ELSEVIER

European Journal of Cancer 41 (2005) 1140–1149

European Journal of Cancer

www.ejconline.com

Effects of chemotherapy on endogenous erythropoietin levels and the pharmacokinetics and erythropoietic response of darbepoetin alfa: A randomised clinical trial of synchronous *versus* asynchronous dosing of darbepoetin alfa

John Glaspy [a,*], David Henry [b], Ravi Patel [c], Simon Tchekmedyian [d], Steve Applebaum [a], Donald Berdeaux [e], Richard Lloyd [f], Russell Berg [g], Matt Austin [g], Greg Rossi [g], the Darbepoetin Alfa 20010162 Study Group

[a] *UCLA, School of Medicine, 100 UCLA Medical Plaza, Suite 550, Los Angeles, CA 90095-6956, USA*
[b] *Joan Karnell Cancer Center, Philadelphia, PA, USA*
[c] *Comprehensive Blood and Cancer Center, Bakersfield, CA, USA*
[d] *Pacific Shores Medical Group, Long Beach, CA, USA*
[e] *Great Falls, MT, USA*
[f] *Virginia K. Crosson Cancer Center, Fullerton, CA, USA*
[g] *Amgen, Thousand Oaks, CA, USA*

Received 11 October 2004; received in revised form 5 January 2005; accepted 27 January 2005
Available online 8 April 2005

Abstract

The introduction of longer-acting erythropoietic agents into the practice of oncology has demanded an understanding of the interaction of chemotherapy with the pharmacokinetics and haematological effects of these erythropoietins. We report results of a randomised trial comparing the haematological effects of darbepoetin alfa, 6.75 μg/kg, administered once every 3 weeks to anaemic cancer chemotherapy patients on either an asynchronous (day 15) or synchronous (day 1) schedule relative to their every-3-week chemotherapy. A total of 81 patients were randomised and received the study drug (43 asynchronous; 38 synchronous). No difference was observed between groups in the primary endpoint of mean haemoglobin change after 6 weeks of therapy ($P = 0.45$) and change scores were similar to those observed with standard weekly darbepoetin alfa therapy. In a subset of patients evaluated with intensive pharmacokinetic sampling, an increase in endogenous erythropoietin concentration (up to 4-fold) lasting approximately 1 week following chemotherapy administration was observed in both groups. Synchronous administration of darbepoetin alfa was associated with a 1.3-fold increase in the area under the darbepoetin alfa concentration–time curve compared with asynchronous administration. Our data suggest that darbepoetin alfa is effective administered every 3 weeks regardless of timing of administration with respect to chemotherapy and that receptor-mediated uptake by the erythron may be an important clearance mechanism for erythropoietic proteins.

Keywords: Darbepoetin alfa; Pharmacokinetics; Chemotherapy; Erythropoietin synchronous; Asynchronous

1. Introduction

Anaemia due to chronic illness and chemotherapy is frequently observed in patients with cancer and may

* Corresponding author. Tel.: +1 310 794 4955; fax: +1 310 794 1460.
E-mail address: jglaspy@mednet.ucla.edu (J. Glaspy).

0959-8049/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ejca.2005.01.021

be treated with erythropoietic agents, such as darbepoetin alfa (Aranesp®) and epoetin alfa [1]. Erythropoietic therapy increases haemoglobin concentrations, decreases the incidence of red blood cell (RBC) transfusions, and improves health-related quality of life in patients with chemotherapy-induced anaemia [2–12]. Darbepoetin alfa has the unique structure of increased sialic acid content compared with epoetin alfa. This structure appears to confer a longer serum half-life and may allow for less frequent dosing with similar efficacy as epoetin alfa [13].

The pharmacokinetics and pharmacodynamics of endogenous and recombinant human erythropoietin (eEPO and rHuEPO, respectively) are of clinical interest but are not well understood. A few studies have attempted to elucidate these characteristics, especially regarding the clearance mechanism of eEPO. One hypothesis supports a two-step clearance process [14] in which blood- or tissue-resident sialidases remove terminal sialic acids from eEPO. The asialoerythropoietin then interacts with an asialoglycoprotein receptor (ASGR) in the liver, which causes uptake and catabolism [15–17]. Some limitations, however, exist for this hypothesis, as no evidence of this asialyation for the recombinant human erythropoietin molecule in humans and no sialidase activity or specificity for glycoproteins (rather than gangliosides) have been demonstrated [18]. Furthermore, rHuEPO does not preferentially accumulate in the liver [14] and hepatectomy does not affect the rate of clearance in sheep [19]. These data suggest that the liver is not a route of clearance for erythropoietin. Another hypothesis is that EPO receptor-bearing target cells may be a route of clearance, through the mechanism of binding, internalisation, and degradation [20–26]. Evidence that serum eEPO levels increased transiently in humans after administration of high-dose chemotherapy suggested that the bone marrow may clear erythropoietic proteins [27]. For darbepoetin alfa, pharmacokinetic data [28] indicated that the terminal half-life increased with standard-dose chemotherapy (compared with the half-life calculated in dialysis patients), presumably through decreased clearance.

Since the bone marrow may be a key route of clearance of erythropoietic agents, the timing of administration of these agents relative to that of myelosuppressive chemotherapy may be critical to producing the maximum erythropoietic response. In a randomised, placebo-controlled clinical trial investigating the dose response relationship of darbepoetin alfa in patients with chemotherapy-induced anaemia, darbepoetin alfa was effective when administered every 3 weeks [29]. As many chemotherapy regimens are administered every 3 weeks, darbepoetin alfa administered every 3 weeks represents an opportunity to synchronise dosing with chemotherapy. However, while synchronous dosing of erythropoietic agents with chemotherapy is convenient, the efficacy of the agent may not be maximised, as the marrow may be too myelosuppressed by the cytotoxic chemotherapeutic agents to enable a maximum response to erythropoietin.

Pre-clinical data using a murine model of carboplatin chemotherapy/radiotherapy (CRT)-induced anaemia indicate that pre-treatment (7 d prior to administration of CRT) with darbepoetin alfa represented the most effective dosing approach compared with same day dosing or post-treatment (10 d post-CRT) dosing algorithms [30]. However, changes in the pharmacokinetic profiles were also observed; which included a reduction in clearance (CL/F) and an increase both in overall exposure ($AUC_{(0-\infty)}$) and in time to peak concentration (T_{max}) in animals in the same day dosing group compared with animals in the pre-treatment group. These findings indicated that clinical investigation into the effect of synchronisation of chemotherapy and erythropoietic therapy is warranted.

To evaluate the effect of the timing of administration of darbepoetin alfa with that of chemotherapy, we conducted a randomised clinical trial in anaemic patients with cancer receiving chemotherapy once every 3 weeks, who received darbepoetin alfa once every 3 weeks, either asynchronously (day 15) or synchronously (day 1) with chemotherapy. Our goals were to determine any differences in erythropoietic efficacy between schedules, to characterise the effects of chemotherapy on serum eEPO concentrations and on darbepoetin alfa pharmacokinetics, and to study the temporal effects of chemotherapy on haemoglobin concentrations.

2. Methods

2.1. Study population

Institutional review boards approved the protocol and patients gave written informed consent before entry. Eligible patients were $\geqslant$18 years old, had non-myeloid malignancies, received cyclic chemotherapy once every 3 weeks, were anaemic (haemoglobin concentration $\geqslant$9.0 and $\leqslant$11.0 g/dl), had an Eastern Cooperative Oncology Group (ECOG) performance status of 0–2, and had adequate renal and hepatic function. Patients with histories of seizures, significant cardiac or inflammatory disease, primary haematological disorders that could cause anaemia, or received any rHuEPO or more than two RBC transfusions within 4 weeks of randomisation were excluded.

2.2. Study drug

Darbepoetin alfa (Aranesp®, Amgen Inc., Thousand Oaks, California, USA) was supplied in vials containing 1 mg/ml darbepoetin alfa.

2.3. Study design

This was a multi-centre, open-label, randomised trial conducted from January 2002 to October 2002. Patients receiving chemotherapy every 3 weeks were randomly assigned 1:1 to receive subcutaneous (s.c.) darbepoetin alfa 6.75 μg/kg once every 3 weeks asynchronously or synchronously with chemotherapy. Randomisation was stratified by baseline haemoglobin concentration (<10.0 *versus* ⩾10.0 g/dl), study centre (UCLA Medical Center *versus* all others), and optional participation in the intensive pharmacokinetic study.

The primary endpoint assessment was after 6 weeks of darbepoetin alfa therapy, to allow sufficient time to detect differences in haematological response while ensuring that most patients retained chemotherapy cycles of once every 3 weeks. Since chemotherapy cessation, delays and modifications of cycle length were likely to occur after 2 cycles, assessment of haemoglobin change beyond 6 weeks may have confounded results of synchronicity.

Darbepoetin alfa was withheld from patients if haemoglobin concentrations were >15.0 g/dl for men or >14.0 g/dl for women. When haemoglobin concentration decreased to ⩽13.0 g/dl, darbepoetin alfa was restarted at 66% of the previous dose. Patients receiving chemotherapy remained in the study for up to 16 weeks. After 6 weeks, darbepoetin alfa dose could be doubled for patients with <1-g/dl increase in haemoglobin concentration from baseline. Blood samples for eEPO, darbepoetin alfa pharmacokinetics, and safety and efficacy analyses were obtained at predefined time points throughout the study.

2.4. Pharmacokinetic assessments

All patients had baseline eEPO measured. A subset of patients from each treatment group signed an additional consent form and participated in the optional pharmacokinetic study. After the first dose of darbepoetin alfa, blood samples were collected over the 3-week dosing interval at frequent predefined points up to 504 h (21 d) after administration. Weekly samples were collected thereafter. Darbepoetin alfa and eEPO concentrations were determined in all of these samples by separate methods.

Darbepoetin alfa concentrations were measured using the Quantikine IVD human erythropoietin enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN, USA) by MDS Pharma Services (Montreal, Canada). The standard curve concentrations range was 5.00–0.125 ng/ml and the lower limit of quantification was generally 0.14 ng/ml. The assay was validated [31] and demonstrated recovery of spike experiments, parallelism, accuracy, inter-assay precision (coefficient of variation for darbepoetin alfa was 1–4%) and stability. Endogenous EPO cross-reacted in this ELISA.

An additional EPO-specific assay was used to determine eEPO concentrations when darbepoetin alfa was present. This assay used a different erythropoietin-specific monoclonal antibody (F12) as the capture antibody [32,33]. This antibody does not bind to darbepoetin alfa at concentrations ⩽5000 ng/ml (maximum tested). The detection antibody was the same anti-rHuEPO rabbit polyclonal antibody used in R&D Systems rHuEPO ELISA kit. This assay used rHuEPO for standard and quality control samples. The standard curve range was 333.3–5.2 mU/ml and the lower limit of quantification was generally 6 mU/ml. This research assay was developed at Amgen Inc. and validated and performed by MDS Pharma Services.

2.5. Study endpoints

The primary efficacy endpoint was change in haemoglobin concentration after 6 weeks of therapy (study week 7). Secondary efficacy endpoints included the proportion of patients who had a ⩾1.0 g/dl increase in haemoglobin concentration by week 7, time to ⩾1.0 g/dl increase in haemoglobin concentration, change in haemoglobin concentration over treatment, haematopoietic response (increase in haemoglobin concentration of ⩾2.0 g/dl over baseline or haemoglobin concentration ⩾12.0 g/dl without RBC transfusions during the preceding 28 d), time to haematopoietic response, and transfusion requirements (week 5 through end of treatment phase (EOTP) and week 1 through EOTP).

Standard non-compartmental pharmacokinetic parameters were estimated for darbepoetin alfa in the pharmacokinetic subset, including peak serum concentration (C_{max}), time of peak serum concentration (T_{max}), area under the serum concentration–time curve from time 0 to infinity (area under the curve, AUC) ($AUC_{(0-\infty)}$), terminal half-life ($t_{1/2,z}$), mean residence time to infinity ($MRT_{(0-\infty)}$), and relative clearance (CL/F). Serum eEPO concentrations were summarised relative to baseline for the same subset of patients.

Safety was assessed by incidence and severity of adverse events by treatment group. Events were categorised and graded according to the World Health Organization system. Another safety endpoint was the proportion of patients in whom anti-darbepoetin alfa antibodies were detected.

2.6. Statistical analysis

The planned sample size was 80 patients randomised 1:1 to 1 of the 2 treatment groups. This sample size was chosen to achieve a minimum of 30 evaluable patients in each treatment group at week 7 of treatment. The standard deviation (SD) of change from baseline

haemoglobin measurement was assumed to be approximately 1.4 g/dl. The study had approximately 90% power using a one-sided *t*-test ($\alpha = 0.05$) to detect a difference between treatment groups, assuming the true difference was $\geqslant 1.0$ g/dl. Since this was the first clinical investigation that evaluated the superiority of one dosing schedule over another (i.e., asynchronous dosing over more standard synchronous dosing of darbepoetin alfa), a one-sided *t*-test was appropriate.

Analyses of all endpoints except transfusions from week 5 to EOTP were conducted on patients who received at least 1 dose of darbepoetin alfa (primary analysis data-set). To handle missing haemoglobin values, both the available data and the last value carried forward (LVCF) approaches were used. Available data analyses included only values at a specified period not within 28 d of an RBC transfusion; missing values were excluded. The LVCF approach imputed missing haemoglobin values or values within 28 d of a transfusion using the last available value (last value carried forward) that was not within 28 d of RBC transfusion. This LCVF approach accounted for all patients randomly assigned to treatment who received study drug and reduced chances of selection bias inherent in a methodology that often excludes more anaemic patients from mean haemoglobin calculation at any given point. Samples drawn outside a 10% window from protocol-specific sampling times were excluded from all summary statistics but not from individual analyses.

The incidence of transfusions was analysed for the subset of patients who received at least 1 dose of darbepoetin alfa and remained in the study after 4 weeks. Previous studies evaluating transfusion requirements have suggested that treatment effects are not expected until after 4 weeks of erythropoietic treatment [34]. The Kaplan–Meier estimate was calculated for the proportion of patients with a haematopoietic response and the proportion transfused from week 5 to EOTP. Approximate 95% confidence intervals (95% CI) for Kaplan–Meier estimates of proportions were calculated using Greenwood's estimate of the variance [35].

To determine the association of asynchronous/synchronous darbepoetin alfa administration and greater haemoglobin increase at week 7, a one-sided Wilcoxon procedure [36] was used stratified by baseline haemoglobin concentration (<10.0 *versus* $\geqslant 10.0$ g/dl) and by study centre. Point estimates (95% CI) of mean change for each schedule and point estimate (with a one-sided 95% CI) for difference in means were presented for both analyses.

Pharmacokinetic analyses of darbepoetin alfa were conducted on data generated by ELISA. Concentrations less than the limit of quantification were given a value of zero. To account for the baseline eEPO that cross-reacts in the assay, baseline-corrected values for each patient were calculated by subtracting the measured pre-study eEPO concentration on day 1, as assessed in the darbepoetin alfa assay, from all subsequent values for that patient. No correction was necessary when the pre-dose value was less than the limit of quantification of the assay. When baseline correction resulted in a negative value, it was converted to zero. This correction does not account for fluctuations in eEPO throughout the course of the study, but eliminates 1 confounding variable.

Pharmacokinetic parameters after a single subcutaneous dose of darbepoetin alfa were estimated by standard non-compartmental methods using WinNonlin Professional Version 1.5 (Pharsight Corp., Mountain View, CA, USA). Data points were included in the regression if they were the last 3 (or more) non-increasing concentrations, and if these concentrations were greater than twice the limit of quantification. This method was used to prevent over-interpretation of data in the region where relative contribution of eEPO was greatest. Actual sampling times and doses were used for estimation of all parameters. For eEPO data, measured concentrations were summarised.

3. Results

3.1. Patient demographics and baseline characteristics

Eighty-four patients were enrolled (Fig. 1). Three patients (all assigned to synchronous dosing) did not receive study drug; thus, 81 were included in the primary analysis set. A total of 74 patients (41 asynchronous, 33 synchronous) completed 6 weeks of study (time of the primary endpoint). The median duration of therapy was 10 weeks. Baseline demographics were well balanced between treatment groups, with slight differences in the proportion of patients with breast and gynaecologic malignancies observed (Table 1). Mean baseline haemoglobin concentration was slightly lower in the asynchronous (10.0 g/dl) than the synchronous group (10.5 g/dl); however, the baseline value of the asynchronous group was taken mid-cycle (7 d before the next chemotherapy administration) rather than immediately before the start of the next cycle as in the synchronous group. This difference may be the result of the impact of chemotherapy.

3.2. Efficacy

Mean increases in haemoglobin concentration after 6 weeks were similar between groups (1.0 g/dl (95% CI 0.6–1.3) asynchronous, 1.0 g/dl (95% CI 0.6–1.5) synchronous) with a *P*-value of 0.45 (Table 2). Over the treatment period, no major differences were observed in either the magnitude or rate of haemoglobin change (Fig. 2). However, an impact of each chemotherapy cycle on haemoglobin concentration was clearly seen in

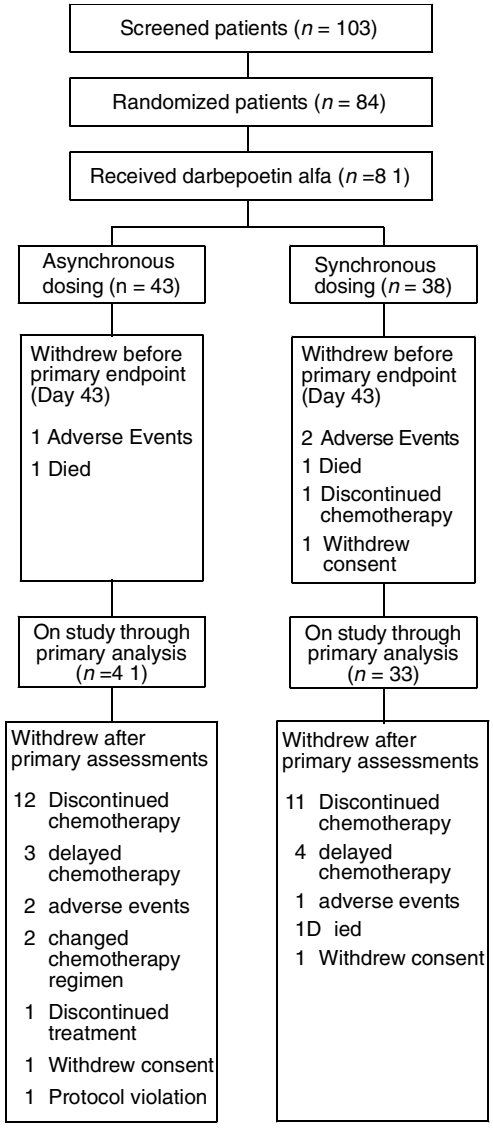

Fig. 1. Patient disposition. CONSORT diagram.

both groups throughout treatment, with a slight decline or stabilisation during the week immediately after chemotherapy administration followed by an increase over the remainder of the chemotherapy cycle length.

Table 1
Baseline demographic and clinical characteristics

Characteristics	All patients[a]	
	Asynchronous	Synchronous
Number of patients	43	38
Sex, *n* (%)		
Women	32 (74%)	28 (74%)
Men	11 (26%)	10 (26%)
Age (years)		
Mean (SD)	61.4 (13.9)	62.2 (13.8)
Primary tumour type, *n* (%)		
Breast	20 (47%)	12 (32%)
Gastrointestinal	2 (5%)	3 (8%)
Genitourinary	4 (9%)	4 (11%)
Gynaecological	4 (9%)	9 (24%)
Lung	3 (7%)	5 (13%)
Other	10 (23%)	5 (13%)
Chemotherapy		
Platinum-containing	12 (28%)	14 (37%)
ECOG performance status, *n* (%)		
0	16 (37%)	13 (34%)
1	24 (56%)	24 (63%)
2	3 (7%)	1 (3%)
Baseline haemoglobin (g/dl)		
Mean (SD)	10.03 (1.15)	10.47 (0.97)
Serum endogenous EPO (mU/ml)		
n	41	38
Median	33.58	25.38
Ferritin (μg/l)		
n	41	38
Median	242.60	202.85
Range (min, max)	19.6, 2976.0	25.4, 1659.0
Transferrin saturation (%)		
n	40	37
Median	23.00	23.00
Range (min, max)	8.0, 60.0	5.0, 57.0

ECOG, Eastern Cooperative Oncology Group.
[a] All patients who were administered at least 1 dose of darbepoetin alfa.

No significant between-group differences were observed in any secondary haematological and clinical efficacy endpoints (Table 2, Fig. 3). The haematological response rates for both groups (69% asynchronous, 81% synchronous) compare favourably to rates reported with standard, more frequently administered erythropoietic therapy [8,11,12].

Table 2
Summary haemoglobin results for each dose group and both groups combined (overall)

	Asynchronous	Synchronous	Overall
Mean (95% confidence (CI)) change in haemoglobin after 6 weeks of treatment (week 7) (g/dl)	1.0. (0.6–1.3)	1.0 (0.6–1.5)	1.0 (0.7–1.3)
[a]K–M proportion (95% CI) of patients with ⩾1 g/dl increase in haemoglobin after 6 weeks of treatment	68% (54–83)	64% (48–80)	66% (56–77)
K–M proportion (95% CI) of haematopoietic response	69% (52–86)	81% (61–100)	74% (61–87)
K–M median (95% CI) time to haematopoietic response (d)	50 (36–92)	43 (36–92)	49 (36–58)
K–M proportion (95% CI) of transfusions (week 5 to EOTP)	19% (6–33)	18% (5–31)	19% (9–28)
K–M proportion (95% CI) of transfusions (week 1 to EOTP)	33% (18–48)	22% (9–36)	28% (18–39)

[a] Kaplan–Meier proportion.

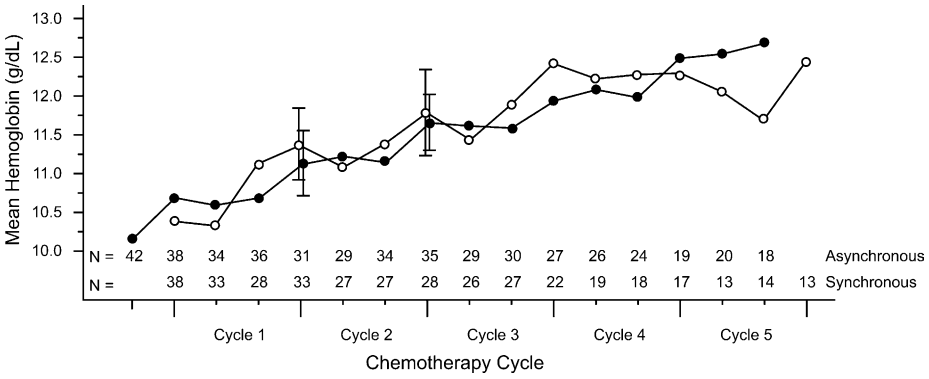

Fig. 2. Mean haemoglobin over time by chemotherapy cycle (available data analysis). Filled circles = asynchronous administration; empty circles = synchronous administration. Error bars represent 95% confidence intervals with point estimates shown for end of chemotherapy cycle 1 and 2 for each dose schedule (asynchronous and synchronous).

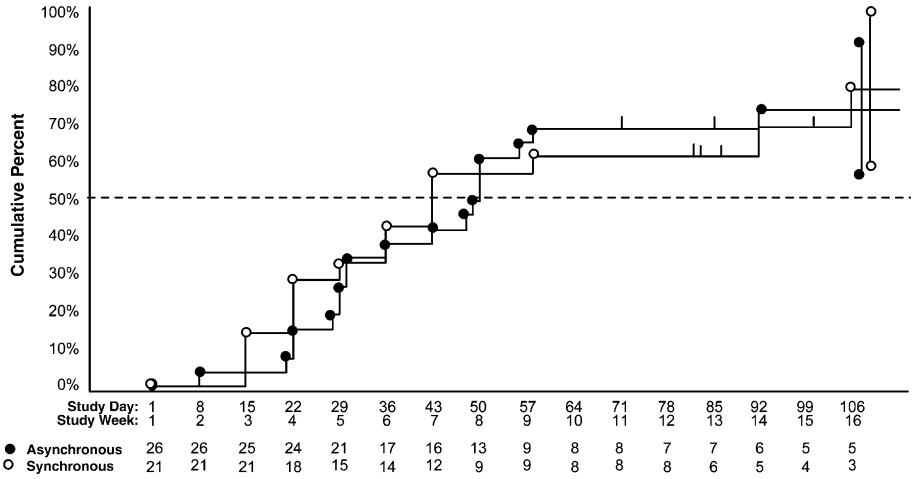

Fig. 3. Haematopoietic response from baseline through the end of study (Kaplan–Meier proportions). Filled circles = asynchronous administration; empty circles = synchronous administration.

3.3. Pharmacokinetic evaluations

Thirty-three patients were in the pharmacokinetic subset, of which 25 patients (12 asynchronous, 13 synchronous) had evaluable profiles after the first dose. Eight patients contributed only to the weekly sampling and 1 was excluded because of high baseline serum eEPO concentration. Demographic and baseline characteristics were generally balanced between groups, with a few exceptions. In the asynchronous group, there were more men than women (7 (44%) asynchronous; 5 (29%) synchronous). The median baseline eEPO levels were 25.2 mU/ml for the asynchronous group and 31.6 g/dl for the synchronous group.

Endogenous EPO concentrations before and during chemotherapy were estimated for the pharmacokinetic subset. The mean (SD) baseline eEPO concentration for asynchronous patients was 81.2 (235) and 34.0 (29.6) mU/ml for synchronous patients. One asynchronous patient, however, had a high baseline eEPO concentration of 1523 mU/ml. This patient also had a severely low baseline haemoglobin level (<6 g/dl), which may have influenced the baseline eEPO value. After exclusion of this patient from the analysis, mean (SD) baseline eEPO concentration for the asynchronous group was 45.2 (47.6) mU/ml.

Increase in eEPO concentrations was observed in the week after chemotherapy administration. Peak concentration was observed 48 h after chemotherapy administration in both groups. In the synchronous group, mean eEPO concentrations were elevated approximately 5-fold over baseline. The individual ratio of 48-h value to baseline ranged from 2 to 32, with mean and median ratios for the group of 8.6 and 6.4, respectively. The eEPO of most patients in the synchronous returned to near baseline values by the 168-h point (end of week 1). In the asynchronous group, eEPO concentrations over the week before chemotherapy administration were relatively constant. In the synchronous group, mean eEPO concentrations rose to a peak value 48 h after chemotherapy (i.e., day 9) with a 4-fold increase in mean eEPO concentration compared with the pre-chemotherapy value. The

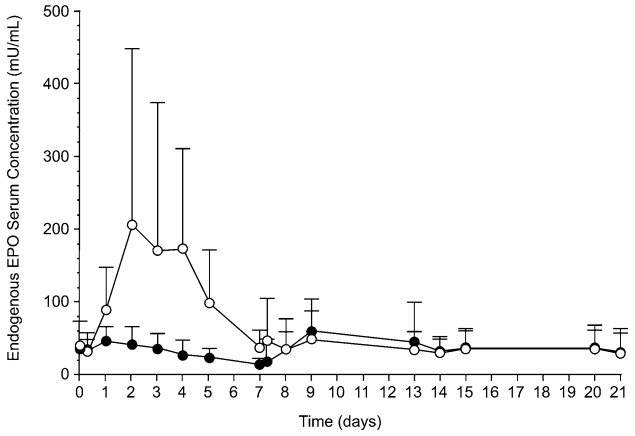

Fig. 4. Mean endogenous erythropoietin (eEPO) concentrations during the first 3 weeks of study for the asynchronous (7–17 patients) and synchronous groups (8–15 patients). Filled circles = asynchronous administration; empty circles = synchronous administration. Error bars represent standard deviations.

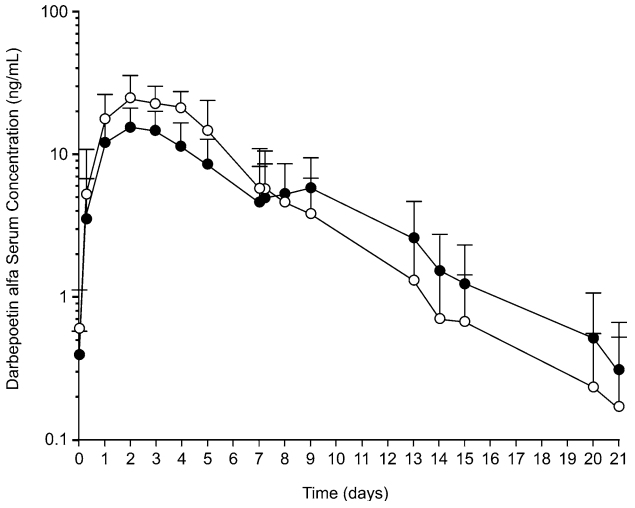

Fig. 5. Mean darbepoetin alfa concentrations after a subcutaneous dose of 6.75 μg/kg during the first 3 weeks of study for the asynchronous (10–13 patients) and synchronous (10–13 patients) groups. Filled circles = asynchronous administration; empty circles = synchronous administration. Error bars represent standard deviations.

individual ratio of 48-h post-chemotherapy to baseline value ranged from 1 to 4, with mean and median ratios for the asynchronous group of 2.6 and 2.8, respectively (see Fig. 4).

Same-day dosing of darbepoetin alfa and chemotherapy was associated with an increase in maximal concentration (1.7-fold increase in mean C_{max}), and area under the serum concentration–time curve (1.3-fold increase in mean $AUC_{(0-\infty)}$) compared with asynchronous dosing (Table 3, Fig. 5). After peak concentration, serum darbepoetin alfa concentrations declined in the expected monophasic manner for patients in the synchronous group. However, for the asynchronous group, the decline in mean serum concentration was interrupted after chemotherapy administration (i.e., 7 d after darbepoetin alfa administration) for 3 d, then declined at a rate similar to that observed in the synchronous group (Table 3).

3.4. Safety

The types of adverse events reported were consistent with those observed in clinical trials of darbepoetin alfa [7,29] and were generally associated with malignant disease and toxic effects of chemotherapy. The safety profile of darbepoetin alfa was similar between groups. Two patients (2%) had thrombotic adverse events: one had pulmonary embolism with disease progression, including spinal cord compression and the other had subclinical left-arm deep-vein thrombosis (DVT). The second patient also had an excess rate of rise in haemoglobin within ±14 d of the thrombotic event; however, this patient also received a transfusion within these 14 d (haemoglobin = 8.2 g/dl before receiving the transfusion). The DVT event occurred 9 d after the transfusion. The rapid rise in haemoglobin reached a maximum level of 10.9 g/dl during these 14 d. Despite the investigator's initial possible association of the rapid rate of haemoglobin rise with darbepoetin alfa, this haemoglobin rise was associated with the transfusion.

No evidence of anti-darbepoetin alfa antibodies was detected in any patient.

4. Discussion

In clinical practice, erythropoietic agents and chemotherapy have been administered synchronously when-

Table 3
Summary pharmacokinetic parameters after a single subcutaneous dose of darbepoetin alfa

Parameter	Asynchronous	Synchronous	Overall
	Mean (SD) (min, max)	Mean (SD) (min, max)	Mean (SD) (min, max)
C_{max} (ng/ml)	15.5 (5.25) (7.39, 26.1)	26.5 (9.67) (9.76, 47.3)	21.2 (9.52 (7.39, 47.3)
T_{max} (h)	59.8 (16.6) (27.5, 85)	70.5 (25.3) (47.1, 120)	65.4 (21.9) (27.5, 120)
$AUC_{(0-\infty)}$(ng h/ml)	2570 (1110) (955, 5070)	3280 (1280) (1090, 6080)	2940 (1230) (955, 6080)
$t_{1/2,z}$ (h)	87.7 (26.0) (58.3, 144)	60.9 (22.3) (23.5, 111)	73.7 (27.3) (23.5, 144)
$MRT_{(0-\infty)}$ (h)	159 (38.1) (118, 247)	111 (23.0) (81.1, 155)	134 (39.3) (81.1, 247)
CL/F (ml/h/kg)	3.18 (1.59) (1.34, 7.14)	2.45 (1.29) (1.11, 6.24)	2.80 (1.46) (1.11, 7.14)

AUC, area under the curve; MRT, mean residence time; CL/F, relative clearance.

ever possible, but without a clear understanding of the underlying biology or guidance from randomised trials addressing the issue. The assumption is that the practice is safe and as effective as asynchronous administration. As longer-acting erythropoietic agents are developed, moving treatment toward less-frequent administration, studies elucidating the potentially complex interaction of chemotherapy and erythropoiesis have become increasingly important. This randomised trial provided several important and relevant insights.

The results from this trial did not provide sufficient evidence to suggest that asynchronous dosing was superior to synchronous dosing when administering darbepoetin alfa every-3-weeks. The two schedules of darbepoetin alfa had similar haematological efficacy and safety profiles, despite some differences in the pharmacokinetic and pharmacodynamic profiles between the two schedules. In the synchronous group, either the increased drug exposure was of insufficient magnitude to produce a detectable difference in efficacy or the target organ, the marrow, did not remain fully responsive during the immediate post-chemotherapy period. Notably, after each dose of myelosuppressive chemotherapy, the rate of haemoglobin rise was consistently and repeatedly impacted in both groups for approximately 1 week, a time frame similar to that observed for the post-chemotherapy increases in eEPO levels.

The pharmacokinetic and pharmacodynamic (PK/PD) findings provide insight into the mechanism of clearance of eEPO and darbepoetin alfa. The increase in eEPO concentrations in both groups occurring as soon as 6 h after chemotherapy and lasting approximately 1 week afterwards suggest that the clearance of eEPO was impaired by chemotherapy rather than that the production of eEPO was increased. Previous studies have shown that eEPO serum concentrations associated with chemotherapy increased up to 7-fold [37–39]. Similarly, chemotherapy may be associated with interruption of clearance of darbepoetin alfa, as a larger AUC for this agent with synchronous administration relative to that of asynchronous administration was observed. One might predict that from this increased drug exposure, the pharmacodynamics (haematopoietic efficacy) of darbepoetin alfa may be enhanced by synchronous dosing, especially if the bone marrow remained responsive to this agent during the immediate post-chemotherapy period.

One possible explanation for our PK/PD findings is that chemotherapy alters the volume of distribution of all erythropoietins and/or the activity of ASGR- based, non-receptor-mediated clearance. However, we believe that the hypothesis that best fits the totality of our data is that the receptor-bearing cells in the bone marrow contribute to the clearance of both eEPO and darbepoetin alfa through binding, internalisation and catabolism of these hormones. This would explain the apparent temporal association of the elevated eEPO levels and relative marrow unresponsiveness observed in our patients, both lasting approximately 1 week. A study in sheep administered busulfan and rHuEPO verified that the clearance, and not the volume of distribution, was significantly decreased by chemotherapy [39]. *In vitro* work has supported the model that rHuEPO is internalised and degraded by target cells [21], and it has been postulated that saturable uptake by bone marrow is mediated by the EPO receptor [40,41]. Reduced clearance of rHuEPO has been reported in patients with myelodysplastic syndromes, who have reduced bone marrow activity [42]. Finally, this hypothesis is consistent with the observed inverse relationship between receptor affinity and serum half-life as well as *in vivo* potency of different erythropoietic proteins.

A few differences in baseline characteristics between the asynchronous and synchronous groups were noted, which based on recent clinical findings may suggest a better prognosis for response for one group over the other. A large study of approximately 1500 patients by Vadhan-Raj *et al.* [43] analysed potential covariates of response. Tumour type (breast and colorectal cancer), non-platinum-containing therapy, and lower baseline haemoglobin levels were identified as important independent variables that conferred better transfusion-based and haematological responses. In our study, baseline characteristics favoured the asynchronous group for better prognosis for response *versus* the synchronous group (breast cancer: 47% *versus* 32%, respectively; platinum-containing chemotherapy: 28% *versus* 37%, respectively; baseline haemoglobin levels: 10.0 *versus* 10.5 g/dl, respectively). However, despite a possible bias in favour of the asynchronous group, no clinically relevant difference in haematological response was observed between the groups. Thus, we feel that our data do not suggest a benefit of asynchronous dosing over synchronous dosing.

We note a few limitations to our study. First, we did not carry out formal drug disposition studies to confirm our suspicion that chemotherapy changes the clearance as opposed to the distribution of darbepoetin alfa, and we did not obtain repeated blood and/or marrow samples for progenitor cell studies to further explore our hypothesis that the responsiveness of the marrow is impaired in the immediate post-chemotherapy period. Therefore, the mechanisms by which chemotherapy impacts on serum eEPO concentrations, darbepoetin alfa pharmacokinetics and haematopoietic responsiveness have not been fully elucidated. Future studies to elucidate the exact role of the bone marrow in the mechanism of erythropoietin clearance need to be conducted using repeated blood and/or marrow samples. Also, the effects of different chemotherapeutic regimens that may influence the pharmacokinetic and pharmacodynamic properties of darbepoetin alfa were not studied and need to be addressed in future studies.

Another important limitation is that the measured drug concentrations were actually a composite of endogenous EPO and darbepoetin alfa because the assay recognises both proteins, albeit differentially. The reported darbepoetin alfa concentrations have been corrected individually for the baseline eEPO concentrations, as measured by cross-reactivity in the darbepoetin alfa assay; standard methodology for recombinant proteins [44]. Corrections were not made for the fluctuations in endogenous erythropoietin levels resulting from chemotherapy because direct subtraction of one from another is not feasible when different assays, with different affinities for the ligand, are used. However, as endogenous erythropoietin contributes no more than approximately 15% to the overall signal up to 216 h after administration of darbepoetin alfa, these fluctuations are insufficient to alter the overall pharmacokinetic conclusions of the study.

Our findings support the hypothesis that the bone marrow is an effector site and clearance site for erythropoietins. With synchronous administration of darbepoetin alfa, transient decreased marrow responsiveness is precisely offset by the observed increased concentration and AUC associated with synchronous administration. The net result is a nearly identical overall erythropoietic benefit between the two dosing schedules. This hypothesis provides a mechanism explaining the observed lack of decrease in efficacy with synchronous every-3-week darbepoetin alfa administration. Therefore, since no advantage appears to be gained with asynchronous dosing as initially expected, synchronous dosing with every-3-week darbepoetin alfa is preferable and offers greater convenience to the patients.

We conclude the following: myelosuppressive chemotherapy is associated with a rapid increase in the concentration of eEPO, probably due to decreased clearance by progenitor cells in the bone marrow. Maximal concentrations and AUC of darbepoetin alfa are increased by synchronous compared with asynchronous chemotherapy administration, again probably due to decreased clearance. Erythropoiesis is compromised during the days after chemotherapy, offsetting the pharmacokinetic advantages of synchronous dosing. Therefore, darbepoetin alfa administered every 3 weeks has similar effectiveness whether given synchronously or asynchronously with chemotherapy, and is highly effective in producing haematopoietic responses that are indistinguishable from those observed with more frequent dosing.

Conflict of interest statement

Russell Berg, Matt Austin and Greg Rossi are employees of Amgen Inc.

None declared for John Glaspy, David Henry, Ravi Patel, Simon Tchekmedyian, Steve Applebaum, Donald Berdeaux and Richard Lloyd.

Acknowledgements

Anne C. Heatherington, PhD, conducted the pharmacokinetic and pharmacodynamic studies. Sameer Doshi and Jose Rodriguez assisted with the analysis of the pharmacokinetics data. Catherine Allen, BS, assisted with managing the study. Christopher Davidson, BS; and MaryAnn Foote, PhD, assisted with the writing of the manuscript.

This study was supported financially by Amgen Inc. Thousand Oaks, CA, USA.

References

1. Groopman JE, Itri LM. Chemotherapy-induced anemia in adults: incidence and treatment. *J Natl Cancer Inst* 1999, **91**, 1616–1634.
2. Gabrilove JL, Cleeland CS, Livingston RB, *et al*. Clinical evaluation of once-weekly dosing of epoetin alfa in chemotherapy patients: improvements in hemoglobin and quality of life are similar to three-times-weekly dosing. *J Clin Oncol* 2001, **19**, 2875–2882.
3. Littlewood TJ, Bajetta E, Nortier J, *et al*. Effects of epoetin alfa on hematologic parameters and quality of life in cancer patients receiving nonplatinum chemotherapy: results of a randomized, double-blind, placebo-controlled trial. *J Clin Oncol* 2001, **19**, 2865–2874.
4. Mirtsching B, Charu V, Vadhan-Raj S, *et al*. Every-2-week darbepoetin alfa is comparable to rHuEPO in treating chemotherapy-induced anemia. *Oncology* 2002, **16**(10 (Suppl 11)), 31–36.
5. Vadhan-Raj S, Mirtching B, Charu V, *et al*. Assessment of hematologic effects and fatigue in cancer patients with chemotherapy-induced anemia given darbepoetin alfa every two weeks. *J Support Oncol* 2003, **1**, 131–138.
6. Glaspy J, Bukowski R, Steinberg D, *et al*. Impact on therapy with epoetin alfa on clinical outcomes in patients with nonmyeloid malignancies during cancer chemotherapy in community oncology practice. *J Clin Oncol* 1997, **15**, 1218–1234.
7. Glaspy JA, Jadeja JS, Justice G, *et al*. Darbepoetin alfa given every 1 or 2 weeks alleviates anaemia associated with cancer chemotherapy. *Brit J Cancer* 2002, **87**, 268–276.
8. Vansteenkiste J, Pirker R, Massuti B, *et al*. Double-blind, placebo-controlled, randomized phase III trial of darbepoetin alfa in lung cancer patients receiving chemotherapy. *J Natl Cancer Inst* 2002, **94**, 1211–1220.
9. Hedenus M, Hansen S, Taylor K, *et al*. Randomized, dose-finding study of darbepoetin alfa in anaemic patients with lymphoproliferative malignancies. *Brit J Haematol* 2002, **119**, 79–86.
10. Hedenus M, Adriansson M, San Miguel J, *et al*. Efficacy and safety of darbepoetin alfa in anaemic patients with lymphoproliferative malignancies: a randomized, double-blind, placebo-controlled study. *Brit J Haematol* 2003, **122**, 394–403.
11. Osterborg A, Brandberg Y, Molostova V, *et al*. Randomized, double-blind, placebo-controlled trial of recombinant human erythropoietin, epoetin beta, in hematologic malignancies. *J Clin Oncol* 2002, **20**, 2486–2494.
12. Demetri GD, Kris M, Wade J, *et al*. Quality-of-life benefit in chemotherapy patients treated with epoetin alfa is independent of

disease response or tumor type: results from a prospective community oncology study. *J Clin Oncol* 1998, **16**, 3412–3425.
13. Egrie JC, Dwyer E, Browne JK, *et al*. Darbepoetin alfa has a longer circulating half-life and greater in vivo potency than recombinant human erythropoietin. *Exp Hematol* 2003, **31**, 290–299.
14. Spivak JL, Hogans BB. The in vivo metabolism of recombinant human erythropoietin in the rat. *Blood* 1989, **73**, 90–99.
15. Briggs DW, Fisher JW, George WJ. Hepatic clearance of intact and desialylated erythropoietin. *Am J Physiol* 1974, **227**, 1385–1388.
16. Fukuda MN, Sasaki H, Lopez L, *et al*. Survival of recombinant erythropoietin in the circulation: the role of carbohydrates. *Blood* 1989, **73**, 84–89.
17. Nielsen OJ, Egfjord M, Hirth P. The metabolism of recombinant erythropoietin in the isolated perfused rat liver. *Liver* 1990, **10**, 343–349.
18. Achyuthan KE, Achyuthan AM. Comparative enzymology, biochemistry and pathophysiology of human exo-alpha-sialidases (neuraminidases). *Comp Biochem Physiol B: Biochem Mol Biol* 2001, **129**, 29–64.
19. Widness JA, Veng-Pedersen P, Schmidt RL, *et al*. In vivo 125I-erythropoietin pharmacokinetics are unchanged after anesthesia, nephrectomy and hepatectomy in sheep. *J Pharmacol Exp Ther* 1996, **279**, 1205–1210.
20. Heatherington A, Rovetti R, Kotasek D, *et al*. Predictability of pharmacokinetic properties of NESP administered once every 3 weeks in patients with nonmyeloid malignancies receiving cyclic chemotherapy. *Proc ASCO* 2001, **20**, 119a. [Abstract 471].
21. Sawyer ST, Krantz SB, Goldwasser E. Binding and receptor-mediated endocytosis of erythropoietin in Friend virus-infected erythroid cells. *J Biol Chem* 1987, **262**, 5554–5562.
22. Kinoshita H, Ohishi N, Kato M, *et al*. Distribution of recombinant human erythropoietin following multiple intravenous administration and effects of age on the distribution in rats. *Arzneimittel-Forschung* 1992, **42**, 579–584.
23. Kato M, Kamiyama H, Okazaki A, *et al*. Mechanism for the nonlinear pharmacokinetics of erythropoietin on rats. *J Pharmacol Exp Ther* 1997, **283**, 520–527.
24. Chapel SH, Veng-Pedersen P, Hohl RJ, *et al*. Changes in erythropoietin pharmacokinetics following busulfan-induced bone marrow ablation in sheep: evidence for bone marrow as a major erythropoietin elimination pathway. *J Pharmacol Exp Ther* 2001, **298**, 820–824.
25. Chapel SH, Veng-Pedersen P, Schmidt RL, *et al*. Receptor-based model accounts for phlebotomy-induced changes in erythropoietin pharmacokinetics. *Exp Hematol* 2001, **29**, 425–431.
26. Kato M, Okano K, Sakamoto Y, *et al*. Pharmacokinetics and pharmacodynamics of recombinant human erythropoietin in rats. *Arzneimittel-Forschung* 2001, **51**, 91–95.
27. Sawabe Y, Takiguchi Y, Kikuno K, *et al*. Changes in levels of serum erythropoietin, serum iron and unsaturated iron binding capacity during chemotherapy for lung cancer. *Jpn J Clin Oncol* 1998, **28**, 182–186.
28. Heatherington AC, Schuller J, Mercer AJ. Pharmacokinetics of novel erythropoiesis stimulating protein (NESP) in cancer patients: preliminary report. *Brit J Cancer* 2001, **84**, 11–16.
29. Kotasek D, Steger G, Faught W, *et al*. Darbepoetin alfa administered every 3 weeks alleviates anaemia in patients with solid tumours receiving chemotherapy; results of a double-blind, placebo-controlled, randomised study. *Eur J Cancer* 2003, **39**, 2026–2034.
30. Hartley C, Elliott S, Begley G, *et al*. Kinetics of haematopoietic recovery after dose-intensive chemo/radiotherapy in mice: optimized erythroid support with darbepoetin alpha. *Brit J Haematol* 2003, **122**, 623–636.
31. Allon M, Kleinman K, Walczyk M, *et al*. Pharmacokinetics and pharmacodynamics of darbepoetin alfa and epoetin in patients undergoing dialysis. *Clin Pharmacol Ther* 2002, **72**, 546–555.
32. Elliott S, Lorenzini T, Asher S, *et al*. Enhancement of therapeutic protein in vivo activities through glycoengineering. *Nat Biotechnol* 2003, **21**, 414–421.
33. Elliott S, Lorenzini T, Chang D, *et al*. Fine-structure epitope mapping of antierythropoietin monoclonal antibodies reveals a model of recombinant human erythropoietin structure. *Blood* 1996, **87**, 2702–2713.
34. Abels RI. Use of recombinant human erythropoietin in the treatment of anemia in patients who have cancer. *Semin Oncol* 1992, **19**, 29–35.
35. Kalbfleish JD, Prentice RL. *The statistical analysis of failure time data*. 1st ed. New York, NY, John Wiley and Sons, 1980.
36. Lehmann EL. *Nonparametrics: statistical methods based on ranks*. San Francisco, CA, Holden-Day Publications, 1975.
37. Piroso E, Erslev AJ, Caro J. Inappropriate increase in erythropoietin titers during chemotherapy. *Am J Hematol* 1989, **32**, 248–254.
38. Ludwig H, Pecherstorfer M, Leitgeb C, *et al*. Recombinant human erythropoietin for the treatment of chronic anemia in multiple myeloma and squamous cell carcinoma. *Stem Cells* 1993, **11**, 348–355.
39. Veng-Pedersen P, Chapel S, Al-Hunitia NH, *et al*. Pharmacokinetic tracer kinetics analysis of changes in erythropoietin receptor population in phlebotomy-induced anemia and bone marrow ablation. *Biopharm Drug Dispos* 2004, **25**, 149–156.
40. Jelkmann W. The enigma of the metabolic fate of circulating erythropoietin (Epo) in view of the pharmacokinetics of the recombinant drugs rhEpo and NESP. *Eur J Haematol* 2002, **69**, 265–274.
41. Dunphy FR, Harrison BR, Dunleavy TL, *et al*. Erythropoietin reduces anemia and transfusions: a randomized trial with or without erythropoietin during chemotherapy. *Cancer* 1999, **86**, 1362–1367.
42. Bowen D, Ehmer B, Neubert P, *et al*. The clearance of a single i.v. bolus of recombinant human erythropoietin from the serum of patients with myelodysplastic syndromes and its effects on erythropoiesis. *Exp Hematol* 1991, **19**, 613–616.
43. Vadhan-Raj S, Mirtsching B, Gregory SA, *et al*. Baseline (BL) covariates of response to darbepoetin alfa (DA) every 2 weeks (Q2W) in patients (pts) with chemotherapy-induced anemia (CIA. *Proc ASCO* 2004, **23**, 8061a.
44. Heatherington AC. Clinical pharmacokinetic properties of rHu-EPO: a review. In Molineux G, Foote MA, Elliot SG, eds. *Erythropoietins and erythropoiesis molecular, cellular, preclinical, and clinical biology*. Basel, Switzerland, Birkhauser Verlag, 2003. pp. 87–112.