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Abstract

Chronic renal failure (CRF) is a widespread medical problem commonly accompanied by a hypoproliferative anemia ("renal anemia") due to erythropoietin deficiency. Anemia greatly contributes to reduced quality of life (Hr-QoL) and high morbidity and mortality in CRF patients. Recombinant human erythropoietin (rHu-Epo) was introduced to medical practice some 20 years ago. It enables correction of anemia (hemoglobin levels, Hb) with dramatic immediate (Hr-QoL improvement) and long-term effects (reduced morbidity and mortality). Newer experimental data suggest that long-term benefits could be due not only to antianemic effect, but also to a direct organoprotective effect of (rHu)-Epo mediated through a receptor complex different from the "erythropoietic" erythropoietin receptor. During the last decade, two alternative treatments for renal anemia have been approved: darbepoetin and CERA. Both are direct agonists of the "erythropoietic" receptors and both were derived from rHu-Epo. Molecularly, they differ from rHu-Epo in that they are much larger molecules (darbepoetin is genetically modified rHu-Epo with a higher sugar content and CERA is pegylated rHu-Epo) with lower affinity for the erythropoietin receptor but with a longer circulating time. In terms of renal anemia correction, they are non-inferior to rHu-Epo and allow for less frequent dosing. They have never been compared to rHu-Epo regarding the long-term outcomes. It is hypothesized that regarding the long-term outcomes (morbidity, mortality), rHu-Epo might be superior to those larger molecules. The hypothesis is based on two types of observations. First, experimental data emphasize the role of small, erythropoietically less valuable rHu-Epo isoforms in its organoprotective effects. Second, clinical observations suggest that rHu-Epo enables for less variable Hb correction than the larger molecules, and pronounced within-subject Hb variability has been suggested as an independent predictor of poor long-term outcomes of renal anemia management.

Introduction

Virtually all patients suffering from chronic renal failure (CRF) eventually become anemic. The condition is termed “anemia associated with CRF” or simply “renal anemia”. Although it is multifactorial in nature, the major etiological factor of this normochromic normocytic anemia is erythropoietin (Epo) deficiency: considering the level of anemia, CRF patients have inappropriately low serum Epo levels due to abolished Epo gene expression (the prototypical hypoxia-sensitive gene) in the kidney [1].

Anemia dramatically reduces exercise tolerance, cognitive abilities, appetite and a number of other aspects of daily living subsumed within the concept of health-related quality of life (Hr-QoL). It also greatly increases morbidity and mortality, particularly cardiovascular. The only clinically relevant way of treating renal anemia is to enhance the signal that committed erythroid progenitors receive from the erythropoietin receptor (Epo-R). This signal promotes their survival and differentiation resulting in correction of anemia [1-3].

Erythropoisis stimulating agents: recombinant human erythropoietin and other approved treatments for renal anemia

Stable expression of the human Epo gene in mammalian host cells was accomplished in 1985 resulting in a pharmaceutical product containing recombinant human erythropoietin (rHu-Epo). Decades of research preceding the actual production of rHu-Epo resulted in extensive understanding of (patho)physiology of Epo, CRF and the kidney-bone marrow communication that enabled rapid progress to clinical trials and a fast-track regulatory approval of rHu-Epo for treatment of renal anemia in the late 1980s [2,3]. The extensive use and research over the past 20 years resulted in great improvements in clinical use of rHu-Epo in this setting, particularly regarding dosing, immediate therapeutic goal, i.e., target

hemoglobin (Hb) levels, and factors interfering with response to treatment. In the early days, rHu-Epo was delivered at high individual and total weekly doses by the intravenous (i.v.) route, three times a week (e.g., 3x10000 IU/kg/week i.v.) [4]. Subsequent research demonstrated that equal therapeutic effect (Hb control) could be achieved with much lower individual/total weekly doses (e.g., 3-fold lower), and with less frequent dosing, e.g., twice or once/week (not affecting drug utilization). It has been further demonstrated that subcutaneous (s.c.) administration (particularly convenient for patients not yet on dialysis) allows for further reduction in drug utilization (by 20-30%) with additional flexibility in dosing, showing equivalent therapeutic effect and drug utilization with thrice, twice or once a week dosing (tiw, biw, qw, respectively), or even with once every 2 weeks (q2w) or every 4 weeks (q4w) dosing [5-7]. This flexibility allows for individualization of treatment in agreement with the (desired) therapeutic effect, rationalization of drug utilization and also contributes to lower occurrence of adverse effects [5-7]. The debate about the preferred Hb levels that should be achieved/maintained for the optimum short-term and long-term benefits is still on-going. The strongest evidence support the view that Hb levels in the range between 10 g/dL and 12-13 g/dL improve Hr-QoL and reduce the cardiovascular risk (as compared to lower Hb levels), whereas higher levels do not further improve immediate outcomes and increase the cardiovascular and cerebrovascular risk [8]. For patients starting treatment at Hb <10 g/dl, Hb increase should be gradual (e.g., not exceeding 1 g/dL/month) [9]. Optimizing the entire patient care contributes to a successful treatment, but keeping appropriate iron availability (to “fuel” the enhanced erythropoiesis) and controlling inflammation, as much as possible, are major factors contributing to the overall treatment success [9]. Accounting for these elements, rHu-Epo is very safe and highly effective in the vast majority of patients with marked immediate (anemia correction, Hr-QoL improvement)

and long-term benefits (reduced morbidity/mortality), and with protective effects on the heart and the kidney [9].

Over the years, new treatments have emerged as alternatives to rHu-Epo for erythropoiesis stimulation, not only in renal anemia but also in other indications to which rHu-Epo has expanded (e.g., cancer, autologous blood donation; not subject of this paper). While there are numerous new molecules in various stages of development, two have been granted regulatory approvals at the turn of the millennium: darbepoetin alfa (initially named NESP, *novel erythropoiesis stimulating protein*) and CERA, *continuous erythropoietin receptor activator*. Both these molecules are Epo-analogues derived from rHu-Epo. Together with rHu-Epo they are called erythropoiesis stimulating agents (ESA) [10].

rHu-EPO

Just as the endogenous hormone, rHu-Epo is a glycoprotein and hence not a single molecule but a family of isoforms: Epo gene encodes the protein part (165 amino acids), whereas glycosilation is a rather variable posttranslational process susceptible to various influences. The protein backbone has 4 sugar docking sites: 3 asparagins (Asn) for N-glycosilation and 1 serine (Ser) for O-glycosilation. The variability of gylcosilation manifests as variable number of glycosilated docking sites and attached sugars vary in the number of branches (1 to 4 for N-sugars, 1-2 for O-sugars) and the content of sialic acid as the outermost sugar. The number of attached sugar chains, their branching and sialic acid content influence the mass and charge of the molecule. Hence, Epo is defined as a glycoprotein with Mw 30-34 kDa, where the protein part is 18.2 kDa and isoforms vary in mass and charge (“glycoforms”). The largest isoforms have all 4 docking sites occupied by sugars, all N-sugars are tetra-antennary and the O-sugar is di-antennary and they have 14 sialic acid residues; smaller isoforms are

consecutively less glycosilated and/or contain less branched sugars/sialic acid residues [2,11].

The “set” of isoforms output by an Epo producing cell depends on the expression/activity of glycosilation enzymes and this may vary. Relative composition of isoforms produced within one same individual may differ on daily basis or depending on (patho)physiological status [12]. rHu-Epo is a product of the human gene expressed in cultured mammalian cell lines that typically differ from endogenous human Epo-producing cells in respect to glycosilation pattern and its isoform profile could be distinguished from the circulating endogenous Epo [12]. Isoform composition in rHu-Epo is affected by the cell line, cell feeding process, harvesting, purification and other technological procedures [13].

The structure-activity relationship of (rHu)-Epo has been well established. The protein part forms the binding sites for Epo-R and is essential for biological activity. Sugars, however, have an important role. “Smaller and less acidic” isoforms (less N-sugars; less sialic acid) have higher affinity for Epo-R, whereas “larger and more acidic” ones have lower affinity for Epo-R. *In vitro*, erythropoietic potency of smaller isoforms is greater than that of larger isoforms. In fact, the core protein part (“naked Epo”) is the most potent. *In vivo*, however, the situation is completely opposite. Smaller isoforms, due to higher affinity, bind more tightly to Epo-R on erythroid progenitors in the bone marrow and are more susceptible to clearance by internalization of the Epo-Epo-R complex. Additionally, they are more susceptible to up-take and clearance by the asialo-glycoprotein receptor in the liver. Hence, their elimination half-life is very short and erythropoietic effect is weak (“naked Epo” is cleared within minutes and has no erythropoietic effect *in vivo*). Conversely, larger isoforms have longer circulating time and correspondingly more pronounced erythropoietic effect (circulating time compensates for reduced receptor affinity) [2,11,13].

Extensive research in *in vitro* and animal models as well as human data clearly demonstrate that (rHu) Epo is a pluripotent hormone that directly affects a variety of tissues and cell types besides the erythropoietic lineage. It influences several intracellular signaling mechanisms and transcription factors; affects fundamental cellular events such as mitochondrial preservation, balance of the oxidant-antioxidant system, cell differentiation, proliferation and apoptosis; modifies inflammatory responses to various noxious stimuli and modulates the immune system by affecting activation of T-lymphocyte (sub)populations. Consequently, it has been implicated in tissue-repair and protection in various organs systems, e.g., the central nervous system and the cardiovascular system [14,15]. In this context, the smaller, erythropoietically less valuable isoforms, might have an important biological/pharmacological role. Specifically, the long-recognized organoprotective effect of Epo (rHu-Epo), initially ascribe solely to correction of anemia, can be separated from the anti-anemic effect [16]. Namely, besides the "classical" or "erythropoietic" Epo-R, in non-hematological tissues Epo binds to another receptor structure. The tissue protective effects appear to require co-expression of Epo-R and CD131, a peptide structure (β-chain) that is a common element of the receptors for several cytokines and is called "beta common receptor" (βcR). Epo binds to an Epo-R-βcR complex. Activation results in inhibition of apoptosis and stimulated proliferation. Small Epo (rHu-Epo) isoforms bind with high affinity to Epo-R-βcR and in animal models display marked organoprotective effects (including neuroprotection) despite their extremely short half-life and no erythropoietic effect [16].

Darbepoetin alfa and CERA

Both molecules are direct Epo-R agonists and, although technologically and structurally different, both result from a concept that larger, longer-circulating molecules are preferred for erythropoiesis [10].

Development of darbepoetin was a direct extension of the knowledge about the role of sugars, particularly N-linked sugars in the Epo molecule. Human Epo-gene is point-mutated so that 5 amino acids in the rHu-Epo primary sequence are substituted – a manipulation allowing for insertion of two extra Asn residues, providing two extra N-sugar docking sites into the molecule. Hence, while rHu-Epo can have a maximum of 3 N-linked sugars and a total of 14 sialic acid residues, darbepoetin can have a maximum of 5 N-linked sugars and a total of 22 sialic acid residues. Consequently, while rHu-Epo is around 30 kDA (18.2 kDA protein + sugars), darbepoetin is a larger molecule (or a family of larger molecules/isoforms) with molecular mass of around 40 kDa (18.2 kDA protein + more sugars) [17]. In line with expectations, darbepoetin has considerably lower affinity for Epo-R than rHu-Epo, the ligand-receptor dissociation time is shorter and it is less potent in *in vitro* erythropoietic assasys (in some assays it behaves as a partial Epo-R agonist with lower affinity vs. rHu-Epo) [17,18]. On the other hand, its elimination is slower - human elimination half-life after i.v. injection is around 25 hours vs. around 8 hours for rHu-Epo – and it allows for a comparable effect on Hb but with less frequent administration [10,17].

CERA is pegylated rHu-Epo – large polyethyleneglycol (PEG) chains are added to the rHu-Epo molecule, so that molecular mass of CERA is 60 kDa (protein 18.2 kDA + 12.2 kDa sugars + 30 kDa PEG). Its receptor affinity is further reduced, ligand-receptor dissociation time shortened, whereas elimination half-life is extended (around 130 hours in humans), allowing for further reduction in dosing frequency [19].

In the pre-clinical development, neither drug showed any relevant distinction vs. rHu-Epo regarding toxicity/safety. The clinical data package resulting in approval of darbepoetin alfa comprised non-inferiority trials designed to show that it (i.v. or sc.; qw, q2w or q4w) corrected/maintained Hb level no worse than rHu-Epo with a comparable safety profile, but with less frequent dosing (rHu-Epo tiw or biw; i.v. or s.c.). Similarly, the CERA clinical data package comprised non-inferiority trials designed to show that the drug (q2w, q4w) corrected/maintained Hb no worse than rHu-Epo (tiw, biw or qw) or darbepoetin alfa (qw, q2w) but with less frequent dosing and with a comparable safety profile [10,17,19].
Based on their key clinical trials, darbepoetin alfa and CERA do not seem to relevantly differ from rHu-Epo in terms of their immediate effects and short-term benefits (anemia correction, Hr-QoL improvement) and safety/tolerability, but these compounds have never been soundly compared to rHu-Epo regarding long-term outcomes of the renal anemia treatment.

Hypothesis

As a treatment for renal anemia, rHu-Epo could be superior to darbepoetin alfa and CERA regarding long-term morbidity and mortality. Being a smaller and a more dynamic molecule (or a set of molecules) with a great flexibility of dosing on individual basis, it's effect could be more manageable and appropriate for a tighter control of Hb within the desired range than in the case of the lager "more inert" molecules. Patients would experience less variability in Hb level over time – a factor suggested to impact the long-term outcomes [20,21]. Also, content of smaller and eryhtropoietically less valuable isoforms might be a factor contributing to greater direct organoprotective effects of rHu-Epo as compared to darbepoetin or CERA.

Observations supporting the hypothesis

Two groups of observations support the stated hypothesis: a) detailed *in vitro* and animal studies in a variety of models indicate that the Epo-R-βcR-mediated organoprotective effect of Epo is mainly due to the action of "smaller", less sialylated isoforms that have no practically relevant antianemic effect [16]; b) clinical trials and observational data indicate that rHu-Epo enables keeping Hb within the target range with less within-subject variability than darbepoetin alfa or CERA. Considering the latter, it should be noted that ESA-treated CRF patients experience the phenomenon of within-subject Hb oscillations that are more extensive than in patients not needing ESA treatment. The causes are not fully understood, but changes of ESA doses have an impact [22]. This is understandable since ESA dosing is based on titration according to the actual Hb level. Within-subject variability in Hb levels has been indicated as an independent predictor of mortality in CRF patients [20,21].

Two almost identically designed phase III darbepoetin randomized controlled trials (RCTs) compared it to rHu-Epo for Hb maintenance [23,24]: dialyzed patients with corrected Hb due to previous rHu-Epo treatment were randomized to continue rHu-Epo or to receive darbepoetin alfa. A 21-24-week dose-adjustment period was followed by an 8-week evaluation period. Data on within-subject variability are reported as a ratio of within-subject variances darbepoetin/rHu-Epo for the evaluation period. In the first trial [23], drugs were delivered i.v., rHu-Epo tiw (n=240) and darbepoetin qw (n=121), and Hb variability was by 18% greater in the darbepoetin group (ratio 1.18, 95% CI 0.96-1.46). In the second trial [24] drugs were delivered either s.c. or i.v., rHu-Epo (n=112) tiw 46%, biw 34% and qw 20% and darbepoetin (n=224) qw 80% and q2w 20%. Ratio of within-subject variances for Hb levels was 1.03 (95% CI 0.86-1.24). Pooled estimate of the within-subject variance ratio from these

two trials obtained by a conventional meta-analytic technique is 1.09 (95% CI 0.95-1.26; p=0.207).

Two identically designed phase III CERA RCTs compared it to rHu-Epo regarding Hb maintenance [25,26]: dialysis patients with corrected Hb (previous rHu-Epo treatment) were randomized to continue rHu-Epo or to receive CERA. A 28-week dose-adjustment period was followed by an 8-week evaluation period. Data on within-subject Hb variability are reported as mean±SD within-subject standard deviation for the titration and the evaluation period. In the first trial [25], drugs were delivered i.v., rHu-Epo (n=225) tiw 86%, biw 7% and qw7%, and CERA q2w (n=220) or q4w (n=221). Since q2w and q4w CERA regimens are considered equivalent, I present data for pooled CERA arms: variability during the 28-week titration period was higher with CERA (0.91±0.42 g/dL) than with rHu-Epo (0.79±0.43 g/dL) and the difference was statistically significant (0.12, 95% CI 0.05-0.18; p=0.001) indicating by 14% higher within-subject variability. No relevant differences were observed during the 8-week evaluation period. In the second trial [26] drugs were delivered s.c., rHu-Epo (n=191) tiw 25%, biw 29% and qw 46% and CERA q2w (n=190) or q4w (n=191). Again, during the titration period variability was higher with CERA (0.85±0.42 g/dL) than with rHu-Epo (0.78±0.43 g/dL) with statistically borderline significant difference (0.07, 95% CI 0.00-0.14, p=0.06) indicating by 10% higher within-subject variability. No relevant differences were observed for the evaluation period. Combining these two studies by conventional meta-analytical technique indicates larger within-subject SD with CERA for the 28-week titration period: mean difference 0.10 g/dL (95% CI 0.05-0.15, p<0.001).

This potential of rHu-Epo to provide Hb maintenance with less within-subject variability observed in strict phase III RCTs apparently translates into daily practice. In a recent study [27], patients from the Australian Renal Anemia Management database with corrected Hb

levels and at least 5 control Hb measurements over a 1-year period were retrospectively analyzed. There were 2596 patients on rHu-Epo and 1023 on darbepoetin, the two groups being comparable regarding age, sex, average Hb level at the beginning of the observational period and proportion of diabetics. Within-subject variance in Hb levels was significantly higher ($p<0.001$) in the darbepoetin group (by 24% higher; 95% CI 18-31). Another observational study embraced 6165 pre-dialysis CRF patients from Australia and several European countries that were followed-up for a minimum of 6 months and had at least 3 repeated Hb measurements [21]. Patients needing ESA for Hb maintenance showed more within-subject variability in Hb than the patients not needing ESA treatment (as one would expect). Specifically, patients treated with rHu-EPO had higher odds of showing high variability than non-treated patients (OR 2.53, 95% CI 2.00-3.19), just as did patients treated with darbepoetin (OR 4.12, 95%CI 3.32-5.12) [21]. The report did not relate rHu-Epo-treated and darbepoetin-treated patients [21], but since both groups were compared to "no treatment" under similar conditions, an indirect comparison of rHu-Epo vs. darbepoetin is feasible ⟨as $exp[ln(OR\ rHu\text{-}EPO) - ln(OR\ darbepoetin)]$⟩. It indicates a considerably lower risk of high Hb variability (defined as within-subject SD >0.7 g/dL [21]) with rHu-Epo than with darbepoetin: OR 0.61 (95% CI 0.48-0.84).

Consequences of the hypothesis and discussion

Evaluation of the stated hypothesis seems to be a worthwhile effort. Due to the high incidence and prevalence of CRF and improvements in the overall care for these patients that have prolonged their life-expectancy, anemia management has turned into a long-term therapy. Under such conditions, effects that would otherwise be considered as minor would yield considerable actual benefits. For example, absolute risk reduction regarding mortality

over a 2-year period as low as 0.5% would, in absolute terms, mean thousands of saved lives. Combined with potentially reduced morbidity and hospitalizations, it would represent a considerable healthcare and pharmacoeconomic benefit.

Well designed clinical experiments (RCTs) comparing rHu-Epo and darbepoetin and/or CERA for long-term "hard" outcomes (cardiovascular and all-cause moribidity and mortality) would be needed for evaluation of the hypothesis. Using Hb variability as a surrogate outcome would not be appropriate. Although some epidemiological data suggest it as a predictor of poor long-term outcomes, the relationship between Hb variability and morbidity and mortality in CRF patients needs yet to be characterized. Furthermore, the hypothesized difference between rHu-Epo and darbepoetin or CERA might not be due solely to the ability of less variable Hb control. On the other hand, trials based on surrogate cardiovascular or renal markers (e.g., left ventricular mass/remodeling or ejection fraction, renal function) would not fully suffice, as they may not be fully predictive of the hard outcomes and they may not evaluate all possible differences between treatments relevant for the outcomes of interest.

Performing such trials would be logistically highly demanding. The trials would need to be long, sequential (for a timely recognition of an effect) and large (to ascertain the needed power). Also, a number of confounding factors would need to be considered, particularly the existing cardiovascular burden and related interventions, likely requesting stratified randomization. Considering that data exclusivity and patent protection for proprietary rHu-Epo has already expired (e.g., in the EU) or is about to expire (e.g., in the USA), it does not seem likely that pharmaceutical industry would have any interest in sponsoring such trials and it does not seem likely that such trials would ever be performed. An alternative approach could include meta-analysis of the existing comparative trials, particularly

individual patient data meta-analysis that could account for the starting patients' cardiovascular burden and other comorbidity, as well as for potential differences in targeted Hb levels in different trials. However, if faces two obstacles: the number of comparative trials is small and there seems to be no trial longer than 1 year. Therefore, under the circumstances, minutely planned stratified observational studies appear to be the only realistic and most feasible approach for evaluation of the stated hypothesis.

Conflict of interest / role of funding source

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References

[1] Lankhorst CE, Wish JB. Anemia in renal disease: diagnosis and management. Blood Rev 2010; 24:39-47.

[2] Jelkman W. Molecular biology of erythropoietin. Intern Med 2004; 43:649-659.

[3] Jelkman W. Eryhtorpoietin after a century of research: younger than ever. Eur J Haematol 2007; 78:183-205.

[4] Eschbach JW, Abdulahadi MH, Browne JK, Delano BG, Downing MR, Egrie JC et al. Recombinant human erythropoietin in anemic patients with end-stage renal disease. Resulst of a phase III multicenter clinical trial. Ann Intern Med 1989; 111:992-1000.

[5] Besarab A. Optimizing anaemia management with subcutaneous administration of epoetin. Nephrol Dial Transplant 2005; 20 [Suppl 6]: vi10-vi-15.

[6] Spinowitz B, Germain M, Benz R, Wolfson M, McGowan T, Tang KL et al. A randomized study of extended dosing regimens for initiation of epoetin alfa treatment for anemia of chronic kidney disease. Clin J Am Soc Nephrol 2008; 3:1015-1021.

[7] Pergola PE, Gartenberg G, Fu M, Sun S, Wolfson M, Bowers P. A randomized controlled study comparing once-weekly to ever-2-weeka and ever-4-week dosing of epoetin alfa in CKD patients with anemia. Clin J Am Soc Nephrol 2010; 5:598-606.

[8] Palmer SC, Navaneethan SD, Craig JC, Johnson DW, Tonelli M, Garg AX et al. Meta-analysis: erythropoiesis-stimulating agents in patients with chronic kidney disease. Ann Intern Med 2010; 153:23-33.

[9] Locatelli F, Aljama P, Barany P, Canaud B, Carrera F, Eckardt KU et al. Revised European best practice guidelines for the management of anaemia in patients with chronic renal failure. Nephrol Dial Transplant 2004; 19 [Suppl 2]:ii1-47.

[10] Macdougall IC. Novel erythropoiesis-stimulating agents: a new era in anemia management. Clin J Am Soc Nephrol 2008; 3:200-207.

[11] Fisher JW. Eythropoietin: physiology and pharmacology update. Exp Biol Med 2003; 228:1-14.

[12] Skibelli V, Nissen-Lie G, Torjesen P. Sugar profiling proves that serum erythropoietin differs from recombinant human erythropoietin. Blood 2001; 98:3626-3634.

[13] Storring PL, Tiplady RJ, Gaines Das RE, Stenning BE, Lamikanra A, Rafferty B et al. Epoetin alfa and beta differ in their erythropoietin isoform compositions and biological properties. Br J Haematol 1998; 100:79-85.

[14] Maise K, Chong ZZ, Li F, Shang YC. Erythropoietin: elucidating new cellular targets that broaden therapeutic strategies. Prog Neurobiol 2008; 85:194-213.

[15] Lisowska KA, Debska-Slizien A, Radzka M, Witkowski JM, Rutkowski B, Bryl E. Recombinant human erythropoietin treatment of chronic renal failure patients normalizes altered phenotype and proliferation of CD4-positive T lymphocytes. Artif Organs 2010; 34:E77-84.

[16] Brines M, Cerami A. Erythropoietin-mediated tissue protection: reducing collateral damage from the primary injury response. J Intern Med 2008; 264:405-432.

[17] Egrie JC, Browne JK. Development and characterization of novel erythropoiesis stimulating protein (NESP). Nephrol Dial Transplant 2001; 16[Suppl 3]:3-13.

[18] Jamal NM, Krzyzanski W, Cheung W, Lau CY, Messner HA. Evaluation of epoetin alpha (rHuEPO) and darbepoetin alpha (DARB) on human burst-colony formation (BFU-E) in culture. J Int Med Res 2006; 34:42-51.

[19] Panchapakesan U, Sumual S, Pollock C. Nanomedicines in the treatment of anemia in renal disease: focus on CERA (continuous erythropoietin receptor activator). Int J Nanomedicine 2007; 2:33-38.

[20] Yang W, Israni RK, Brunelli SM, Joffe MM, Fishbane S, Feldman HI. Hemoglobin variability and mortality in ESRD. J Am Soc Nephrol 2007; 18:3164-3170.

[21] Boudville NC, Djurdjev O, Macdougall IC, de Francisco ALM, Deray G, Besarab A. Hemoglobin variability in nondialysis chronic kidney disease: examining the association with mortality. Clin J Am Soc Nephrol 2009; 4:1176-1182.

[22] Fishbane S, Berns JS. Hemoglobin cycling in hemodialysis patients treated with recombinant human erythropoietin. Kidney Int 2005; 68:1337-1343.

[23] Nissenson AR, Swan SK, Lindberg JS, Soroka SD, Beatey R, Wang Ch et al. Randomized controlled trial of darbepoetin alfa for the treatment of anemia in hemodialysis patients. Am J Kindey Dis 2002; 40:110-118.

[24] Vanrenterghem Y, Barany P, Mann JFE, Kerr PG, Wilson J, Baker NF et al. Randomized trial of darbepoetin alfa for treatment of renal anemia at a reduced dose frequency compared with rHuEPO in dialysis patients. Kindey Int 2002; 62:2167-2175.

[25] Levin NW, Fishbane S, Canedo FV, Zeig S, Nassar GM, Moran JE et al. Intravenous methoxy polyethylene glycol-epoetin beta for haemoglobin control in patients with chronic kidney disease who are on dialysis: a randomized non-inferiority trial (MAXIMA). Lancet 2007; 370:1415-1421.

[26] Sulowicz W, Locatelli F, Ryckelynck JP, Balla J, Csiky B, Harris K et al. Once-monthly subcutaneous C.E.R.A. maintains stable hemoglobin control in patients with chronic kidney disease on dialysis converted directly from epoetin one to three times weekly. Clin J Am Soc Neprhol 2007; 2:637-646.

[27] Walker R, Pussell BA. Fluctuations in haemoglobin levels in hemodialysis patients receiving intravenous epoetin alfa or intravenous darbepoetin alfa. Nephrology 2007; 12:448-451.