. A further 4 patients in the HX575 group and 2 patients in the Erypo®/Eprex® group showed positive RIP results during the 6-month follow-up period of the study. The corresponding NAb results were negative in all cases.

Case reports of patients with NAbs

Case 1

The first patient to develop NAbs was an 81-year-old male who weighed 63.5 kg. He

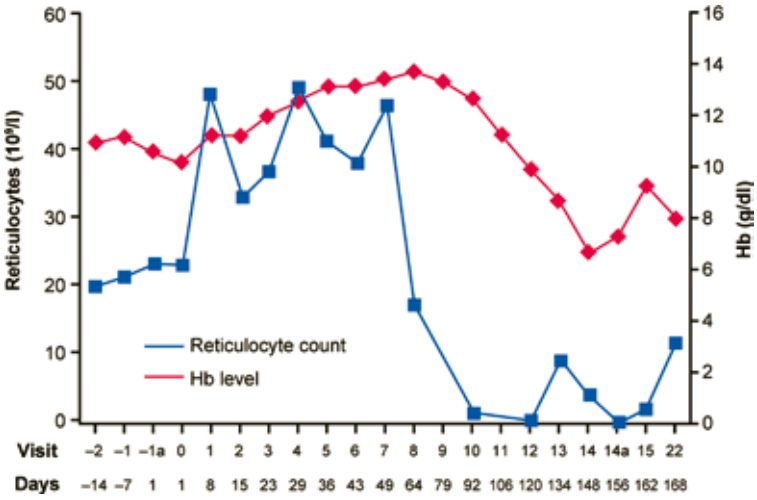

Figure 4. Time course of Hb level and reticulocyte count (Case 1).

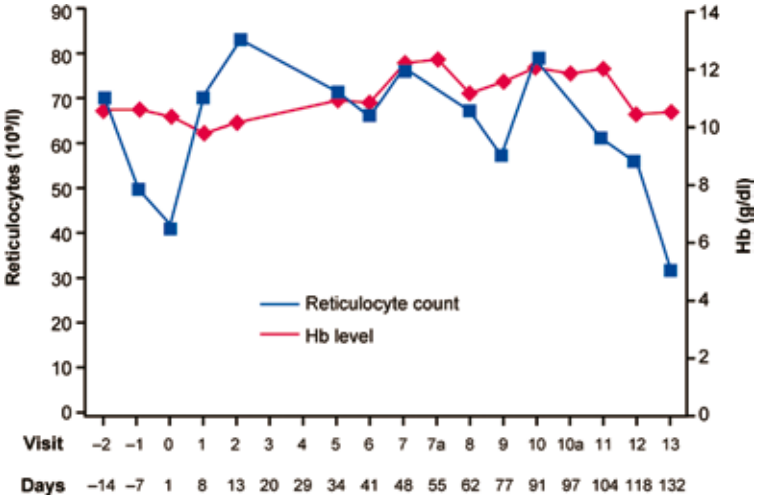

Figure 5. Time course of Hb level and reticulocyte count (Case 2).

had CKD due to hypertensive nephropathy, and reported multiple co-morbidities including heart failure (NYHA II), carcinoma of the prostate in remission, osteoarthritis in the right knee, hypothyroidism after radioiodine therapy, secondary hyperparathyroidism, vitamin D deficiency, and previous infection with parvovirus (with high titres of IgG antibodies against parvoB19 (2.4, normal < 0.9), IgM 0.2 (normal < 0.9)). He was in receipt of several concomitant medications (including intravenous iron sucrose) and was ESA-naïve.

The patient commenced study treatment with HX575 at an initial dose of 5,000 IU/week for ~8 weeks. The time course of Hb level and reticulocyte count are shown in Figure 4. Treatment with HX575 was interrupted (as per protocol) following a Hb increase above 13.0 g/dl, and reintroduced 2 months later at a dose of 3,000 IU/week and a Hb level of 9.9 g/dl. Subsequently, Hb levels did not increase and dropped to 6.6 g/dl despite the dose being increased to 6,000 IU/week. Upon restarting treatment with HX575, the patient complained of generalized pruritus; this was treated with prednisone. Of note, the patient had a history of food allergies. The patient was hospitalized 1 month later due to profound anemia (Hb level 6.3 g/dl). Despite protocol-compliant epoetin treatment, the patient's reticulocyte counts were very low ($< 5 \times 10^9$/l). HX575 treatment was stopped and the patient received blood transfusions. Possible causes of the persistent refractory anemia were investigated according to the diagnostic algorithm, and antibody assays were performed.

The pre-study screening RIP assay was negative for anti-epoetin antibodies. A subsequent on-study RIP assay detected low levels of binding antibodies, but the NAb assay was negative indicating that the detected antibodies were not neutralizing. After 5 months in the study, both the RIP and NAb anti-epoetin assays were positive. PRCA was subsequently confirmed by bone marrow examination at a recognized reference laboratory (University of Freiburg, Germany).

Case 2

The second patient to develop NAbs was a 79-year-old male who weighed 65.4 kg. This patient had CKD Stage 4 and had multiple cardiovascular co-morbidities, including angina pectoris, three previous myocardial infarctions with ongoing coronary artery disease, ventricular extrasystoles, chronic heart failure (NYHA II) and essential hypertension. He was receiving several concomitant medications, including previous treatment for mercury intoxication, and was ESA-naïve.

The patient commenced treatment with HX575 at an initial dose of 5,000 IU/week for 1 month. The time course of Hb level and reticulocyte count are shown in Figure 5. The pre-study screening RIP assay was negative for anti-epoetin antibodies. Three months following commencement of HX575, the RIP assay became positive but the NAb assay was negative. Subsequently, both the RIP and NAb assays were positive in a routine

Table 2. Summary of adverse events during the treatment and safety follow-up periods.

			HX575 (n = 174, treatment period; n = 151, safety follow-up)			Erypo®/Eprex® (n = 163, treatment period; n = 119, safety follow-up)		
			n	%	events	n	%	events
Treatment period	All TEAEs	Overall	137	78.7	574	132	81.0	615
		Non-serious	125	71.8	462	119	73.0	463
		Serious*	50	28.7	112	70	42.9	152
	Drug-related TEAEs	Overall	28	16.1	43	25	15.3	40
		Non-serious	28	16.1	39	25	15.3	40
		Serious	3	1.7	4	0	0.0	0
Safety follow-up	AEs	Overall	87	57.6	200	58	48.7	130
		Non-serious#	80	53.0	165	46	38.7	89
		Serious	22	14.6	35	24	20.2	41
	Drug-related AEs	Overall	2	1.3	2	2	1.7	2
		Non-serious	2	1.3	2	2	1.7	2
		Serious	0	0.0	0	0	0	0

AE = adverse event; TEAE = treatment-emergent adverse event; *p = 0.0087 Erypo®/Eprex® vs. HX575; #p = 0.0202 HX575 vs. Erypo®/Eprex® (Fisher's exact-test).

blood sample drawn ~5 months after starting HX575; the Hb level at the same time was 10.5 g/dl. The patient died 6 days later as a result of a fourth myocardial infarction, which was considered unrelated to the development of antibodies by the treating physician and the DSMB. No bone marrow examination could be performed; however, reticulocyte count decreased from 56 to 32 × 10^9/l, and a subsequent RIP assay was positive with a weakly positive NAb assay. Of note, there was no clinical evidence of PRCA before the patient died; however, there was an evolution to a positive RIP assay and a weakly positive NAb assay, coinciding with a decline in reticulocyte levels.

Adverse events

The overall incidence of adverse events (AEs) was similar in the two treatment groups (Table 2). During the treatment period, one or more AEs were experienced by 78.7% (137/174) of patients in the HX575 group and 81.0% (132/163) of patients in the Erypo®/Eprex® group. Serious AEs were experienced by 28.7% (50/174) and 42.9% (70/163) of patients in the HX575 and Erypo®/Eprex® groups, respectively. Six patients in the HX575 group and 14 patients in the Erypo®/Eprex® group died during the treatment period. No deaths were considered to be related to the study drug.

The incidence of drug-related AEs was similar in the HX575 group (16.1%, 28/174) and Erypo®/Eprex® group (15.3%, 25/163). Hypertension was the most frequently reported drug-related AE.

In the 6-month safety follow-up period, AEs were experienced by 57.6% (87/151) of patients in the HX575 group and 48.7% (58/119) of patients in the Erypo®/Eprex® group. Serious AEs were experienced by 14.6% (22/151) and 20.2% (24/119) of patients in the HX575 and Erypo®/Eprex® groups, respectively.

Root-cause investigation

A thorough root-cause evaluation was initiated immediately by Sandoz internal personnel and guided by an external international advisory board; full results have been reported by Seidl et al. [2]. This investigation identified increased tungsten concentrations precipitated during the manufacture of prefilled syringes; this elevated tungsten stimulated denaturation of epoetin molecules and the subsequent formation of immunogenic aggregates.

Discussion

In this study, s.c. HX575 was shown to be therapeutically equivalent to the compara-

tor epoetin-α with respect to mean absolute change in Hb levels and weekly epoetin dose.

However, 2 elderly male CKD patients developed NAbs during treatment with s.c. HX575 in this study. Of the 2 cases, one patient developed PRCA while the other died from unrelated cardiovascular disease at a time when his Hb level was not severely decreased. PRCA is a rare adverse effect of treatment with ESAs, caused by the development of NAbs to the endogenous and recombinant hormone [3]. The incidence of PRCA associated with epoetin therapy peaked in 2002 [4]. This peak was due to an increased incidence rate in patients who were treated with epoetin-α (Erypo®/Eprex®) administered by the s.c. route [5]. Although the exact cause of the increased immunogenicity in these cases could not be definitively proven, the common underlying cause is hypothesized to be related to formation of aggregates. The Erypo®/Eprex® label was changed to permit only i.v. use, measures were taken to improve the cold-chain management, and uncoated rubber stoppers were replaced with Teflon-coated stoppers in pre-filled syringes. Subsequently, the incidence of PRCA rapidly declined and s.c. use of Erypo®/Eprex® was reinstated. However, background PRCA persists at a low rate and cases have been reported in patients receiving most ESAs [4, 6]. Until recently, there were no confirmed reports of PRCA in patients treated exclusively by i.v. administration; however, a very recent report from Japan describes what appears to be the first such case, with the patient receiving both epoetin-α and darbepoetin-α on separate occasions [7]. There are no reported cases in patients with cancer receiving s.c. epoetin for chemotherapy-induced anemia or to reduce exposure to allogeneic blood transfusions.

There have also been sporadic cases of antibody-mediated PRCA reported following the use of locally manufactured epoetins in Asia and Latin America [8]. It is important to distinguish between these alternative biologic products (manufactured outside of Europe and the United States) and true biosimilar epoetins, which are approved under a strict regulatory pathway such as the one set out by the EMA [9, 10, 11, 12]. The products manufactured in Asia and Latin America required only bioequivalence data to gain marketing approval (in the same way as conventional generic medicines), with no requirement for clinical trial data or formal post-marketing surveillance [13]. In addition, analytical studies of these products have shown them to vary widely in composition, suggesting that some manufacturers do not have adequate control over their production processes [14, 15]. In contrast, biosimilar epoetins approved under the EMA regulatory pathway are required to undergo extensive physicochemical characterization to demonstrate similarity to the originator product [16]. The EMA biosimilar regulatory pathway also mandates clinical trial data in at least one representative indication and a comprehensive risk management plan [9, 10, 11, 12].

The incidence of anti-epoetin antibody formation (RIP assay) in the present study was 0.0437 per patient-year with HX575 and 0.0196 per patient-year with Erypo®/Eprex®, although this may be misleading since most of these cases were shown to be non-neutralizing. The incidence of neutralizing anti-epoetin antibody formation (NAb assay) was 0.0175 per patient-year with HX575 and 0.000 per patient-year with Erypo®/Eprex® since no case was reported in this study. Based on published data, the background incidence of epoetin-associated PRCA is 0.002 – 0.005 per patient-year [17, 18].

There is continuing debate around which factors can induce an immune response to epoetin, but both patient- and product-related factors may play a role [19]. Product-related factors possibly include the presence of impurities and structural modifications resulting from the manufacturing process, formulation of the product, inappropriate storage or handling and route of administration. Patient-related factors may include the disease being treated, the presence of concomitant illness, the patient's immune status and genetic factors [19, 20, 21].

A full clinical and analytical investigation was undertaken by the study sponsor and a group of external experts (including nephrologists, hematologists, physiologists and immunologists) to identify any potential root cause for the findings of this study. This comprehensive evaluation is reported in more detail elsewhere; briefly, this study found that tungsten species in the pre-filled syringes caused the HX575 protein to unfold, which subsequently

resulted in formation of aggregates. At least a proportion of these appeared to be irreversible and held together by disulphide bonds [2], and such species may represent an important marker of conditions that are sufficient to stimulate immunogenicity. The most likely source of the tungsten is heat-resistant pins used in the manufacture of glass syringes. Tungsten from this source has also been implicated in the aggregation of other biopharmaceuticals [22, 23, 24]. It is important to note that no cases of NAbs or PRCA have been reported in association with the use of commercially available i.v. HX575 (estimated exposure 71,000 patient-years as of August 2010) (Sandoz data on file). No unexpected side effects or immunogenicity were reported in a Phase III study of HX575 in 478 patients receiving hemodialysis, a Phase IV study with 1,697 patients, or among 2,235 hemodialysis patients currently under observation in post-marketing surveillance studies [25, 26, 27, 28, 29].

Aside from these 2 cases of NAbs, reported AEs were typical for patients with Stage III or V CKD and comparable in both treatment arms, and the overall safety profile of HX575 was comparable with that of Erypo®/Eprex®. HX575 and Erypo®/Eprex® were equally effective in the correction of anemia, with an equivalent Hb response achieved with similar doses. After successful correction of anemia, Hb levels remained stable and comparable with both treatments. No patient discontinued the study due to an inadequate response to treatment.

The regulatory requirements laid down by the EMA for biosimilars based on recombinant erythropoietin are more stringent than for biosimilar versions of other recombinant proteins [30]; therapeutic equivalence with the reference product must be demonstrated in a minimum of two randomized, parallel-group (and preferably double-blind) clinical trials, ideally in patients with anemia due to CKD [12]. In these trials, equivalence must be shown for both predialysis CKD patients and those on hemodialysis, and by the i.v. and s.c. routes of administration. The clinical trial requirements are, however, abbreviated in comparison with originator products, with no Phase II studies required. It is important to note that the abbreviated program required by the EMA proved sufficient to detect a relevant safety issue.

In conclusion, this study demonstrated the efficacy and therapeutic equivalence of s.c. HX575 compared with the reference epoetin-α. Two patients developed NAbs during treatment with s.c. HX575 in this study. Extensive clinical and analytical investigations have suggested aggregation caused by tungsten (used in the manufacture of glass syringes) as the most likely root cause of the immunogenic response, and these findings are currently being discussed with health authorities. There have been no reports of NAbs or PRCA associated with the use of HX575 in accordance with the current label (i.v. administration).

Acknowledgments

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Conflict of interest

Andrea Vetter and Karsten Roth are employees of Hexal AG, a division of Sandoz Biopharmaceuticals. Simon Roger and Walter Hörl have acted as advisors to Sandoz Biopharmaceuticals. Marianne Haag-Weber and Kai-Uwe Eckardt have acted as investigators on studies sponsored by Sandoz Biopharmaceuticals/Hexal AG.

Registration

ClinicalTrials.gov, NCT00701714.

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