

Nanomedicines in the treatment of anemia in renal disease: focus on CERA (Continuous Erythropoietin Receptor Activator)

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Abstract: Anemia is a common complication of chronic kidney disease (CKD), with erythropoietin deficiency being the major contributing factor. The availability of erythropoiesis-stimulating agents (ESAs) has been a seminal advance in the treatment of anemia related to chronic kidney disease. Over the course of the last decade and a half, newer generations of ESAs have become available. The first-generation ESAs or epoetins have a relatively shorter half-life and have traditionally been administered up to 3 times per week intravenously or subcutaneously to maintain adequate hemoglobin (Hb) levels. At the turn of the century, darbepoetin alfa, a hyperglycosylated form, became available for clinical use. It conferred greater metabolic stability in vivo owing to two additional N-linked carbohydrate chains attached to the protein backbone and has a half-life 3 times longer than that of epoetin (Macdougall et al 1999). Recently developed and undergoing phase III clinical trials is the third-generation ESA, Continuous Erythropoiesis Receptor Activator (CERA), which has a methoxy-polyethylene glycol polymer chain integrated and has a longer elimination half-life than the first- and second-generation ESAs. Its receptor binding characteristics also differ from those of previous ESAs. Its major advantage is that extended dosing intervals are possible in the management of anemia related to erythropoietin deficiency.

Keywords: CERA, epoetin, anemia, chronic kidney disease

Introduction

Anemia is common in chronic kidney disease (CKD) and although the cause is usually multifactorial, it is primarily related to the lack of erythropoietin (EPO) production. Mortality data from non-randomized studies show an association between anemia and risk of all-cause and cardiovascular death (Madore et al 1997; Locatelli et al 1998; Ma et al 1999; Xia et al 1999). However, randomized controlled trials targeting different hemoglobin (Hb) levels in hemodialysis patients with cardiac disease show conflicting results. Besarab et al (1998), in a study that was prematurely terminated, showed that in patients with pre-existing cardiac disease, treatment of anemia resulting in higher targeted Hb led to an increased risk of death. This study was prematurely halted prior to reaching the pre-specified statistical stopping boundary. Normalization of Hb levels in incident hemodialysis patients does not have a beneficial effect on cardiac structure (Parfrey et al 2005). Recent data suggest an association between high Hb levels and reduced mortality (Rayner et al 2004; Parfrey et al 2005). Trials in the pre-dialysis population are not powered to detect such a difference (Pollock and McMahon 2005). There is emerging evidence to suggest that the adverse effects of anemia are not only related to Hb levels but also to the low levels of EPO. EPO stimulates the proliferation and migration of endothelial cells, promotes angiogenesis (Anagnostou et al 1990; Yamaji et al 1996; Ribatti et al 1999, 2003) and prevents apoptotic injury (Kawakami et al 2001; Sekiguchi et al 2004). It is becoming evident that the benefits of EPO treatment may extend above and beyond the treatment of anemia.

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Anemia in renal failure and traditional treatment options

Prior to the availability of erythropoiesis-stimulating agents (ESAs), the treatment of anemia related to CKD was limited to recurrent blood transfusions. Transfusion dependence was associated with the risks of blood-borne infections, iron overload, and HLA sensitization, subsequently limiting renal transplantation opportunities. The development of ESAs has been a seminal advance in the treatment of anemia. This is coupled with the use of intravenous iron. The European Best Practice Guidelines (EBPG) (Locatelli et al 2004) suggest target Hb levels of >11g/dL within 4 months of starting therapy. Although there is a lack of consensus surrounding the desired upper limit of the Hb level due to a lack of evidence, the EBPG suggest limiting Hb levels to <14 g/dL in hemodialysis patients and to <12 g/dL in patients with severe cardiovascular disease and in patients with diabetes and peripheral vascular disease. The CARI (Caring for Australians with Renal Impairment) Guidelines recommend Hb levels between 12 and 14 g/dL in those without proven or likely significant cardiovascular disease and should not exceed 12 g/dL in those with proven or significant cardiovascular disease (Pollock and McMahon 2005). The main concerns with higher Hb targets include the risk of hypertension and vascular thrombosis. Iron deficiency is common in patients with CKD and the cause is usually multifactorial. Once ESAs are commenced there is a rapid depletion of iron stores due to erythropoiesis. Failure to correct iron deficiency commonly leads to EPO resistance. Iron is usually replaced parenterally and therapy is individualized based on the results of iron studies. Sufficient iron should be administered to maintain a transferrin saturation of >20% and a minimum serum ferritin of >100 µg/L, although the majority would accept that ferritin should be maintained well above this level. This often involves ongoing regular doses of intravenous iron.

EPO, a hematopoietic growth factor, was purified from the urine of patients with aplastic anemia in 1977 (Miyake et al 1977), which led to the subsequent cloning of the human EPO gene in 1985 (Jacobs et al 1985) and the use of recombinant human EPO (Eschbach et al 1987). It is a sialoglycoprotein hormone that is immunologically and biologically indistinguishable from the endogenous form (Faulds and Sorokin 1989). The first ESAs were recombinant human EPO alpha and beta (epoetins) which differed in their glycosylation. They were initially administered 2–3 times per week on the basis of their relatively short biological half-life (Halstenson et al 1991). However more recently it is recognized that the dosing interval may be extended to at

least once per week (Barre et al 2004). Darbepoetin alfa, a second generation ESA, became available at the turn of the century. Due to its hyperglycosylated structure it has a longer elimination half-life (Macdougall et al 1999) allowing for an extended dosing interval. The latest ESA, a third-generation drug known as CERA (Continuous Erythropoietin Receptor Activator), has a higher molecular weight at 60 kDa and has a methoxy-polyethylene glycol chain integrated via amide bonds between the N-terminal amino group of lysine using a succinimidyl butanoic acid linker (Bailon et al 2003). It has a significantly longer elimination half-life in vivo and allows for a further extension of dosing intervals.

Mechanism of action and pharmacokinetics

Receptor binding characteristics as well as the pharmacokinetics of CERA differ quite significantly from first and second generation ESAs (Macdougall et al 2003). CERA has a lower affinity for its receptor and dissociates faster from the soluble EPO receptor compared with epoetin β, and has a reduced activity with respect to cellular proliferation in vitro. It is considered that the binding of CERA to its receptor is too brief to allow for internalization of the molecule therefore the repeated binding, stimulation and dissociation leads to prolonged activity in vivo and an extended elimination half-life. Pharmacokinetic properties have been studied in rodent and non-rodent models and the systemic clearance of CERA has been found to be much lower than that of epoetin, contributing to an extended elimination half-life and prolonged stimulation of erythropoiesis (Bailon et al 2003). The pharmacokinetic and pharmacodynamic profile of CERA in anemic (Hb ≤12 g/dL) CKD patients on peritoneal dialysis have been examined in i.v. and s.c. dosing schedules. These studies demonstrated prolonged and comparable half-life for CERA (i.v. or s.c.), suggesting feasibility of extended dosing intervals in this population (Macdougall et al 2005). In healthy patients, the pharmacokinetic properties of s.c. CERA are unaffected by administration site (arm, abdomen, thigh) (Fishbane et al 2005), allowing for flexibility in the injection site. An in vitro model showed that there was no effect of hemodialysis or hemofiltration on serum concentrations of CERA (Dougherty et al 2003).

Efficacy and safety studies

Preclinical studies in vitro

The in vitro erythropoietic activity of CERA has been assessed by measuring its effect on the proliferation of a

human acute myeloid leukaemia cell line (UT-7 cell line) that expresses the EPO receptor. Compared with epoetin, CERA stimulated less proliferation of UT-7 cells across a dose range 0.003–3 U/mL. However, in normocythemic mice, CERA induces greater reticulocytosis compared to epoetin β (Haselbeck et al 2002). This is possibly attributed to the binding characteristics and pharmacokinetics as described above.

Preclinical studies in vivo

Preclinical studies in animal models using intravenous (i.v.) and subcutaneous (s.c.) CERA, in single and multiple doses, in the range 0.75–20 $\mu\text{g}/\text{kg}$ demonstrate CERA to be a more potent stimulator of erythropoiesis than epoetin with respect to the magnitude and duration of response (Fishbane et al 2003).

Phase I clinical studies in healthy volunteers

Phase I studies using single and multiple ascending doses via intravenous and subcutaneous routes resulted in dose-dependent increments of reticulocyte counts. In the randomized single ascending dose studies, healthy subjects received i.v. CERA in the dose range 0.4–3.2 $\mu\text{g}/\text{kg}$ or s.c. CERA in the dose range 0.1–3.2 $\mu\text{g}/\text{kg}$ vs placebo respectively (Reigner et al 2003). In the randomized multiple ascending dose studies, healthy subjects received 3 i.v. doses of CERA in the dose range 0.4–3.2 $\mu\text{g}/\text{kg}$ every 3 weeks or 4 s.c. doses of CERA in the same dose range every fortnightly vs placebo, respectively. Peak effects in reticulocyte counts were seen at 7 and 10 days after i.v. and s.c. administration respectively. Repeated dosing had no apparent clinically relevant effect on pharmacokinetics and accumulation did not occur with 3-weekly i.v. dosing and was small with fortnightly s.c. dosing. CERA was generally well tolerated and no serious adverse events were reported (Dougherty et al 2004).

Phase II clinical studies in anemic patients

The use of CERA for the treatment of anemia in CKD has been studied in Phase II clinical trials, both in ESA naïve dialysis and non-dialysis patients for the correction of anemia and the maintenance of Hb levels. de Francisco et al (de Francisco et al 2003) studied the efficacy of s.c. CERA in 61 dialysis patients (≥ 18 years; Hb 8–11 g/dL; on hemodialysis ≥ 1 month and on peritoneal dialysis ≥ 2 months). Three dose levels were tested in sequence, with three dosing intervals in

each sequence. Following a 4-week run-in period, patients in the first sequence were randomized to receive CERA (0.15 $\mu\text{g}/\text{kg}$ per week, 0.30 $\mu\text{g}/\text{kg}$ per 2 weeks, or 0.45 $\mu\text{g}/\text{kg}$ per 3 weeks). Patients in the second sequence received 0.3 $\mu\text{g}/\text{kg}$ per week and those in the third 0.45 $\mu\text{g}/\text{kg}$ per week. After 6 weeks, dose adjustments were permitted according to defined Hb criteria and patients were followed for 12 weeks. A response was noted in all three dosing intervals and with all three starting doses. Mean increases in Hb were 0.84, 1.15, and 1.11 g/dL during the first 6 weeks of constant dosing and 1.15, 2.50, and 2.35 g/dL during the entire study, in the low, intermediate, and high dose groups, respectively. Faster response time was associated with increasing dose with the median time to response in the low dose group of 52 days, in the intermediate dose group of 38 days, and in the high dose group of 30 days. No major adverse effects occurred. One patient experienced a pruritic rash. Hence this study showed CERA had a potent erythropoietic activity with a favorable safety profile. Provenzano et al in the BA16528 study examined the efficacy of subcutaneous CERA in 65 epoetin-naïve non-dialysis patients with CKD. After a 2 week run-in period, patients were randomized to receive CERA (0.15 $\mu\text{g}/\text{kg}$ per week or 0.30 $\mu\text{g}/\text{kg}$ per week or 0.60 $\mu\text{g}/\text{kg}$ per week) once weekly, once every 2 weeks, or once every 3 weeks. Patients were followed for 18 months. Hb increases occurred in each dose group with mean Hb increases of 0.31, 0.70, and 1.76 g/dL during the first 6 weeks in the 0.15, 0.30, and 0.60 $\mu\text{g}/\text{kg}$ per week groups respectively. The Hb change from baseline was dose dependent but was independent of the frequency of administration and was sustained until the end of the study. The effect on Hb was independent of the frequency of administration and responses were sustained until the end of the study (Provenzano et al 2004). This extended to a long-term Phase II study over a 54-week period where patients continued to receive their original dose of s.c. CERA weekly, fortnightly, or once every 3 weeks with the aim of maintaining Hb levels at 11–12 g/dL. During this study period, mean Hb levels which were measured monthly were 11.3 g/dL with once weekly dosing, 11.4 g/dL with fortnightly dosing, and 11.7 g/dL with once every 3 weeks dosing. CERA was generally well tolerated. Hence CERA showed sustained and stable control of anemia in non dialysis patients with CKD.

Phase II studies have also been conducted in anemic dialysis patients to determine the efficacy of CERA in maintaining Hb levels (Hb 10–13 g/dL). In the first study, patients on 3 times a week i.v. epoetin alfa (dose 80–250 IU/kg per week) were changed to i.v. CERA. Three conversion

factors were used to calculate dose, based on the estimated equi-effective dose of CERA to epoetin: 0.25 µg CERA/150 IU epoetin, 0.4/150, and 0.6/150. Once weekly and once every 2 weeks administration schedules were assessed for each conversion factor and patients were followed for 19 weeks. The results from the primary efficacy analysis showed that the 0.6/150 conversion factor was adequate for providing stable Hb levels in patients treated once a fortnight. These studies showed that fortnightly i.v. CERA may maintain stable Hb levels in dialysis patients (Besarab et al 2004). In the second study by Locatelli et al 137 dialysis patients previously on s.c. epoetin (1–3 x/wk) were randomized to one of three arms receiving CERA, with the dose administered estimated to be equivalent to the previous epoetin dose and data from CERA exposure in healthy volunteers. The median CERA doses per week in each group were 19 µg/kg, 32 µg/kg, and 48 µg/kg. Once weekly, once every 3 weeks, and once every 4 weeks dosing schedules were assessed in each group and patients were followed for 19 weeks (21 weeks for once every 4 weeks cohorts) (Locatelli et al 2004). There was a dose-dependent effect on Hb change from baseline with mean Hb change of –0.63, –0.10, and 0.48 g/dL in each of the groups from lowest to highest doses respectively. This dose-finding study showed that s.c. CERA was effective in maintaining Hb levels in dialysis patients when administered every 4 weeks.

Clinical studies in cancer-related anemia

Limited studies to date have been undertaken assessing CERA in patients with cancer-related anemia. An exploratory Phase I–II dose escalation study was performed in patients with multiple myeloma and anemia (Hb ≤11 g/dL). CERA was administered s.c. once every 3 weeks for 6 weeks with an optional extension to 12 weeks. Initially patients were randomized to receive doses of 2.0, 3.5, or 5.0 µg/kg. Following review of efficacy and safety data, additional patients were assigned sequentially to doses of 6.5 and 1.0 µg/kg, then 8.0 and 4.2 µg/kg. CERA (3.5–8.0 µg/kg) produced a dose-related increase in Hb of 1.6–2.3 g/dL, which resulted in a rapid and sustained Hb increase (Dmoszynska et al 2004).

Phase III clinical trials

Four Phase III clinical trials for CERA were recently completed (Roche, data on file). In these maintenance studies, dialysis patients on stable maintenance treatment of anemia with epoetin or darbepoetin were randomized to either continue their treatment or to change over to CERA every 2

weeks or 4 weeks. The primary endpoint was a change in Hb concentration between baseline and the evaluation period. These studies met their primary endpoints and showed that both routes of administration, ie, s.c. and i.v. at extended dosing intervals were effective in maintaining Hb levels. The first study was designed to evaluate i.v. CERA (dosed every 2 weeks or every 4 weeks) for maintenance of Hb in dialysis patients previously on i.v. epoetin (dosed up to 3 times weekly). The second study was similar to the first, but CERA and epoetin were administered s.c. The third study was designed to evaluate i.v. CERA (dosed fortnightly) for maintenance of Hb in dialysis patients previously on i.v. darbepoetin alfa (dosed once a week or once a fortnight). The fourth study was designed to evaluate s.c. or i.v. CERA (dosed once a fortnight) in a pre-filled syringe in the maintenance of Hb in dialysis patients previously on epoetin (dosed up to 3 times weekly). The Phase III correction of anemia studies in dialysis and non-dialysis patients are nearing completion.

Safety profile

CERA has been generally well tolerated with no unexpected safety concerns. In phase II studies in dialysis patients (n=109) administered i.v. or s.c. CERA for 12 months, the most common adverse events were hypotension (8.02%), muscle cramp (4.39%), hypertension (3.05%), headache (2.86%), and nasopharyngitis (2.10%). The most common serious adverse events were hypotension (5 events), myocardial infarction (5 events), cellulitis (4 events) and pancreatitis (4 events). The frequency of the adverse events appeared to be characteristic of the patient population under study, rather than being attributable to CERA (Dougherty and Beyer 2005b). In the Phase III trials the common adverse events in all treatment groups were infections, injuries, procedural complications, and gastrointestinal disorders. Again these events were characteristic of the population under study and not attributable to the study drug. There have been no reports of pure red cell aplasia with CERA as has occurred particularly with the first-generation ESAs. However, this remains a potential or theoretical risk. In contrast to the side-effects reported with the first- and second-generation ESAs, no changes in blood pressure were noted in dialysis patients after 12 months of treatment with i.v. or s.c. CERA (Dougherty and Beyer 2005a).

Patient implications

The greatest advantage of CERA over first- and second-generation ESAs is the extended dosing intervals needed

to achieve adequate Hb levels for the treatment of anemia related to CKD and potentially cancer. This has an advantage not only for health care providers but also for patients, especially those who self-administer ESAs. For patients with anemia related to cancers, coinciding chemotherapy and other parenteral medications provides additional benefits. As the pharmacodynamics are unaffected by injection sites, this offers flexibility and convenience to patients, suggesting that compliance would be increased.

Conclusions

ESAs have revolutionized the treatment of anemia in CKD and cancers. Although there remains discussion around the mode of administration and the risk of pure red cell aplasia, the form of delivery of the ESA (prefilled syringe, single- or multi-use dispensers), difference in discomfort at the injection site, and so on, the main differentiating factor between the ESAs relates to their half-life and hence the dosing interval. First-generation ESAs have a relatively short half-life resulting in more frequent administration to maintain Hb levels. Darbepoetin, a second-generation ESA, has a longer elimination half life (Macdougall et al 2003) both in the i.v. and s.c. routes of administration. Darbepoetin is widely used and trials in CKD and dialysis patients have shown that initial one weekly dose is effective and safe (Macdougall et al 2003) both in the i.v. and s.c. routes of administration. Once-weekly darbepoetin alfa is equipotent to thrice-weekly epoetin (Nissenson et al 2002). Stable patients on once a week epoetin can be converted to once a fortnight darbepoetin alfa (Locatelli et al 2003; Brunkhorst et al 2004). Small studies have shown that monthly administration of darbepoetin was effective in the treatment of anemia in dialysis and non-dialysis patients with CKD (Jadoul et al 2004; Ling et al 2005; Theodoridis et al 2005). Hence darbepoetin alfa can be effectively and safely administered less frequently than epoetin. It is more potent at longer dosing intervals and at higher doses (Scott 2002). Initial data from clinical studies suggest that the pharmacokinetic and pharmacodynamic properties of CERA will allow a further increase in the dosing schedule of CERA. The clinical and economic consequences remain to be determined in large-scale clinical trials.

References

- Anagnostou A, Lee ES, Kessimian N, et al. 1990. Erythropoietin has a mitogenic and positive chemotactic effect on endothelial cells. *Proc Natl Acad Sci U S A*, 87:5978-82.
- Bailon P, Pahlke W, Brandt M, et al. 2003. CERA (continuous erythropoiesis receptor activator) for the treatment of renal anemia: a new agent with an innovative mechanism of action [abstract]. *Nephrol Dial Transplant*, 166(Suppl 4).
- Barre P, Reichel H, Suranyi MG, et al. 2004. Efficacy of once-weekly epoetin alfa. *Clin Nephrol*, 62:440-8.
- Besarab A, Bansal V, Fishbane S, et al. 2004. Intravenous CERA (Continuous Erythropoiesis Receptor Activator) administered once weekly or once every 2 weeks maintains haemoglobin levels in haemodialysis patients with chronic renal anemia [abstract]. Abstract Book of the XLI Congress of the ERA-EDTA 230 (Abstract M047).
- Brunkhorst R, Bommer J, Braun J, et al. 2004. Darbepoetin alfa effectively maintains haemoglobin concentrations at extended dose intervals relative to intravenous or subcutaneous recombinant human erythropoietin in dialysis patients. *Nephrol Dial Transplant*, 19:1224-30.
- de Francisco AL, Sulowicz W, Dougherty FC, et al. 2003. Subcutaneous CERA (Continuous Erythropoiesis Receptor Activator) has potent erythropoietic activity in dialysis patients with chronic renal anemia: an exploratory multiple dose study [abstract]. *J Am Soc Nephrol*, 14(27A-28A):(Abstract SA-FC124).
- Dmoszynska A, Kloczko J, Rokicka M, et al. 2004. CERA (Continuous Erythropoiesis Receptor Activator) in patients with multiple myeloma: an exploratory phase I-II dose escalation study [abstract]. *J Clin Oncol*, 22(Suppl 5):14S (Abstract 6552).
- Dougherty FC, Beyer U. 2005a. No changes in blood pressure in dialysis patients after 12 months of treatment with i.v. or s.c. CERA (Continuous Erythropoietin Receptor Activator)[abstract]. *Nephrology*, 10(Suppl.) (A313):Abstract W-PO40131.
- Dougherty FC, Beyer U. 2005b. Safety and tolerability profile of Continuous Erythropoietin receptor Activator (CERA) with extended dosing intervals in patients with chronic kidney disease on dialysis [abstract]. *Nephrology*, 10(A313):Abstract W-PO40130.
- Dougherty FC, Leypoldt JK, Cheung AK, et al. 2003. No effect of hemodialysis or hemofiltration on concentrations of CERA (Continuous Erythropoiesis Receptor Activator): *In Vitro* Study Results [abstract]. *J Am Soc Nephrol*, 14(770A):SU-PO1065.
- Dougherty FC, Reigner B, Jordan P, et al. 2004. CERA (Continuous Erythropoiesis Receptor Activator): dose-response, pharmacokinetics and tolerability in phase I multiple ascending dose studies [abstract]. *J Clin Oncol*, 22(Suppl 15):14S (Abstract 6692).
- Eschbach JW, Egrie JC, Downing MR, et al. 1987. Correction of the anemia of end-stage renal disease with recombinant human erythropoietin. Results of a combined phase I and II clinical trial. *N Engl J Med*, 316:73-8.
- Faulds D, Sorokin EM. 1989. Epoetin (recombinant human erythropoietin). A review of its pharmacodynamic and pharmacokinetic properties and therapeutic potential in anaemia and the stimulation of erythropoiesis. *Drugs*, 38:863-99.
- Fishbane S, Pannier A, Liogier X, et al. 2005. Pharmacokinetic (PK) properties of subcutaneous (s.c.) CERA (Continuous Erythropoietin Receptor Activator) are unaffected by administration site [abstract]. *J Am Soc Nephrol*, 16:SA-PO942.
- Fishbane S, Tare N, Pill J, et al. 2003. Preclinical pharmacodynamics and pharmacokinetics of CERA (Continuous Erythropoietin Receptor Activator), an innovative erythropoietic agent for anemia management in patients with kidney disease [abstract]. *J Am Soc Nephrol*, 14:Abstract SA-FC 123.
- Halstenon CE, Macres M, Katz A, et al. 1991. Comparative pharmacokinetics and pharmacodynamics of epoetin alfa and epoetin beta. *Clin Pharmacol Ther*, 50:702-12.
- Haselbeck A, Bailon P, Pahlke W, et al. 2002. The discovery and characterization of CERA, an innovative agent for the treatment of anemia [abstract]. *Blood*, 100:Abstract 857.
- Jacobs K, Shoemaker C, Rudersdorf R, et al. 1985. Isolation and characterization of genomic and cDNA clones of human erythropoietin. *Nature*, 313(6005):806-10.

- Jadoul M, Vanrenterghem Y, Foret M, et al. 2004. Darbepoetin alfa administered once monthly maintains haemoglobin levels in stable dialysis patients. *Nephrol Dial Transplant*, 19:898-903.
- Kawakami M, Sekiguchi M, Sato K, et al. 2001. Erythropoietin receptor-mediated inhibition of exocytotic glutamate release confers neuroprotection during chemical ischemia. *J Biol Chem*, 276:39469-75.
- Ling B, Walczyk M, Agarwal A, et al. 2005. Darbepoetin alfa administered once monthly maintains hemoglobin concentrations in patients with chronic kidney disease. *Clin Nephrol*, 63:327-34.
- Locatelli F, Aljama P, Barany P, et al. 2004. Revised European best practice guidelines for the management of anaemia in patients with chronic renal failure. *Nephrol Dial Transplant*, 19(Suppl 2):ii1-47.
- Locatelli F, Canaud B, Giacardy F, et al. 2003. Treatment of anaemia in dialysis patients with unit dosing of darbepoetin alfa at a reduced dose frequency relative to recombinant human erythropoietin (rHuEpo). *Nephrol Dial Transplant*, 18:362-9.
- Locatelli F, Conte F, Marcelli D, et al. 1998. The impact of haematocrit levels and erythropoietin treatment on overall and cardiovascular mortality and morbidity--the experience of the Lombardy Dialysis Registry. *Nephrol Dial Transplant*, 13:1642-4.
- Locatelli F, Villa G, et al. 2004. CERA (Continuous Erythropoietin Receptor Activator) maintains hemoglobin levels in dialysis patients when administered subcutaneously up to once every 4 weeks [abstract]. *J Am Soc Nephrol*, 15:SU-PO051.
- Ma JZ, Ebben J, Xia H, et al. 1999. Hematocrit level and associated mortality in hemodialysis patients. *J Am Soc Nephrol*, 10:610-9.
- Macdougall IC, Bailon P, Tare N, et al. 2003. CERA (Continuous Erythropoiesis Receptor Activator) for the treatment of renal anemia: an innovative agent with unique receptor binding characteristics and prolonged serum half-life [abstract]. *J Am Soc Nephrol*, 14:Abstract SU-PO1063.
- Macdougall IC, Gray SJ, Elston O, et al. 1999. Pharmacokinetics of novel erythropoiesis stimulating protein compared with epoetin alfa in dialysis patients. *J Am Soc Nephrol*, 10:2392-5.
- Macdougall IC, Matcham J, Gray SJ, et al. 2003. Correction of anaemia with darbepoetin alfa in patients with chronic kidney disease receiving dialysis. *Nephrol Dial Transplant*, 18:576-81.
- Macdougall IC, Robson R, Opatrna S, et al. 2005. Pharmacologic Profile of CERA (Continuous Erythropoietin Receptor Activator) in chronic kidney disease (CKD) patients (pts) following intravenous (i.v.) and subcutaneous (s.c.) administration [abstract]. *J Am Soc Nephrol*, 16:SA-PO926.
- Madore F, Lowrie EG, Brugnara C, et al. 1997. Anemia in hemodialysis patients: variables affecting this outcome predictor. *J Am Soc Nephrol*, 8:1921-9.
- Miyake T, Kung CK, Goldwasser E, et al. 1977. Purification of human erythropoietin. *J Biol Chem*, 252:5558-64.
- Nissenson AR, Swan SK, Lindberg JS, et al. 2002. Randomized, controlled trial of darbepoetin alfa for the treatment of anemia in hemodialysis patients. *Am J Kidney Dis*, 40:110-8.
- Parfrey PS, Foley RN, Wittreich BH, et al. 2005. Double-blind comparison of full and partial anemia correction in incident hemodialysis patients without symptomatic heart disease. *J Am Soc Nephrol*, 16:2180-9.
- Pollock C, McMahon, L. 2005. The CARI Guidelines: Haemoglobin. *Nephrology*, 10(S4):S108-S115.
- Provenzano R, Besarab A, Macdougall IC, et al. 2004. CERA (Continuous Erythropoietin Receptor Activator) administered up to once every 3 weeks corrects anemia in patients with chronic kidney disease not on dialysis [abstract]. *J Am Soc Nephrol*, 15:Abstract SU-PO056.
- Rayner HC, Pisoni RL, Bommer J, et al. 2004. Mortality and hospitalization in haemodialysis patients in five European countries: results from the Dialysis Outcomes and Practice Patterns Study (DOPPS). *Nephrol Dial Transplant*, 19:108-20.
- Reigner B, Jordan P, Pannier A, et al. 2003. Phase 1 studies of the new erythropoietic agent, CERA (continuous erythropoiesis receptor activator): demonstration of a dose-dependent response [abstract]. *Nephrol Dial Transplant*, 18(Suppl 4):167 (Abstract M527).
- Ribatti D, Presta M, Vacca A, et al. 1999. Human erythropoietin induces a pro-angiogenic phenotype in cultured endothelial cells and stimulates neovascularization in vivo. *Blood*, 93:2627-36.
- Ribatti D, Vacca A, Roccaro A M, et al. 2003. Erythropoietin as an angiogenic factor. *Eur J Clin Invest*, 33:891-6.
- Scott SD. 2002. Dose conversion from recombinant human erythropoietin to darbepoetin alfa: recommendations from clinical studies. *Pharmacotherapy*, 22:160S-165S.
- Sekiguchi N, Inoguchi T, Kobayashi K, et al. 2004. Effect of erythropoietin on endothelial cell apoptosis induced by high glucose. *Diabetes Res Clin Pract*, 66(Suppl 1):S103-7.
- Theodoridis M, Passadakis P, Kriki P, et al. 2005. Efficient monthly subcutaneous administration of darbepoetin in stable CAPD patients. *Perit Dial Int*, 25:564-9.
- Xia H, Ebben J, Ma JZ, et al. 1999. Hematocrit levels and hospitalization risks in hemodialysis patients. *J Am Soc Nephrol*, 10:1309-16.
- Yamaji R, Okada T, Moriya M, et al. 1996. Brain capillary endothelial cells express two forms of erythropoietin receptor mRNA. *Eur J Biochem*, 239:494-500.