Early suppression of glomerulosclerosis in rats with progressive glomerulonephritis

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Introduction and Aims: C.E.R.A. is a long-acting erythropoiesis stimulating agent widely used to treat anemia in patients with chronic kidney disease. Although C.E.R.A. shows clinical benefit with respect to kidney disease in diabetic nephropathy models, the therapeutic effects on the progression of kidney disease have not been fully elucidated. In this study we aimed to investigate the renoprotective effect of C.E.R.A. in rats with progressive glomerulonephritis induced by anti-Thy-1.1 antibody and ureteronephrectomy (chronic glomerulonephritis: CGN).

Methods: CGN rats (F344, male, 6 wk old) were established by injection of anti-Thy-1.1 monoclonal antibody (mAb: OX-7; 0.6 mg/kg, i.v.) after ureteronephrectomy (Day 0). C.E.R.A. (25 μg/kg, i.v.) was injected 24 h after the injection of the mAb. We evaluated changes over time in hemoglobin (HB), blood urea nitrogen (BUN), and urinary total protein (uTP) after the induction of kidney disease. At Wk 20, we measured wet kidney weight (KW) and creatinine clearance (CCR). Renal cortical tissue was harvested at days 1, 4, 8, and 28 (each n=8) for histological and RT PCR analysis. Glomerulosclerosis expressed as glomerulosclerosis index (GSI) was evaluated using 50 randomly selected glomeruli in PAS-stained sections. In addition, RT PCR analysis for extracellular matrix fibronectin and connective tissue growth factor (CTGF) were performed.

Results: First, we examined the effects of C.E.R.A. on changes in biochemical parameter of kidney function. In CGN rats (disease control: DC n=12), compared with sham-operated rats (Sham, n=6), significant increases in uTP and BUN and significant decrease in HB was observed. On the other hand, C.E.R.A. treatment (C.E.R.A., n=12) significantly improved all of these parameters (DC vs. C.E.R.A., 2-way ANOVA followed by Bonferroni post hoc test). Furthermore, at Wk 20 there was a significant increase in kidney weight per body weight (KW/BW) and a significant decrease in CCR in the DC group compared with the Sham group. C.E.R.A. treatment significantly suppressed the increase in KW/BW (DC 6.7±0.6; C.E.R.A. 5.4±0.4, p<0.05 vs. DC) and decrease in CCR (DC 19.7% of Sham vs. C.E.R.A. 60.5% of Sham). Next, we assessed changes over time in GSI and mRNA expressions of CTGF and extracellular matrix fibronectin in the kidney. Elevated GSI was observed from Day 4 to 28. In contrast, C.E.R.A. treatment significantly inhibited GSI at Days 8 and 28. C.E.R.A. treatment significantly inhibited mRNA expressions of CTGF and fibronectin at Day 4. However, there were no differences between the DC group and the C.E.R.A. group in these mRNA levels after Day 8.

Conclusions: These results suggested that the treatment with C.E.R.A. inhibited progression of chronic kidney disease, resulting in improvement of anemia in rats with progressive glomerulonephritis. The amelioration of kidney function by C.E.R.A. might be in part due to early suppression of glomerulosclerosis.

Continuous erythropoietin receptor activator (C.E.R.A.) treatment prevents glomerulosclerosis and suppresses activation of alternatively activated macrophages in a rat model of acute glomerulonephritis

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Introduction and Aims: Mesangial proliferative glomerulonephritis (GN) shows features typical of glomerular pathology, including expansion of mesangial cells and extracellular matrix, a critical cause of glomerulosclerosis. Macrophages infiltrating into the glomerulus are implicated in promoting kidney fibrosis, and alternatively activated (M2) macrophages play a critical role in the progression of glomerulosclerosis. C.E.R.A., a long-acting erythropoiesis-stimulating agent (ESA) used to treat anemia, shows renoprotective effects in several models. However, the protective effect of C.E.R.A. on mesangial proliferative GN is not yet clear. We assessed the renoprotective effect of C.E.R.A. in an anti-Thy-1.1 antibody-induced GN (Thy1-GN) rats, a model of mesangial proliferative GN, and investigated whether C.E.R.A. suppresses the infiltration of macrophages.

Methods: Thy1-GN rats (F344, male, 6 wk old) were established by injecting anti-Thy-1.1 monoclonal antibody (OX-7: 0.6 mg/kg, i.v.). C.E.R.A. or darbepoetin-α (DA) (25 μg/kg, i.v.) was injected 4 h before induction of GN (day 0). At day 6, blood and urine was sampled for analysis of blood urea nitrogen (BUN), plasma creatinine, urinary protein and N-acetyl-b-(D)-glucosaminidase (NAG). To evaluate the effects of C.E.R.A., kidneys were harvested for histological analysis and RT-PCR analysis.

Results: At day 6 after induction of GN, C.E.R.A. and DA significantly suppressed proteinuria in Thy1-GN rats (Normal, 2.6±0.2; Thy1-GN: 72.1±5.6; Thy1-GN+C.E.R.A., 48.8±4.1; Thy1-GN+DA, 47.4±2.5 mg/day; mean±SE, n=5–10). The increased BUN, plasma creatinine, and NAG in Thy1-GN rats were also significantly suppressed by C.E.R.A. and DA. Histologically, C.E.R.A. and DA significantly suppressed glomerulosclerosis index and expression of α-smooth muscle actin protein. C.E.R.A. also prevented upregulation of mRNA of extracellular matrix proteins such as collagen-1 and fibronectin in isolated glomeruli. Infiltration of macrophages, evaluated by immunohistochemistry staining of ED-1 in glomeruli, was significantly prevented by treatment with C.E.R.A. Expression of monocyte chemotactic protein-1 mRNA in glomeruli was also inhibited by C.E.R.A. Expression of TNF-α mRNA (an M1 macrophage marker) was decreased in Thy1-GN rat glomeruli and not changed by C.E.R.A.; conversely, arginase-1 mRNA (an M2 macrophage marker) was markedly upregulated in Thy1-GN rats and significantly suppressed by C.E.R.A. (arginase-1/ GAPDH, Normal, 0.2±0.01; Thy1-GN, 1.5±0.07; Thy1-GN+C.E.R.A., 0.8±0.1; mean±SE, n=5–8).

Conclusions: These results suggest the long-acting ESA, C.E.R.A., has a renoprotective effect in mesangial proliferative GN. Also, C.E.R.A. significantly prevented production of extracellular matrix proteins in the glomeruli of Thy1-GN rats. Suppression of macrophage infiltration in glomeruli, particularly M2 macrophages, may contribute to the renoprotective effect induced by C.E.R.A.
Methods: Sixty patients with chronic kidney disease on hemodialysis (15M/45F, 60±14 years) and on peritoneal dialysis (17M/15F, 59±15 years) without clinical manifestations of inflammation as well as twenty-nine healthy controls (10M/19 F, 66±16 years) were recruited. Serum hepcidin levels were measured by competitive ELISA method and high-sensitivity CRP by nephelometry.

Results: In PD patients, hepcidin levels were significantly higher than in NC (313.7±32.7 vs. 131.4±55.9 ng/ml, mean ±SD, p<0.001), but did not differ from those of HD patients (300.1±38.6). CRP concentrations were significantly higher (p<0.001) in patients than in NC (1.35±0.14 mg/l vs. 4.28±3.70 in HD and 4.89±3.69 in PD). A Hepcidin in HD and PD patients was positively correlated to CRP (HD group: r=0.513, p=0.038 and PD group: r=0.384, p=0.048).

Conclusions: Elevated hepcidin concentration in serum of peritoneal and hemodialysis patients may be associated to inflammation.

MP195
SUPERIORITY OF DARBEPOETIN-ALPHA TO ERYTHROPOIETIN IN ERYTHROPOETIC ACTIVITY BY HEPcidIN-25 RESPONSE
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Introduction and Aims: Hepcidin is the key regulator of iron metabolism and is suppressed by erythropoietic activity (Pak M. et al. Blood 108:3735, 2006). The present study was conducted to demonstrate the better effect of longer-acting darbeopoetin-alpha (DPO) than that of short-acting recombinant human erythropoietin (EPO) in hemodialysis (HD) patients from the standpoint of hepcidin metabolism.

Methods: To assess the potency of DPO to mobilize iron from body stores in comparison with EPO in HD patients without apparent inflammation or infection, serial serum iron, transferrin saturation (TSAT), ferritin, and hepcidin-25 were measured serially. The present study included (i) a long-term crossover study for 3 years to compare the effects of the two ESA on serum iron, TSAT, and ferritin, and (ii) a short-term crossover study for 8 weeks to examine their effects on serum hepcidin-25 in HD patients. Twenty-eight uremic patients maintained on HD were enrolled in the long-term study. For the first year, all patients were maintained on trice weekly EPO injection with the target Hb level set between 10.0 and 11.0 g/dL. Then trice weekly EPO injection was replaced with a weekly DPO injection for two years thereafter. The exchange ratio of administration doses between EPO and DPO was based on equivalent peptide mass: 200 units (U) EPO to 1 µg DPO, as previously described. In the short-term study, six hemodialysis patients with stable Hb levels by EPO (3000 units x 3/week) were enrolled. After 4 weeks, EPO was switched to DPO (40 mcg weekly). No iron administration was done during the experiment. At each 6, 2nd, 4th, 7th and 28th days, serum Hb, Fe, TIBC, ferritin, Hepcidin-25 (by liquid chromatography tandem mass spectrometry), hs-CRP and IL-6 were measured.

Results: The long-term study demonstrated that the change of ESA from EPO to DPO significantly decreased serum ferritin while serum iron and TSAT remained unchanged, while DPO as well as EPO maintained hemoglobin level in the target range between 10.0 and 11.0 g/dL. In the short-term study, there was no significant change in
Hb and ferritin in the EPO administration period. In the DPO period, Hb was increased (11.07±0.84 to 11.68±1.14 g/dL) and ferritin was decreased (105.7±61.8 to 52.5±36.9 ng/mL) significantly after 28 days. No significant change of hs-CRP and IL-6 was observed in both period. Area under the percent change in serum hepcidin-time curve in DPO period was significantly greater than that in the EPO period (348.0±91.24 vs. 178.4±131.5, p=0.030).

Conclusions: DFO-alpha seems to be superior to EPO in erythropoietic activity by hepcidin-25 response.

Introduction and Aims: Renal anemia results from a combination of inadequate stimulation of erythropoiesis, iron deficiency and inflammation-induced defective iron mobilization from macrophages. As the interplay between erythropoietin (Epo), hepcidin and inflammation driven by the declining renal function increases in severe renal failure, higher serum ferritin can be attributed either to replete iron stores or to inflammation. We aimed to evaluate the relationships between hepcidin, erythropoietin, inflammation (C-reactive protein - CRP) and serum ferritin in anemic non-dialysis chronic kidney disease (CKD) patients.

Methods: One hundred sixty two non-dialysis patients with renal anemia, iron and erythropoietin free (52% males, 25% diabetes mellitus) entered this prospective single center study. Serum hepcidin and erythropoietin were measured by ELISA, and ferritin, transferrin and CRP, by immunoturbidimetric methods. TSAT was calculated as the percentage of serum iron from total serum iron binding capacity. Data are presented as mean (median) and 95% confidence intervals of the mean (median) and were logarithmized as appropriate for regression analysis.

Results: Hepcidin was higher in this cohort of an old age (67 [63-70] years), anemic (9.4 [9.2-9.8] g/dL) with advanced CKD (eGFR 12.0 [10.8-18.0] mL/min) and moderate inflammation (CRP 7.6 [6.0-10.0] mg/mL) than reported in the general population (83.3 [76.3-94.5] vs. 18-22 ng/mL). Hepcidin levels were not influenced by gender and in bivariate analysis (Spearman’s rs test) were inversely related to renal function (eGFR) and to hepcidin levels, and directly to iron stores (ferritin) and iron available for erythropoiesis (transferrin), but were non-significantly related with inflammation (CRP) (rs = 0.32; rho = -0.29; 0.29; 0.19 and 0.16, respectively). However, the correlations were not impressive. Thus, renal function, directly or via suppressed erythropoiesis production, and iron status seem more important than inflammation in defining hepcidin levels. The independent predictors of hepcidin were the decline in renal function (LnGFR -0.30 [-0.56 to -0.05]), the decrease in erythropoietin levels (LnEpo -0.36 [-0.57 to -0.14]) and the increase in serum ferritin (LnFerritin 0.28 [0.10 to 0.66]) in a model of logistic regression which explained only 23% of hepcidin variability. To note, CRP was not retained in that model.

Conclusions: The increase in hepcidin levels in CKD patients is related to the decline in renal function and is probably mediated by the decreased erythropoietin production. Hepcidin seems to be inadequately to iron stores, and as this reaction is independent of inflammation, high ferritin levels in CKD patients with moderate inflammation suggest iron store repletion rather than inflammation.

Introduction and Aims: Iron deficiency anemia (IDA) and anemia of inflammation (AI) frequently compound renal anemia. Although the distinction between IDA and AI is therapeutically important, peripheral iron indices were not reliable in all cases. We thought to investigate if hepcidin – the key regulator of iron metabolism – and erythropoietin (Epo) – the key regulator of erythropoiesis – measurements could add useful information to differentiate IDA from AI in renal anemia, using bone marrow (BM) iron as reference.

Methods: One hundred sixty seven anemic, iron and erythropoietin free, non-dialysis CKD patients entered this prospective single center study. BM examination (aspiration, Perls’ stain) was normal in 4 patients, 97 patients had IDA, 65 had AI and 1 had erythrodisplasia. Only IDA and AI patients were retained in the final analysis (N=162; 52% males, median age 67 years, eGFR 14.2 mL/min, Hb 9.4±g/dL, 23% with diabetes mellitus). Serum hepcidin and Epo were measured by ELISA, and ferritin, transferrin and CRP by immunoturbidimetric methods. Transferrin saturation index (TSAT) was calculated as the percentage of serum iron from total serum iron binding capacity. Data are presented as mean (median) and 95% confidence intervals of the mean (median).
EMBASE (2004 to August 2012); the Cochrane Library (Issue 9, 2012) without restrictions. Two reviewers extracted data on participant characteristics, interventions, and risk of bias. We summarized treatment effects on mortality, doubling of serum creatinine, need for renal replacement therapy, reduction in GFR (mL/min) and withdrawal of treatment due to adverse events using random-effects meta-analysis.

Results: We included 13 trials including 7854 participants enrolling any patient with CKD stages 1 to 4. By current methodological standards, trial quality was variable. There was no evidence that aiming higher Hemoglobin (Hb) targets effects on mortality (Risk Ratio [RR] 1.10 [CI 0.89–1.37], 13 trials, n=7854 patients). No statistically significant differences in the risks for end-stage kidney disease (RR, 1.03 [CI 0.81–1.29]), doubling of serum creatinine (RR, 1.33 [CI 0.90–1.95]), and kidney transplantation (RR, 1.10 [CI 0.70–1.73], 6 trials, n=1778 patients) or number of patients with at least one adverse event during the study (RR, 1.01 [CI 0.99–1.04], 8 trials, n=918 patients) showed no difference between groups. Of the 13 trials included only two small studies showed doubling of serum creatinine, both with positive results on the higher target arm (RR, 0.38 [CI 0.15 to 0.95], n=213 patients). The data available about hypertension and hospitalization were not consistent reported.

Conclusions: Our analysis of existing trials published after 2004 in patients with CKD treated with ESA have not been found to have significant impact on end-of-treatment GFR or need for renal replacement therapy. On the basis of the evidence that we have marshalled, we do not think it is very likely that small alterations in Hb/haematocrit will influence CKD progression one way or another. No evidence for low dose chronic ESA having any cytoprotective effect.

**Introduction and Aims:** Chronic kidney disease (CKD) patients under recombinant human erythropoietin (rEOPO) therapy, usually, present an anemia associated with alterations in iron metabolism, which are enhanced in patients who develop resistance to rEOPO therapy. This study intended to evaluate iron metabolism, at biochemical and molecular levels, in a model of chronic renal failure (CRF) and of resistance to rEOPO due to formation of anti-EOPO antibodies.

**Methods:** Three groups (n=7 each) of male Wistar rats (280 g), were studied during 12 wks: Sham: CRF: S/nephrectomy; CRF+rEOPO(200): CRF treated with 200 IU/kg/week (s.c.) of beta-EOPO (Recomine®). Blood samples were collected to monitor hematological and biochemical data, including IL-6 and serum iron metabolism markers (iron, transferrin and ferritin). Liver expression of EPO, EPO receptor (EPCR) and iron related genes (by RT-qPCR) was evaluated. Anti-EOPO antibodies were measured by ELISA.

**Results:** CRF rats developed anemia, with a significant decrease in RBC count and Hb concentration, rEOPO(200) treatment in CRF animals corrected anemia until the 9 wks, and, afterwards the rats developed anemia due to production of anti-EOPO antibodies. Furthermore, CRF animals showed a significant increase in BUN and creatinine levels, which were further aggravated in the last 3 weeks; that deterioration was slightly prevented by rEOPO (200IU). Serum iron content decreased significantly in CRF rats, and rose in CRF+rEOPO(200) group. Furthermore, the CRF+rEOPO(200) group presented several significant changes in liver gene expression: increased EPO, TR4α, HJV, HFE and DMT1, decreased SLC40 and TMPRSS6, and a trend towards lower expression of Hamp gene. We also observed an overexpression of liver IL-6 mRNA.

**Conclusions:** Our study showed that the correction of anemia in CRF is closely dependent on rEOPO therapy. Since the formation of anti-EOPO antibodies lead to pure red cell aplasia, triggering hypoxia, it would be expected to observe changes in iron metabolism and mobilization to improve erythropoiesis; indeed, the reduction in Hb, which is not in accordance with the changes observed in the other up-regulating hepcidin gene expression, can be attributed to the presence of high levels of EPO. Therefore, it seems that EPO immunocomplexes could have an inhibitory effect on hepcidin (Hamp gene).

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**Introduction and Aims:** High level of ferritin is reported to be associated with increased incidence of stroke in pregnancy and post-acute Myocardial infarction. Very little data in the literature on the effect of high level of ferritin on cardiovascular disease or mortality in pre-dialysis chronic kidney disease (CKD) patients. Objective: To study the relationship of serum ferritin and the incidence of cardiac myocardial infarction (MI), stroke and mortality in pre-dialysis patients who were treated with IV and on erythropoietin therapy (Aranesp).

**Methods:** Hospital based retrospective case-control study of 345 patients treated with IV and Aranesp for renal anaemia. Data on ferritin level, Aranesp doses, incidence of MI, stroke and all causes mortality was collected from computerised system and discharge summaries, between 2010 and 2012.

**Results:** Baseline characteristics of both groups: Mortality, CVD and cancer incidence between 2010 and 2012.

**Conclusions:** Higher incidence of myocardial infarction has been noted in pre-dialysis CKD patients with higher level of blood ferritin. However, there was no significant difference in stroke or cancer incidence or all causes mortality in both groups.

**EVALUATING THE MAINTENANCE DOSE CONVERSION RATIO (DCR) IN ADULT HAEMODIALYSIS (HD) PATIENTS SWITCHING FROM DARBEPOETIN ALFA (DA) TO PEG EPOETIN BETA**

**Introduction and Aims:** Long acting erythropoietin–stimulating agents (ESAs) available for treatment of anaemia associated with CKD include darbeapoetin alfa (DA) and pegylated epoetin beta (PEG epoetin beta). There is no published literature on the dose outcome of the conversion from DA to PEG epoetin beta, in a non-interventional setting. This retrospective observational study was designed to estimate a population mean maintenance dose conversion ratio (DCR) in adult haemodialysis (HD) patients converted from DA to PEG epoetin beta.

**Methods:** Eligible patients had been receiving HD for ≥12 months and DA for ≥7 months. Data were collected from 7 months prior to, until 7 months after, the date of conversion from DA to PEG epoetin beta with 2 evaluation periods (EP): pre-conversion (months 1 and 2 prior to conversion) and post-conversion (months 6 and 7 post-conversion). The DCR (mean weekly dose equivalent of PEG epoetin beta during the post-conversion EP, divided by the mean weekly dose equivalent of DA in the pre-conversion EP) was calculated for patients who remained on PEG epoetin beta for 7 months post-conversion with ≥1 Hb value and an ESA dose in each EP. Hb could not differ by more than +/- 0.5 g/dL between the 2 EPs. Linear and quadratic regression were used to explore the relationship between mean weekly ESA dose pre- and post-conversion.

**Results:** Of 302 eligible patients enrolled at 12 sites in France, Germany, Spain and the UK, 206 were included in the DCR subgroup (mean age 62 years, 62% male; 67% with a history of cardiovascular disease and 32% with diabetes). The mean maintenance DCR was 1.17 (95% CI 1.05, 1.39), rising to 1.21 (95% CI 1.09, 1.35) when excluding patients who received a red cell transfusion within 90 days or during either EP. Best fit was obtained with a quadratic regression indicating a non-linear relationship between pre- and post-conversion ESA EP doses.
Conclusions: In an HD patient population converted from DA to PEG epoetin beta, the dose of PEG epoetin beta required to achieve comparable Hb was ~20% greater than the DA dose pre-conversion.

**MP203**

**ANAEMIA IN HAEMODIALYSIS PATIENTS AND ULCER-LIKE ABNORMALITIES OF THE RED BLOOD CELLS MEMBRANE: BIOPSY OF PERIPHERAL BLOOD FILMS WITH THE ATOMIC-FORCE AND SCANNING-ELECTRON MICROSCOPES**

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**Introduction and Aims:** Three major factors contribute to anemia in haemodialysis patients (HDp); reduced production of erythropoietin, iron deficiency and reduced lifespan of red blood cells (RBCs). The uraemic environment is considered responsible for the decreased lifespan of RBCs and overall worsening of anemia. Since the biochemical interference between the uraemic toxins and the biomolecular constituents of cell membrane can probably motivate structural instabilities, in the present work we performed biopsy of intact RBCs (iRBCs) of HDp in comparison to healthy donors.

**Methods:** RBCs of 14 HDp (N=863) subjected to 4-hour dialysis thrice a week and of 14 healthy donors (N=910) were studied with advanced microscopes. The iRBCs refer to freshly collected RBCs (in EDTA tubes) that are deposited onto glass slides in single-layered form with only minor treatment. The iRBCs were surveyed by means of two advanced microscopes, the Atomic Force Microscope (AFM) and Scanning Electron Microscope (SEM). Both can selectively focus on the iRBCs membrane and reveal information at the nanometer level (1 nm ~ 10 nm). Biochemical and hematological data were obtained with the standard clinical methods.

**Results:** The AFM and SEM data consistently revealed that the iRBCs membrane displays morphological abnormalities that have mainly circular shape and typical size ranging within 100 to 200 nm, in both the HDp and healthy donors. These local morphological abnormalities of the iRBCs membrane have the form of ulcer-like deteriorations, thus are termed ulcer morphological abnormalities (UMA). The observation of UMA in the RBCs membrane of healthy donors indicates that they possibly relate to physiological aging of RBCs. The population of UMA per iRBC is 3.4 and 5.0 in the healthy donors and HDp, respectively, evidencing a pronounced increase, 47% (p=0.00041) in the latter group. This indicates that in the HDp the aging of RBCs is accelerated by mechanisms that relate to the underlying disease. To resolve if there is any connection with the clinical data we performed a straightforward comparison of the AFM and SEM data with the basic uraemic indices. It turned out that the population of UMA per iRBC exhibits a statistically significant correlation (p<0.05) with all Ur, Cr, Ca, P and CaXP.

**Conclusions:** The correlation of the population of UMA per iRBC with all basic uraemic markers is a strong indication, if not proof, that uraemia motivates/promotes the structural deterioration of the membrane and as a consequence the premature elimination of these detected RBCs from the circulation, ultimately worsening anemia.

**MP204**

**COMPARABLE DOSES OF FG-4592 HAVE SIMILAR PK/PD IN HEALTHY CAUCASIAN AND JAPANESE SUBJECTS**

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**Introduction and Aims:** Anemia affects >10 million patients with chronic kidney disease (CKD) worldwide. Only ~10% of anemic CKD patients are treated. FG-4592 is a novel oral hypoxia inducible factor prolyl hydroxylase domain inhibitor being developed in the US, Japan, China, and Europe for treatment of CKD anemia. We compared pharmacokinetic (PK) and pharmacodynamic (PD) profiles of FG-4592 in Caucasian and Japanese subjects in individual phase 1 open-label, single-arm, dose-escalation studies.

**Methods:** Healthy adults received single oral doses of FG-4592: Caucasian and Japanese subjects received weight-adjusted doses ranging from 0.3–4.0 mg/kg (n=20, and 30 respectively). Blood was sampled before dosing and 0.5–96 h after dosing. Results: FG-4592 was well tolerated, treatment emergent adverse events (AEs) were mild to moderate, and no serious AEs were reported. Tables show FG-4592 PK parameters and PD effect of single dose FG-4592 on endogenous erythropoietin (eEPO). (Note: 1.0 and 2.0 mg/kg FG-4592 have been shown to result in robust hemoglobin response in patients with CKD anemia).

**Conclusions:** The results suggest overall similar drug and circulating eEPO exposures in Caucasians and Japanese, slightly higher in Japanese subjects, compared to Caucasian subjects. The PK parameters of FG-4592 after repeat dosing thrice weekly (TIW) is comparable to single dose, suggestive of a lack of drug accumulation, and comparative results will be reported in this presentation. Phase 2 and Phase 3 studies are underway globally in Asia Pacific, the US, and Europe.

**MP205**

**FACTORS INFLUENCING THE FIRST DARBEPOETIN ALFA DOsing FREQUENCY CHANGE IN CKD PATIENTS NOT ON DIALYSIS**

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**Introduction and Aims:** The primary objective of EXTEND, a European/Australian observational study, was to assess the effectiveness of DA administered subcutaneously at extended dosing intervals (every 2 wk [Q2W] or every month [QM]) in CKD patients not on dialysis (ND-CKD). This subanalysis was performed to identify which factors may be associated with the first dose frequency change occurring after the first 3 months on extended dosing.

**Methods:** Multivariate logistic regression was performed to explore inter-current events occurring ≥120 days prior to the first DA dose frequency increase or decrease occurring ≥90 days after commencement of extended dosing with Q2W or QM. Analyses were performed for patients who were receiving ESAs in the 6 months before commencement of extended DA dosing (ESA prior) and those who were not (ESA naive).

**Results:** Of 6037 subjects enrolled, 2231 had sufficient covariate information to be included in the analysis. Independent factors influencing the first DA dose frequency increase (e.g. Q2W to QW, QM to Q2W) in ESA-naive and ESA-prior patients are presented in the Table. The results for factors influencing dose frequency decrease are reciprocal to the ones presented and are not shown.

**Conclusions:** Hb concentration <10 g/dL, low eGFR, hospitalization and RRT commencement were all positively associated with the first dose frequency increase. Low mean weekly dose and occurrence of ≥1 transfusion were also positively associated with dose frequency increase in ESA-naive subjects; receiving iron therapy was negatively associated with dose frequency increase in ESA-prior subjects.
MP205  

**ANAEMIA MANAGEMENT IN CHRONIC KIDNEY DISEASE PATIENTS NOT ON DIALYSIS IN CLINICAL PRACTICE FOLLOWING THE RECOMMENDATIONS OF THE ANAEMIA WORKING GROUP OF THE EUROPEAN RENAL BEST PRACTICE (ERBP)**  

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**Introduction and Aims:** Following the findings of the TREAT study, the Anaemia Working Group of ERBP recommended maintaining Hb levels in the range of 11-12 g/dL, without intentionally exceeding 13 g/dL, and to consider doses of ESA therapy to achieve the target Hb. This study was devised to evaluate the impact of this statement in the clinical practice setting.  

**Methods:** Multicenter, cross-sectional, observational study involving patients with anaemia secondary to chronic kidney disease (CKD) not on dialysis who initiated anaemia treatment (naïve) or were converted from other ESAs since January 2011. Final results are presented.  

**Results:** Of 441 patients evaluated, 67.6% were naïve and 32.4% were converted (mean ESA treatment duration, 20.1±18.4 months). Mean age: 73.1±13.0 years. CKD stages 3/4: 30.1%/50.5%/19.4%. Predominant CKD etiologies: 29.7% vascular and 25.8% diabetes. Mean weekly dose (above vs below median) 0.713 (0.530, 0.959) 0.0254 - - -  

**Conclusions:** These results indicate an appropriate anaemia management in CKD patients not on dialysis, achieving the recommended Hb target range of 11-12 g/dL, without intentionally exceeding 13 g/dL, as stated the last recommendations of ERBP group. In fact, 66.6% of naïve and 72.7% of converted patients required blood transfusions.  

**MP206**  

**COMMON EFFECT OF ACE GENE I/D POLYMORPHISM AND ACE INHIBITION MODULATES ERYTHROPOIETIN IN CKD-5D PATIENTS**  

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**Introduction and Aims:** There is a debate about the association among ACE (angiotensin converting enzyme) gene insertion/deletion (I/D) polymorphism, inhibition of ACE activity and erythropoietin. Therefore, this study aim was to prove the different effect of erythropoietin resistance between dialysed chronic kidney disease (CKD-5D) patients with II and ID genotype. We also tested how ACE inhibitor (ACEI) therapy can influence erythropoietin and how it can modify the effect of ACE gene I/D polymorphism on erythropoietin need in CKD-5D patients.  

**Methods:** It was a retrospective, multicentre observational study among 706 dialyzed patients. We allocated patients (II and ID genotype detected by PCR method) into match pair groups for statistical analysis. Based on patient’s similarities (age, DM, time on dialysis, ACEI therapy) we could find 127 matched patient pairs (II – ID genotype)  

**Results:** In total haemoglobin (Hb) level was higher (p=0.197) in patients with ID genotype and without ACEI therapy (98,8±12,5 vs 95,6±14,1 g/l). Patients with DD genotype on ACEI therapy the Hb level was (p=0,01) significantly low (92,7±12,5 vs 98,2±11,8 g/l), however, total median erythropoietin dose was similar (p=0.314). In erythropoietin and ACEI treated dialysed patients with DD genotype the Hb level (91,9 ±11,6 vs 97,8±12,3 g/l) was lower (p<0.01) and the median erythropoietin resistance index (204,1±175,1) value was higher (p<0.05) than in patients with II genotype. In erythropoietin treated patients without ACEI therapy these associations between subgroups were not significant.  

**Conclusions:** ACEI therapy may be an erythropoietin resistance factor in CKD-5D patients with ACE gene DD genotype. This study was supported by Hungarian Scientific Research Fund T029297.  

**MP207**  

**UTILITY OF INTRAVENOUS IRON CARBOXYMALTOSE IN A SINGLE DOSE IN THE CONTROL OF ANEMIA IN CHRONIC KIDNEY DISEASE STAGE 3-4**  

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**Introduction and Aims:** The presence of anemia of multifactorial cause, with an iron deficiency component in chronic kidney disease is a common fact. Tolerance to oral iron supplementation is irregular, as well as its effectiveness in patients with advanced renal disease. There are several iv formulas with potential side effects and administration discomfort. The iron carboxymaltose allows for a single dosage with few side effects which permits a good control of anemia.  

**Methods:** Patients with chronic kidney disease on an outpatient basis of nephrology were included, with associated anemia and need for iv iron supplements. We proceeded to administer a single dose of intravenous iron carboxymaltose, for 15 minutes in 250 cc of 0.9% sodium chloride solution. The standard dose was 1000 mg, not exceeding 15 mg of iron per Kg of body weight.  

**Results:** 32 patients with administration of IV iron carboxymaltose were analyzed with basal control and 6 months. The mean age was 79 ± 9 years, with the following etiologies of renal disease: 12 nephroangiosclerosis, 8 not known, 5 diabetes, 4 interstitial and 3 glomerular. Eleven patients in turn received erythropoietic stimulating factors (ESF). The mean basal haemoglobin was 10.7 ± 1.2 g / dl at 6 months ±1.8 (p<0.001), with a basal hematocrit of 33.9 ± 4.5% and at 6 months 39.4 ± 6.4 (p<0.011). The baseline ferritin and saturation index of basal transferrin at 6 months were respectively: 95.2 mg / ml, 11.1%, 291, 20 (p: 0.015, p: 0.002). Renal function remained stable, the mean serum creatinine (SCr) basal was 2.1 ± 0.6 mg / dl and estimated glomerular filtration rate measured by MDRD (GFR) of 29.9 ± 11 ml / min, SCr at 6 months was 2.07 ± 0.5 and 29.8 ± 10 (p<0.001). No patient experienced adverse reactions at the time of administration or within 24 hours. ESF necessities decreased but they were not statistically significant. There were no difference in phosphorus level.  

**Conclusions:** The administration of iron carboxymaltose a single iv dose in patients with chronic kidney disease appears safe and effective, allowing for a well-tolerated simple dosage, reducing the number of punctures, with minimal side effects.

**MP208**  

**COMMON EFFECT OF ACE GENE I/D POLYMORPHISM AND ACE INHIBITION MODULATES ERYTHROPOIETIN IN CKD-5D PATIENTS**  

Zoltán Kiss1, Lóránt Kerkovits2, Csaba Ambruš3, Imre Kucskó4, János Szegedi3, Attila Benkő5, Béla Borbás6, Sándor Ferenci7, Mária Hegyesi8, Szívia Kazúp2, Lajos Nagy2, József Németh2, Antal Rozinka2, Tamás Szabó3, Tamás Szentesi4, Eszter Tóth1, Gábor Varga5, Gyula Wagner6, Gábor Zákó4, László Gergely1 and István Kiss1  
1Dialysis Network B.Braun Avitum Hungary, 2CPLC Budapest Hungary  

**Introduction and Aims:** There is a debate about the association among ACE (angiotensin converting enzyme) gene insertion/deletion (I/D) polymorphism, inhibition of ACE activity and erythropoietin. Therefore, this study aim was to prove the different effect of erythropoietin resistance rate between dialysed chronic kidney disease (CKD-5D) patients with II and ID genotype. We also tested how ACE inhibitor (ACEI) therapy can influence erythropoietin and how it can modify the effect of ACE gene I/D polymorphism on erythropoietin need in CKD-5D patients.  

**Methods:** It was a retrospective, multicentre observational study among 706 dialyzed patients. We allocated patients (II and DD genotype detected by PCR method) into match pair groups for statistical analysis. Based on patient’s similarities (age, DM, time on dialysis, ACEI therapy) we could find 127 matched patient pairs (II – ID genotype)  

**Results:** In total haemoglobin (Hb) level was higher (p=0.197) in patients with ID genotype and without ACEI therapy (98,8±12,5 vs 95,6±14,1 g/l). Patients with DD genotype on ACEI therapy the Hb level was (p=0,01) significantly low (92,7±12,5 vs 98,2±11,8 g/l), however, total median erythropoietin dose was similar (p=0.314). In erythropoietin and ACEI treated dialysed patients with DD genotype the Hb level (91,9 ±11,6 vs 97,8±12,3 g/l) was lower (p<0.01) and the median erythropoietin resistance index (204,1±175,1) value was higher (p<0.05) than in patients with II genotype. In erythropoietin treated patients without ACEI therapy these associations between subgroups were not significant.  

**Conclusions:** ACEI therapy may be an erythropoietin resistance factor in CKD-5D patients with ACE gene DD genotype. This study was supported by Hungarian Scientific Research Fund T029297.
Introduction and Aims: Anaemia is a known complication of advanced chronic kidney disease (CKD). In recent years, there has been a growing interest in optimizing iron management for the treatment of renal anaemia, in order to reduce the high cost of erythropoietin stimulating agents (ESAs) in comparison to iron therapy. Furthermore, in a significant proportion of pre-dialysis CKD patients iron therapy is undertaken as the primary treatment of anaemia [1]. Intravenous (IV) iron sucrose (IS) is the agent of choice in most hospitals for the treatment of renal anaemia. However, IV IS is must be administered over 30 minutes requiring multiple IV access. As a suitable alternative, we introduce IV Ferric carboxymaltose (FC) as a single dose agent for the treatment (Rx) of renal anaemia with multiple advantages including reduced hospital visits, patient satisfaction and cost effectiveness.

Methods: We modified our local renal anaemia management policy according to National Institute of Clinical Excellence guideline (NICE CG114, UK) using single dose IV FC. All cases that were classed as CKD renal anaemia received IV FC were included in this audit over a 6 month period. The cohort also included patients on ESAs. Data was collected prospectively, including pre and post haemoglobin (6 weeks post IV FC Rx), iron studies and clinical information regarding pre and post blood pressure (BP) monitoring and adverse effects.

Results: A total of 245 patients with renal anaemia (not on dialysis) were identified and results obtained pre and post IV FC infusion. Among them, 47% of patients were on ESAs. Mean age 71(24-95) with M: F ratio 119:126. There was a mean increase of Hb level > 1 g/dl with single dose IV FC (mean Hb 10.1 g/dl vs. Hb 11.3g/dl post treatment).There was also a significant improvement of the iron profile (Table 1). Median BP pre 144/77 mmHg and post 133/47 mmHg. In patients on ESAs (n=104), 26% (64/4) either had their ESA dose reduced or stopped following IV FC infusion. Only 17% (n=41) of the total patient cohort required 2nd dose of IV FC as their target iron profile iron level was not achieved. We noted no significant incidence of any serious adverse effect. Table1: Pre and Post treatment (Rx) Ib and iron profile with IV FC.

Conclusions: Single dose IV FC was associated with significant improvements in HB and iron profile and was relatively safe. Other possible benefits include reduced hospital visits, reduced interruption in lifestyle and improved patient experience. Furthermore, single dose IV iron replacement with FC has been associated with reduced ESA usage and therefore, overall cost efficient.


MP211 DARBEPETOIN ALFA, ONCE MONTHLY DOSING, CORRECTS ANAEMIA IN CHRONIC KIDNEY DISEASE PATIENTS NOT ON DIALYSIS: A RANDOMIZED PHASE III STUDY

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Introduction and Aims: While darbepoetin alfa (DA) can be administered once monthly (QM) to maintain haemoglobin (Hb) levels in anemia patients with chronic kidney disease not on dialysis (CKD-ND), the monthly use of DA in anemia of CKD-NK disease was not previously investigated in interventional studies.

Methods: In this randomized double-blind, non-inferiority, active-controlled, phase III study, adult patients diagnosed with CKD-stage 3-4 and Hb levels of <10 g/dl who had not been treated with an erythropoiesis-stimulating agent were randomized in a 1:1 ratio to receive DA every 2 weeks (Q2W) or once monthly for 33 weeks (QM) with initial doses of 0.75 µg/kg Q2W or 1.5 µg/kg QM. Patients were treated to target Hb levels of 10-12 g/dl and a 1 g/dl increase from baseline. The primary endpoint was Hb change between baseline and the evaluation period (weeks 29-33). The non-inferiority margin was 0.5 g/dl. Additional endpoints included the proportion of subjects and time to achieve Hb level ≥10.0 g/dl and ≥1.0 g/dl increase in Hb from baseline, DA doses over the duration of the study, and safety.

Results: 155 patients were enrolled (Q2W: n=79; QM: n=180). Of these, n= 141 (Q2W) and n=116 (QM) had ≥1 Hb during the evaluation period. The mean change (95% CI) in Hb from baseline was 2.16 g/dl (1.98, 2.33) for the Q2W group and 1.97 g/dl (1.80, 2.14) for the QM group. The mean (95% CI) difference in Hb change (QM-Q2W) was -0.19 (-0.43, 0.05) g/dl. The majority of subjects (97.9% QM, 98.1% Q2W) achieved both a Hb level ≥10.0 g/dl and ≥1.0 g/dl increase in Hb from baseline; median (Q1, Q3) time to this event was 5 (3, 7) weeks for the Q2W arm and 5 (3, 9) weeks for the QM arm. The mean (SD) weight-adjusted weekly equivalent dose over the evaluation period was 0.02 (0.232) µg/kg/week for the Q2W group and 0.37 (0.306) µg/kg/week for the QM group. Safety profiles were similar between groups.

Conclusions: The results of our study indicate that QM dosing was non-inferior to Q2W dosing for correction of anaemia with similar safety profiles in CKD-ND patients.
MP213 MATRIPTASE-2 GENE MUTATIONS IN HEMODIALYSIS PATIENTS WITH IRON RESISTANT IRON DEFICIENCY ANEMIA IN COMPARISON TO NORMAL POPULATION

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Introduction and Aims: Matriptase-2 (TMPRSS6) is a serine protease that plays an important role in regulating hepcidin expression in the liver and therefore to regulate iron hemostasis. Plasma level of hepcidin is high in patients with chronic renal failure. The aim of this study is to research on Matriptase-2 mutations in patients with chronic renal failure and iron deficiency anemia that could lead to better diagnosis and treatment of anemia in these patients.

Methods: This case-control study was done in 2012 to comparison between hemodialysis patients with refractory anemia and healthy persons. The patients had been referred to Labbafinejad academic hospital, Tehran to hemodialysis. Inclusion criteria were: dialysis experience more than three months, low serum hemoglobin levels is not consensual. This study provide a representative picture of French medical practices before KDIGO recommendations.

Results: 121 patients entered the study that eventually 15 patients were selected as cases. Also of 15 healthy persons were selected as a control group. All of the persons in control group had no mutation in TMPRSS6 gens, but all patients had mutation in one locus (c.1807G>C). 10 cases were heterozygous for this mutation and 5 cases were homozygous.

Conclusions: Finally, the study showed that mutation in the TMPRSS6 gene along with other factors may cause iron deficiency anemia in chronic kidney disease patients.

MP214 EFFECTS OF PARICALCITOL ON HEMOGLOBIN (HB) LEVELS IN CKD PATIENTS: A PILOT RANDOMIZED TRIAL

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Introduction and Aims: Although current activated vitamin D therapies are approved for secondary hyperparathyroidism treatment in chronic kidney disease (CKD), several experimental data in animals confirms that vitamin D effects extend beyond mineral metabolism. Recent studies show that the new vitamin D analog paricalcitol has much more pleiotropic benefits compared to calcitriol. Our study aims to determine whether the use of oral paricalcitol leads to improvement in anemia in CKD, and whether this effect is independent from hyperparathyroidism correction.

Methods: A total of 34 patients with CKD 3-5 stage not on dialysis (eGFR <60 ml/min/1.73 m²) and anemia (HB 10-12.5 g/dl) were enrolled. Patients with iron deficiency (ferritin <100 ng/ml; transferrin saturation <20%), severe hyperparathyroidism (PTH >300 pg/ml) and inflammation (C-reactive protein >1mg/dl) were excluded. The enrolled patients were randomly 1:1 assigned to receive either paricalcitol (CASE) or native vitamin D/calcitriol (CONTROL) for 6 months. The initial paricalcitol dose was 1 mcg/die. Dose adjustments were based on laboratory results for PTH, Calcium and Phosphorus, according to KDIGO guidelines. The primary end point was the difference in HB levels from the basal after 6 months of treatment (T3) in the two groups.

Results: Both groups had similar characteristics at baseline and follow up. The patients (pt) of the case group (n=17) showed a significant increase in HB levels after 6 months of therapy (12.02 g/dl vs 12.96 g/dl respectively at T0 and T3, p=0.03). In control group (n=17; 8 pt in treatment with calcitriol and 9 with native vitamin D), HB progressively decreased (12.03 g/dl vs 11.31 g/dl respectively at T0 e T3, p=0.01). Moreover, after only 2 months (T1) the difference in HB levels between the groups was significant (HB12.43 g/dl vs 11.75 g/dl respectively in case and in control group, p=0.012), and remained stable until the end of the study (12.96 g/dl vs 11.31 g/dl at T3, p=0.015). In case group, 1 pt stopped the terapy with eritropoitin (epo); 1 reduced the epo dose; 1 stopped iron therapy. No significant change was reported in PTH and Cr-reactive protein levels. No change was made in paricalcitol dose.

Conclusions: Oral paricalcitol could improve anemia in CKD patients. The increase in HB levels is likely due to a direct stimulation of erythropoiesis precursors as reported in vitro for calcitriol and it could be no related to hyperparathyroidism correction.