SCIENTIFIC DISCUSSION

1.1 Introduction

Chronic kidney disease (CKD)

Chronic kidney disease is characterised by a progressive reduction of functional renal parenchyma. The clinical course of progressive kidney disease can be divided into a continuum of 5 stages based on the glomerular filtration rate (GFR). According to this classification CKD is defined as either kidney damage or GFR < 60 mL/min/1.73 m2 for ≥ 3 months.

There is a global increase in the number of patients with CKD. Data from the European Renal Association-European Dialysis and Transplant Association (ERA-EDTA) registry, in which roughly 80% of patients were on haemodialysis (HD) and 20% on peritoneal dialysis (PD), showed that the incidence of renal replacement therapy for ESRD had risen from 79 per million population (pmp) in 1990 to 117 pmp in 1999. More recent data from 2000-2003 has revealed an average European rate of 135 pmp.

Anaemia is a common complication in patients with chronic renal failure (CRF), and although its pathogenesis is multifactorial, the decreased production of erythropoetin, a hormone produced primarily in the kidneys, is considered the main etiologic factor. Anaemia is already seen in early stages of the disease and at a later stage, in patients undergoing dialysis, anaemia is present in over 90% of patients. The main impact of anaemia on organ function is reduced oxygen delivery to tissues leading to fatigue and effort intolerance. Other consequences, such as impaired cognitive function, sleep disorder, altered haemostasis, depressed immune function and impaired cardiac function are not uncommon.

Exogenous replacement of erythropoetin by the recombinant hormone epoetin (r-HuErythropoetin) is a well-accepted therapy for treatment of anaemia in patients with CKD. Clinical Guidelines for the treatment of anaemia in CKD were established for dialysis patients in 1997 (United States) and 1999 (Europe). Current clinical practice guidelines in both Europe and the USA recommend earlier initiation of anaemia treatment in patients with renal insufficiency that are not on dialysis. According to the *European Best Practice Guideline* (EBPG [1]) all patients with CKD who develop anaemia (Hb<11 g/dl) should be treated. Currently, regimens for the treatment of these patients require frequent administrations of erythropoiesis stimulating agents (ESAs) once weekly, once every two weeks or, in the maintenance setting, once a month with continuous dose adjustments to maintain a target Hb concentrations from 11g/dL to as high as 14 g/dL. Patients with CKD and anaemia will require life-long treatment with these agents.

Currently, two erythropoietins (epoetin-α and epoetin-β) are available for correction of anaemia associated with chronic kidney disease (human-like recombinant DNA-derived). They are administered once a week or more frequently. In addition, a modified recombinant erythropoietin, darepoetin-α, is available. The modified carbohydrate moiety of darepoetin slows elimination and makes a less frequent administration possible.

About the product

MIRCERA, methoxy polyethylene glycol-epoetin beta, is an ESA which differs from erythropoietin through formation of a chemical bond between an amino group present in erythropoietin beta and methoxy polyethylene glycol (PEG) butanoic acid. MIRCERA has an approximate molecular weight of 60 kDa. Epoetin beta is a glycoprotein of 165 amino acids, produced by recombinant DNA technology in Chinese hamster ovarian cells.

As compared to epoetin beta, MIRCERA shows a different activity at the receptor level characterised by a slower association with and faster dissociation from the receptor, a reduced specific activity in vitro with an increased activity in vivo, as well as an increased half-life. These different

pharmacological properties are relevant in order to achieve a less frequent dosing regimen with MIRCERA.

1.2 Quality aspects

Introduction

Epoetin beta (EPO) is a recombinant form of erythropoietin. The drug substance of MIRCERA (RO0503821 or methoxy-polyethylene glycol-epoietin beta) is synthesized by forming a chemical bond between one linear methoxy-polyethylene glycol molecule to EPO. The EPO used in the manufacturing process is the active substance of the centrally authorised product NeoRecormon (Roche).

The drug substance is formulated as a sterile, preservative-free protein solution for intravenous or subcutaneous administration. MIRCERA is intended to be marketed in two dosage forms; vials and pre-filled syringes (PFS). Seven dosage strengths for the vials and nine dosage strengths for the PFS are being registered.

Drug Substance

EPO is a glycoprotein that consists of 165 amino acids resulting in a molecular mass of 30 kDa. The polypeptide chain is linked through two disulfide bridges, one between cysteines at position 7 and 161 and one between cysteines at position 29 and 33 in the amino acid sequence. Carbohydrates are attached to three asparagines at position 24, 38 and 83 and one serine at position 126. The molecular mass of the protein portion is 18 kDa whereas fully glycosylated EPO amounts to approximately 30 kDa.

The pegylation is carried out through integration of an amide bond between methoxypolyethylene glycol-succinimidyl butanoic acid (PEG-SBA) and either the N-terminal amino group or the ε-amino group of lysine, predominantly Lys 52 and Lys 45. This results in a molecular weight of around 60 kDa.

- Manufacture

Genetic development

For expression of the protein a Chinese hamster ovary (CHO) cell line is used. The cell bank system is established using a CHO-DN2-3α3 cell expressing constitutively the erythropoietin gene.

Cell banking

For production of EPO, cells were adapted to serum-free medium conditions and a master cell bank (MCB) was laid down which is used for establishment of working cell banks (WCB). The cell bank system is tested according to the current guidelines. The strategy and the assays carried out to test for microbial, fungal and viral contamination of the cell banks are considered adequate. The results obtained showed that the cell banks are free from adventitious viruses and microorganisms.

The genetic stability of the WCB is considered adequately demonstrated. Results obtained showed a stable integration of the EPO coding sequence even at generation cycles exceeding the maximum number of generation cycles of the production process by almost 50%.

Fermentation
EPO is produced by recombinant CHO cells in suspension culture, in a serum-free medium.
The fermentation process employed comprises of:
- The seed train,
- The production run,
- The harvest procedure.

Purification of EPO
Each harvest undergoes purification which leads to 10 batches of purified EPO. The purification process consists of five chromatographic steps. This process starts with a capture step using Blue Sepharose chromatography. Further purification is performed using a hydrophobic interaction chromatography on Butyl Toyopearl, an adsorption chromatography on Hydroxyapatite Ultrogel, a Reversed Phase HPLC on Vydac C_4 and finally an anion exchange chromatography on diethylamminoethyl (DEAE) Sepharose. The pool is 0.2 µm filtered into 2 L Teflon bottles and the EPO solution is stored at -60 to -90°C.

Pegylation reagent
The pegylation reagent is an ester of the methoxypoly (ethylene glycol)-butyric acid with molecular weight (MW) centred around 30,000 g/mol. *Pegylation reaction*
Purified EPO is thawed and concentrated for pegylation.
After pegylation, RO0503821 is purified from the mixture of by-products through chromatography steps.

The purified RO0503821 pool is concentrated, diafiltrated filtered into Teflon bottles and stored at -70°C or -20°C.

Process control and validation
The EPO production process has been well validated and its consistency and robustness are supported by the several hundreds batches produced so far.

Validation of the pegylation process and purification of the drug substance was done on the basis of the following criteria:

- Consistency of the manufacturing process
- Removal capability of the process with regard to process related and product related impurities
- Lifetime of the SP Toyopearl column
- Robustness of the process
- Stability of process hold solutions

The results provided show a consistent manufacturing process that is able to reliably eliminate both product-related and process-related impurities. The robustness of the process has been shown by testing a number of parameters that influence the pegylation reaction. Variation of the tested parameters within the established range does not seem to affect the distribution of positional isomers. The report for the lifetime column study has also been provided and considered adequate. The validity of the scaled down model has been shown and the robustness of the chromatography steps has been well demonstrated.

Manufacturing Process Development
The EPO, to be used as starting material, is also marketed under the trade name NeoRecormon and was initially approved under the EMEA centralized procedure in 1996.

The RO0503821 drug substance process has evolved over time in four main steps including e.g. scale-up of the process. This development resulted in different process variants.

Data obtained by IPC testing, impurity profile, release testing, extended characterization, and stability show a high consistency of the production process as well as comparability of material produced with the different process variants.

- Characterisation

The following methods were used to characterize RO0503821 drug substance:

Matrix Assisted Laser Desorption Ionization Mass Spectrometry (MALDI-MS) was used to determine the molecular mass.

The sites as well as the degree of pegylation were analyzed by means of LysC-peptide mapping, showing that the amino terminus, Lys 45 and Lys 52 constitute the major pegylation sites.

The N-linked glycans were enzymatically released from the protein by means of N-glycosidase F treatment, the sialic acids were cleaved off simultaneously by neuraminidase treatment. The released asialo N-linked oligosaccharides were analyzed using high performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD).

O-linked oligosaccharides were investigated by means of LysC peptide mapping with online mass detection, the O-linked glycosylation of the samples was assessed using specific ion current (SIC) chromatograms of the LysC peptide carrying the O-linked oligosaccharides.

The sialic acids were enzymatically released from the protein and analyzed by means of HPAEC-PAD.

The higher order structure was investigated by circular dichroism (CD) spectroscopy. The CD spectra were acquired in the far-UV (185 nm – 260 nm) as well as in the near-UV (250 nm – 350 nm) spectral regions.

Product related impurities were determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis, (SDS-PAGE) (low molecular weight forms), reverse phase-high performance liquid chromatography (RP-HPLC) (oligo forms and RO0503821 related substances), and size exclusion-HPLC (SE-HPLC) (oligo forms, aggregates and low molecular weight forms).

The bioassay used for activity determination is based on the measurement of the increase in reticulocyte production (as a measure of erythrocyte production) after parenteral administration to normal healthy mice. The reticulocyte count is carried out fluorometrically in a flow-cytometer using whole blood from experimental animals. The *in vivo* activity is calculated in comparison with a bioactivity standard substance according to the method of parallel lines.

The presence and content of process related impurities was investigated. The data was found between to be well within the defined specification limit.

- Specification

Specification of EPO
The EPO release specification is divided into three parts namely routine batch control, characterization assays and tests of the unprocessed bulk. The characterization assays are performed on the first and the last batch of each production run. If one of the characterization assays fails, all batches of this production run are tested in the characterization assays. The specifications are considered adequate for control of the quality of EPO.

Specification of methoxy polyethylene glycol-epoetin beta

The specification parameters for routinely testing of RO0503821 drug substance were chosen in order to address all relevant quality attributes of the molecule. The limits were established on the database obtained during process development.
The specifications control the physico-chemical characteristics, impurities, identity and potency of methoxy polyethylene glycol-epoetin beta.

- Stability

Stability studies have been designed in accordance with the relevant guidelines. Material batches have been entered into:

- Real-time study at -70°C and -20°C for 72 months
- Accelerated study at 4°C for 12 months

- Accelerated study at 25°C and 35°C for 3 months

The data presented are considered satisfactory to support the proposed shelf-life, i.e. 36 months at -70°C or 24 months at -20°C. Additional data according to the stability protocol will be provided on an ongoing basis.

For future production a follow-up program will be performed to confirm continuously the stability of the drug substance. According to ICH guideline Q7A one batch per year from the established production facility will be placed on stability.

- Adventitious Agents
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TSE aspects
The information regarding ruminant materials used in the establishment of the MCB is considered compliant with the TSE Note for Guidance if it is already used in an authorized product (Epoetin beta) which is the case. Besides those ruminant components in the MCB, the only materials from ruminant origin are included in media fills and in the manufacture of the recombinant human insulin. They all derive from milk sourced from healthy animals in the same conditions as milk collected for human consumption. Therefore, they are considered in compliance with the current TSE guideline.

Drug Product

MIRCERA is formulated as a sterile, preservative-free protein solution for intravenous (IV) or subcutaneous (SC) administration.

The qualitative and quantitative composition of the drug product (vials & PFS) is justified and contains RO0503821 drug substance and the following excipients:
- L-Methionine
- Sodium sulphate anhydrous
- Sodium dihydrogen phosphate monohydrate
- Mannitol
- Poloxamer 188
- Water for injections

- Pharmaceutical Development

The excipients have been selected on the basis of physico-chemical investigations predominantly addressing the stability of the drug substance and the drug product solution as well as considering physiological tolerability in the patient.
The commercial formulation was used for the pivotal clinical trials.

- Manufacturing process

The manufacturing process consists of three steps: compounding of the drug product solution, sterile filtration and filling into vials or pre-filled syringes. The company has identified the critical steps (performance of sterile filtration and aseptic conditions during filling). They have justified the choice of the material and equipment used. The batch sizes have been presented and are acceptable. The manufacturing process for commercial purpose is validated.

- Control of excipients

All the excipients comply with the Ph. Eur. (BP in the case of sodium dihydrogen phosphate monohydrate) or USP/NF. None of the excipients used are of human or animal origin and no novel excipients are used.

- Product Specification

The drug product specifications were established according to the ICH guidelines and include all tests necessary for the acceptance/rejection of batches on the basis of those features which are important to

assure the identity, strength, quality, purity and potency of the product. Where applicable, they comply with the pertinent monographs of the European Pharmacopoeia.

The assessment criteria or limits for the non-compendial test parameters have been established on the basis of the analytical evidence gained during drug substance and drug product development. The proposed levels are consistent with currently recognized standards. The proposed levels are derived from the historical database of all batches produced used for clinical trials, including the registration batches (vials and pre-filled syringes). The analytical procedures have been validated.

A bioassay is used as a stability-indicating assay. The activity is determined at the level of the drug substance and is proposed not to be done for drug product release as historical data shows no significant difference in bioassay results once the drug substance is formulated into drug product.

- Stability of the Product

Vials

Primary and supporting stability studies, a short term study at room temperature, and a photostability study were performed to support the proposed expiration dating and storage conditions. The setting of the proposed shelf-life of 24 months at 5 ± 3°C including storage at RT for up to one month for convenience of the end user is justified based on real time and accelerated stability data of registration batches as well as real time data of clinical batches.

Pre-filled syringes

Up to date, a total of 25 batches of drug product in PFS have been produced, applying essentially the same process, procedures and equipment as intended for commercial manufacture. The stability studies have been performed focusing on three major areas:

- Formal long-term stability testing of the drug product to establish and justify the proposed storage conditions of 2 to 8°C and shelf life.
- Short-term stability testing (up to 3.5 months) at room temperature to support that for the convenience of the end user the drug product may be stored at up to 25°C for a single period of up to one month.
- Photostability testing is performed on the basis of the pertinent regulatory requirements.

The setting of the proposed shelf-life of 24 months at 5 ± 3°C including storage at RT for up to one month for convenience of the end user is justified based on the results of the above listed studies.

1.3 Non-clinical aspects

Introduction
Toxicology studies were conducted in accordance with international guideline for biotechnology-derived pharmaceuticals (ICH S6, 1997) and all pivotal safety studies were conducted in concordance with Good Laboratory Practices (GLP).

Pharmacology
Biochemical and cellular studies were conducted to analyze the receptor interaction of RO0503821 in comparison to epoetin beta. Pharmacological activity (stimulation of erythropoiesis), dose-effect relationship, route of administration (SC vs. IV) and dosing schedule (once weekly vs. once every two weeks) were evaluated. *In vivo* models included the normocythemic mouse (C57Bl/6J or B6D2F1 mice), Sprague Dawley rats and beagle dogs. Nephrectomized rats were used as an animal model of chronic kidney disease. During the course of each study, haematological parameters, which included reticulocyte and erythrocyte counts, haematocrit values and haemoglobin levels, were measured at regular intervals.

- Primary pharmacodynamics

The main cellular model used was UT-7, a human acute myeloid leukemia cell line that expresses the erythropoietin receptor and is dependent on growth factors, e.g. erythropoietin, for cell proliferation. Approximately a 10-fold higher concentration of RO0503821 (300 – 400 pM) was required to stimulate proliferation of UT-7 cells compared with epoetin beta (30-60 pM). This could be verified also with CD34+ selected human primary cells from bone marrow or cord blood which needed an approximately 40-fold higher concentration of RO0503821 to reach the same level of erythroid cell formation compared with epoetin beta.

Competitive binding experiments using radiolabelled (^{125}I) epoetin in UT-7 cells revealed an approximately 100-fold lower binding affinity of RO0503821 to the erythropoetin-R compared to epoetin beta. Half maximal inhibition of ^{125}I-epoetin beta binding was caused by 1.5 nM epoetin beta or 200 nM RO0503821. With respect to the biochemical receptor interaction study using the extracellular part of the erythropoetin-R, the equilibrium constant was ~2.5 x 10^{-9} mol/L for epoetin beta/erythropoetin-R interaction and ~ 1.2 x 10^{-7} mol/L for RO0503821/erythropoetin-R interaction. The total binding affinity of epoetin beta is about 45-fold higher than RO0503821. A more detailed analysis showed that this significant difference in binding relies mainly on different association rate constants. Epoetin beta associates much faster to erythropoetin-R than RO0503821 whereas RO0503821 dissociates from erythropoetin-R about factor 1.5 faster than epoetin beta.

A functional assay was developed to evaluate the consumption of erythropoietic agents in UT-7 cells. Cells were incubated in a culture medium supplemented with equi-effective concentrations of epoetin beta (100 pM) or RO0503821 (1000 pM) for 72, 92 and 120 h. Epoetin beta levels were reduced in the culture medium in a time dependent manner but no statistically significant reduction was seen in RO0503821 concentrations.

The following *in vivo* pharmacodynamic studies were performed:

1. In female mice after a single injection of 20 ug/kg of RO0503821 or epoetin beta the magnitude of the responses (elevation of reticulocytes, erythrocytes, haematocrit and haemoglobin) was about twice as great for RO0503821 compared to epoetin beta and the duration of the response was about 3 days longer. The response to RO0503821 was dose-dependent at tested doses (1.25, 2.5, 5 and 20 ug/kg) and no differences were found between subcutaneous or intravenous injections. White blood cells and platelets were not significantly affected by the administration of RO0503821.

2. Erythropoietic activity of multiple injections of RO0503821 compared to epoetin beta was investigated in female mice. RO0503821 was injected s.c. at doses of 5 or 2.5 ug/kg once per week for four weeks. A cyclical increase in reticulocytes was seen. Both doses of RO0503821 were more efficient than epoetin beta that was injected at a dose of 5 ug/kg three times per week. 5 ug/kg of RO0503821 once every other week for four weeks showed a smaller increase but it was still more efficient than the epoetin beta treatment. When RO0503821 was injected at a dose of 2.5 ug/ kg s.c. once weekly or bi-weekly erythrocyte numbers showed gradual steady increase; once tri-weekly had an erythrocyte response approximately equivalent to that of mice receiving three 0.9 ug/kg weekly injections of epoetin beta. No significant changes were seen in white blood cell parameters.

3. A 4-week intravenous pharmacodynamic and pilot tolerability study of RO0503821 and epoetin beta was performed in male rats. RO0503821 was administered i.v. once a week at doses 2.5, 7.5 or 25 ug/kg for four weeks and compared to i.v. epoetin beta at 2.5 ug/kg 3 times per week. Reticulocyte and erythrocyte counts were increased in all treated groups. The increase in RO0503821 treated groups was dose-dependent. Effect of RO0503821 at a dosage of 2.5 ug/kg once a week was comparable to epoetin beta 2.5 ug/kg three times a week. No effect was found on white blood cells or platelets.

Tolerability testing following treatment with RO0503821 showed minimal to moderate extramedullary erythropoiesis in the spleen or increase in spleen weights, minimal to moderate dose-dependent increase of haematopoiesis in the bone marrow and ossification of the bone marrow in the form of new trabecular bone formation.

4. A 4-week subcutaneous pharmacodynamic and pilot tolerability study of RO0503821 and epoetin beta was performed in male rats. RO0503821 was administered subcutaneously once a week at doses 2.5, 7.5 or 25 ug/kg for four weeks and compared to i.v. epoetin beta at 2.5 ug/kg 3 times per week. Reticulocyte and erythrocyte counts were increased in all treated groups. The increase in RO0503821 treated groups was dose-dependent. The increase in the group given RO0503821 at a dosage of 7.5 (reticulocytes) or 2.5 (erythrocytes) ug/kg once a week was comparable with epoetin beta 2.5 ug/kg three times a week. No effect was found on white blood cells or platelets.

Tolerability testing following treatment with RO0503821 showed minimal to slight dose-dependent decrease in body weight gain, slight to moderate increase in aspartate aminotransferase, minimal to moderate extramedullary erythropoiesis in the spleen or increase in spleen weights, minimal to moderate dose-dependent increase of haematopoiesis in the bone marrow and ossification of the bone marrow in the form of new trabecular bone formation.

5. Stimulation of erythropoiesis after RO0503821 treatment was assessed in beagle dogs. Treatment with a single injection of 2.5 ug/kg RO0503821 was compared to 2.5 ug/kg epoetin beta administered during 6 consecutive days, both under s.c. and i.v. conditions. Reticulocytosis was enhanced in all treatments by day 4. Intravenous administration of epoetin beta or RO0503821 resulted in a larger increase in reticulocytes than subcutaneous administration. After multiple (six) injections of epoetin beta, the magnitude of the response was greater than after a single injection of RO0503821 while the duration of the response was similar. No drug-induced changes were observed either with regard to general condition or behaviour of the animals.

6. A model of nephrectomized Sprague-Dawley rats was used in order to evaluate erythropoiesis under conditions of chronic renal failure and erythropoietin deficiency. RO0503821 was given s.c. once a week at doses 0.75, 2.5 or 7.5 ug/kg, and compared to epoetin beta given at a dose of 0.75 ug/kg once a week, both treatments for 8 weeks. The total numbers of reticulocytes were lower in nephrectomized rats than in healthy rats. Administration of RO0503821 resulted in a dose-dependent increase in reticulocytes and red blood cells. The duration of the response was longer for RO0503821 than epoetin beta when both at 0.75 ug/kg. No effect was found in white blood cells or platelets. Antibodies were found at the end of the 8 weeks in the higher dose group of RO0503821 (1/9 animals) and also in the epoetin beta treated group (9/ 14 animals). Higher mortality was observed in the highest dose group of RO0503821.

- Secondary pharmacodynamics

No specific studies were performed.

- Safety pharmacology programme

Safety pharmacology was evaluated in one study addressing EGC, general haemodynamics and respiratory rate in anaesthetized beagle dogs. Animals received increasing cumulative doses of 5, 15, and 50 ug/kg of RO0503821 i.v. at 30 min intervals. Haemodynamics data and ECG were captured continuously, starting at least 15 minutes prior (t-15) to administration of the first dose. Data samples for each variable were taken at t-15 and t0 for baseline values and 1, 2, 5, 10, 20 and 30 minutes after the start of each administration. A gradual increase in mean serum concentration (from 104 to 1810 ng/mL) was achieved. The following parameters were recorded: ECG, heart rates, arterial blood pressures, mean femoral arterial blood flow, left ventricular pressure variables, cardiac output, respiration rate, arterial blood gases, base excess/deficient; standard bicarbonate, $\%O_2$ saturation and pH. Total peripheral resistance, femoral arterial conductance, stroke volume, QTcF and QTcV were also calculated. RO0503821 had no notable effects on any of the cardiovascular or respiratory variables measured. ECG waveform analysis showed no abnormalities in gross morphology or rhythm that could be attributed to the administration of either test article or vehicle.

- Pharmacodynamic drug interactions

No specific studies were performed.

Pharmacokinetics

PK assessment in animals was conducted after IV and SC administrations in rats and dogs. Serum concentrations of RO0503821 were determined using an enzyme-linked-immunosorbent assay

(ELISA), and the PK characteristics of RO0503821 were evaluated by non-compartmental analysis using WinNonlin.

- Absorption-Bioavailability

In the rats, after IV injection at 0.25, 2.5 or 25 µg/kg, the systemic CL of RO0503821 was only 2-7% of liver blood flow and 1/10 of epoetin beta value. As a result of the profound reduction in systemic CL, the apparent elimination $t_{1/2}$ of RO0503821 was prolonged by approximately 2-fold when compared to epoetin beta (18-27 vs. 11-17 hours). The Vdz was only 7-10% of total body water volume and 1/5 of epoetin beta value indicating a limited distribution of RO0503821 (the tissue distribution of RO0503821 was confined to extracellular space). Dose proportional increases in AUC were observed over the dose range of 2.5-25 µg/kg for both IV and SC routes of administration. These results suggest a linear kinetics of test compound. After SC administration of the same doses, the increase in C_{max} over the range of 2.5 to 25 µg/kg was also dose proportional. Compared to epoetin beta, T_{max} after SC administration of RO0503821 was delayed by 12 hours, which reflected slower depot absorption. The bioavailability after SC dosing was 31% and 45% in the 2.5 and 25 µg/kg dose groups, respectively. The bioavailability of RO0503821 was still lower than that of epoetin beta (31% vs. 77%).

In dogs, following a single IV and SC administration (3, 7.5 or 10 µg/kg) the CL was relatively constant between the 3 and 7.5 µg/kg dose, i.e. linear kinetics, but decreased at 10 µg/kg, an indication of non-linear kinetics. Compared with epoetin beta, the systemic CL (1-4% of liver blood flow) and terminal phase volume of distribution (6-14% of total body water volume) of RO0503821 reduced to about 1/10 and 1/4, respectively, of epoetin beta values. Terminal $t_{1/2}$ (41-70 hours) was 7- to 11-fold longer than that of epoetin beta. Tmax (48 hours) was about twice longer and the delayed Tmax suggested decreases in the rate of absorption. Following a single SC administration, AUC and C_{max} increased proportionally which was consistent with the linear kinetics observation from the IV study at the doses of 3 and 7.5 µg/kg. Bioavailability after SC injection was about 46-80%. In contrast to the IV study, approximately dose proportional increases in AUC and Cmax were observed in all doses at SC study. A 3.3-fold increase in dose (from 3 to 10 µg/kg) gave rise to a 3.3 and 2.6 fold increase in AUC and Cmax respectively. Because of the high AUC obtained form the 10 µg/kg IV group, the bioavailability of RO0503821 at this dose is rather low (46% vs. 80 % at 3 µg/kg). The bioavailabilities of RO0503821 and epoetin beta at 3 µg/kg are almost identical. The terminal half-life of RO0503821 is comparable after IV and SC injections at 3 µg/kg. When RO0503821 was administered subcutaneously, the serum concentration increased slowly and reached its maximum concentration at 48 hours whereas Tmax was 24 hours for unmodified EOP. This result suggested that pegylation substantially decreased the rate of absorption. The absolute bioavailability in male dogs is higher that that in female dogs (98 and 68% vs. 66 and 36 % at 3 and 10 µg/kg, respectively).

- Distribution

After single and multiple SC administrations of ^{14}C-PEG erythropoetin in rats, radioactivity was detected in all analyzed tissues except oesophagus, medulla and spinal cord. The highest concentrations were observed in lymph nodes, testis, blood, adrenal gland (medulla) and spleen. High levels of radioactivity were also observed at the injection site. Limited amount of radioactivity was detected in cerebellum and cerebrum. These results indicate that drug-derived radioactivity may cross the blood/brain barrier, although at very low levels (<0.3% of administered dose). Following a single dose, the tissue:serum concentration ratios in most organs were less than one at all collection times through 72 hours post-dose. However, at 168 hours post-dose, concentration in many tissues was greater than in serum. Due to the sensitivity of the assay, the doses used in these studies were orders of magnitude higher (mg/kg or mg/animal) than the therapeutic dose (µg/kg).

Female Sprague Dawley rats were used to assess the lacteal excretion and placental transfer of radioactivity following a SC administration of RO0503821. Following a single dose, within a 336 hour collection period, ^{14}C-RO0503821 derived radioactivity in the whole foetus was less than 0.30% of the administered dose and in foetal tissues it never exceeded 0.01% at any given time points. In lactating rats, negligible amount of ^{14}C-RO0503821 derived radioactivity was detected in milk at 4 hours post-dose and in serum at 2 hours post-dose. The milk to serum concentration ratio was ranged from 0.00865 to 0.158. The concentrations in milk and serum rose until they reached Cmax at 48

hours post-dose, with mean values of 1.18 and 12.3 µg equivalents ^{14}C-RO0503821 respectively. Concentrations declined with time, but were still detectable at 168 hours post-dose. On gestation days (GDs) 13 or 18 only a small (3-13%) fraction of radioactivity was excreted in urine and faeces (urine was the primary route of elimination on GD 13 and faeces were the primary route of elimination on GD 18). At both GDs the kinetic profile of the test compound in blood and plasma were comparable to those reported in non-pregnant rats. The tissues with the highest percent of dosed radioactivity in DGs 13 and 18 animals were maternal blood, maternal liver, placenta and uterus. The results indicate that drug-derived radioactivity may cross the blood/placental barriers but at very low levels.

- Metabolism

No specific studies were performed.

- Excretion

To assess the excretion profiles, ^{14}C-RO0503821 was administered to rats by a single (0.674 mg/animal) and multiple (0.674 mg/animal) SC injection as well as a single IV injection. Urinary excretion is the primary mechanism of elimination and excretion of RO0503821 (23% vs. 4% in faeces). Both RO0503821 and a 30 kDa PEG-like moiety were excreted in urine. The median of terminal elimination half-life of RO0503821 in serum after IV dose was 18 – 27 h in rats and 41 to 70 h in dogs, which is 6 times longer than T½ of epoetin beta (6.4 h) in dosage of the same range. Median AUC is about 10 times greater after RO0503821. No signs of accumulation were seen in multiple dose animal studies. However, only 3 consecutive doses were given in rat studies.

- Pharmacokinetic drug interaction

No specific studies were performed.

- Other pharmacokinetic studies

Bioequivalence was studied in rats in order to evaluate if modification of production conditions had any effect of bioavailability of RO0503821. The study was carried out with 7 animals in parallel groups. The study demonstrated a ratio of 0.85 [0.75; 0.96] for the API-PMP (API-Preliminary Manufacturing Process) and API-OMP (API-Optimized Manufacturing Process) and a ratio of 0.94 [0.77; 1.14] for the PMP-PF (Preliminary Manufacturing Process-Preliminary Formulation) and OMP-IF (Optimized Manufacturing Process – Improved Formulation).

Toxicology

- Single dose toxicity

In single-dose acute toxicity studies (see Table 8), doses up to 750 µg/kg administered as IV bolus injections were well tolerated in rats and mice without any adverse clinical sign. Increased red blood cell parameters and enlarged spleen detected in these animals at the end of the 2-week observation period were consistent with the pharmacological effect of RO0503821. The maximum non-lethal dose for RO0503821 by single IV administration in mice or rats was greater than 750 µg/kg.

The highest dose tested was 1250 times the clinical SC or IV starting dose of 0.6 µg/kg once every two weeks in patients not currently treated with an erythropoiesis stimulating agent (ESA), or 125 times the highest clinical starting dose of 360 µg once every four weeks for patients currently treated with an ESA.

Table 8: Summary of the major non-clinical toxicology findings

Species/Strain/ n/sex/group	Dose/route/follow up	Approx. Lethal Dose/Observed Max Non-lethal Dose	Major findings

Mouse/ CRL:CD-1®BR M/F: 5/grp	0, 50, 150, 450, 750 (µg/kg)/ IV (Single bolus)/2-week observation period	> 750 µg/kg / 750 µg/kg	- Elevated reticulocyte count, erythrocyte counts, haematocrit, haemoglobin, red cell distribution width, platelet counts and mean platelet volume. - Decreased MCH and MCHC. - Enlarged spleens - Increased spleen weights (in all treated mice, except 50 µg/kg males) - Splenic extramedullary haematopoiesis
Rat/HsdBrlHan:WIST M/F: 5/grp	0, 50, 150, 450, 750 (µg/kg) IV (Single bolus))/2-week observation period	> 750 µg/kg / 750 µg/kg	- Reduced mean body weight gain in males. - Elevated reticulocyte counts, erythrocyte counts, haematocrit, haemoglobin, red cell distribution width and platelet counts. - Decreased MCH and MCHC. - Enlarged spleens. - Splenic extramedullary haematopoiesis.

- Repeat dose toxicity (with toxicokinetics)

The studies included are the following:
- 2 pivotal, GLP, 13-week toxicity studies with interim and TK evaluations by IV and SC routes in rats.
- 2 pivotal, GLP, 13-week toxicity studies with interim and TK evaluations by IV and SC routes in dogs, with doses selected by a previous range finding. Non-GLP study by IV and SC routes.
- A pivotal, GLP, 26-week toxicity study with TK evaluations by SC route in rats.
- A GLP, 4-week formulation comparability study by SC injection in rats.

Haematological evaluations were performed once weekly except for the 26-week study in which they were performed once monthly. Serum concentrations of RO0503821 were determined by an ELISA validated method and qualitative anti-erythropoietin antibodies were determined by an ELISA method.

Treatment of rats and dogs with RO0503821 once weekly at 1-10 µg/kg/dose for 13 weeks by IV, at 1-30 µg/kg/dose for 4 weeks by IV and SC routes or at 0.3-3 µg/kg/dose for 26 weeks (rats only) by SC route, resulted in several histopathological findings including vascular congestion, haemorrhage, erosion in the glandular mucosa of the stomach, thrombosis, necrosis, and/or inflammation in various organs and tissues including brain, heart, kidney, liver, spleen, stomach, and thymus. These findings were associated with the polycythemic condition due to the exaggerated pharmacological effects, which were manifested as clinical observations, haematologically and at histopathological level. There were no increases in platelet counts. The coagulation parameters Prothrombin Time (PT) and Activated Partial Thrombin Time (APTT) increased when RBC counts increased following treatment. The effects on coagulation parameters were likely artifactual changes and were not considered to be the direct effects of RO0503821 on haemostasis.

Secondary changes to accelerated erythropoiesis included increases in spleen size and weight and a widespread extramedullary haematopoiesis with increased hemosiderin pigment in the spleen and increased Kupffer cell pigmentation in the liver, which were due to the higher red blood cell turnover. A functional iron deficiency was seen mainly in dogs. Specific for dogs in the SC study was a minimal to moderate segmental glomerular sclerosis and/or interstitial fibrosis in the kidneys observed in animals after recovery which were interpreted as scarring of the glomerular thrombi and tubular basophilia present in the terminal sacrifice rather than as a delayed toxicity response. Minimal to moderate myelofibrosis in the bone marrow was seen in dogs treated with high doses of RO0503821 and is thought to be reactive rather than a pre-neoplastic disorder. Additional findings in IV studies in rats included reversible increased trabecular bone formation.

Some rats and dogs developed anti-erythropoetin antibodies and at the same time developed resistance in responding to the administration of RO0503821 resulting in anaemia. Resistance to RO0503821 in animal studies is considered a reflection of the presence of neutralizing antibodies to RO0503821 and endogenous erythropoetin in these animals.

A few deaths, mostly in rats, occurred and were associated with either polycythemia or anaemia. There was no pattern related to the dose or route of administration. In the 26-weeks toxicity study by SC route in rats, no new findings attributed to longer exposure of RO0503821 were noted, in particular no hyperplasic or neoplasic lesions were observed.

Toxicokinetics results in 13-week studies in rats and dogs by IV and SC routes, showed that after the first dose the increase in exposure was generally not linear but higher in the dose range between 1 and 10 µg/kg/dose. This trend was no longer present when the dose was increased from 10 to 30 µg/kg/dose. There was evidence of drug accumulation during the first 4 weeks of dosing except in the rat IV study and the high dose group in the dog IV study. No further accumulation was seen between day 22 and day 85; instead, the exposure levels on day 85 were lower than those of day 22 and/or day 1 in the dog studies. This effect was more pronounced in the dogs receiving 3 or 10 µg/kg/dose RO0503821 by subcutaneous injection. This finding may be related to the antibody-formation. In general, no gender differences trend was seen in any study.

In a 26-week+12-week recovery, SC, rat study serum concentrations peaked at 16 to 36 hours post-dose across all treatment groups. On day 84 greater than or approximately dose-proportional increases in AUC were observed. Generally, there was no accumulation in Cmax for the 0.3 and 1 µg/kg/dose groups (days 84 and 175), however a slight accumulation for the 3 µg/kg/dose group was observed. A small degree of accumulation in AUC was observed across all treatment groups ranging from 1.36 to 3.71. There was no trend for gender differences.

A comparability formulation study performed in rats using SC doses up to 50 µg/kg once a week for 4 weeks showed comparable toxicokinetic profiles, anti-RO0503821 antibody development, pharmacodynamic effects and toxicity profiles of the preliminary formulation of RO0503821 used in single and repeated studies and a final formulation of RO0503821 used in reproductive toxicity studies and in local tolerance studies.

- Genotoxicity

No genotoxicity studies were performed as justified with reference to ICH guideline S6 (1997).

- Carcinogenicity

No carcinogenicity specific studies were performed as justified with reference to ICH guideline S6 (1997). Neither RO0503821 nor epoetin beta stimulated proliferation of erythropoietin receptor positive (HepG2 and K562) or negative (RT112) cell lines. However, both stimulated the growth of UT-7 cells, a cell line dependent on the presence of growth factors (e.g. erythropoietin, GM-CSF). The *in vitro* tissue binding profile of RO0503821 was comparable to that of epoetin beta.

- Reproduction Toxicity

The reproductive and developmental program includes the following studies:
- One pivotal, GLP Fertility and General Reproduction toxicity study by SC route in rats,
- 2 pilot teratology studies in rats and rabbits by SC injection and 2 pivotal GLP teratology and TK studies in rats and rabbits by SC route.
- A developmental and Peri-/post-natal reproduction toxicity study by SC route in rats, including a post-natal behavioural/functional evaluation.

Weekly subcutaneous injections of RO0503821 at 5, 20, and 50 µg/kg/doses to male and female rats did not affect reproductive performance or fertility parameters such as oestrous cycling, mating, fertility, and sperm production. Haematology and necropsy findings in treated animals conformed to the anticipated pharmacological effects (stimulation of erythropoiesis). The reproductive NOEL was greater than 50 µg/kg/.

Teratogenicity was assessed in pregnant rats and rabbits. Rats received SC dosages of 5, 20, or 50 µg/kg/dose of RO0503821 on gestation days (GD) 6, 9, 12, and 15. All dams were Caesarean

sectioned on GD 21 and litters examined for gross anomalies. In general, the PK profile of RO0503821 in pregnant rats was comparable to that of normal female rats. Greater than dose proportional increases in AUC and Cmax were noted in 20 µg/kg but comparable results were not observed for 50 µg/kg probably due to a limited absorption from the injection site. The maternal adverse effects, which were secondary to the exaggerated erythropoiesis (polycythemia), included lower mean body weight and body weight gain observed at 20 and 50 µg/kg/dose, and correlated to decreased fetal body weights. RO0503821 was not teratogenic. The maternal NOEL was less than 5 µg/kg/dose. The developmental (F_1 litters) NOEL was less than 5 µg/kg/dose (based on significant decreases in fetal body weights).

Pregnant NZW rabbits received SC dosages of 5, 20, or 50 µg/kg on GD 6, 9, 12, 15, and 18. Dams were Caesarean sectioned on GD 29 and litters examined for gross anomalies. Greater than dose proportional increases in AUC and Cmax were noted (20 and 50 µg/kg/dose). After repeated dosing, drug accumulation was observed in all dose groups. Exaggerated pharmacodynamic effects resulted in maternal adverse effects (reductions in food consumption and body weight gains). Fetal toxicities included increased resorptions (20 and 50 µg/kg), decreased fetal body weights (all dose levels) and increased number of fetuses with alterations (50 µg/kg/dose). However, there was no evidence of increased incidences of malformation. RO0503821 was not teratogenic in rabbits. The maternal NOEL was less than 5 µg/kg/dose. The developmental (F_1 Litters) NOEL was also less than 5 µg/kg/dose based on decreased fetal body weights.

A developmental and perinatal/postnatal reproduction toxicity study including a postnatal behavioural/functional evaluation was conducted in rats by SC route. Doses of 5, 20, and 50 µg/kg did not adversely affect pregnancy parameters, natural delivery or litter observations. The survival rate of offspring was reduced in the 50 µg/kg maternal dose group. A significant increase in the appearance of abdominal distension was also observed in the offspring of the 50 µg/kg/dose group, and speculated to be due to enlarged spleens. Based on significant reduction in growth rate, especially during lactation and early post-weaning periods, the NOAEL of F1 generation was determined to be less than 5 µg/kg/dose. However, no meaningful changes were observed for reflex and physical development, learning, or memory in F1 generation at any maternal dose groups. Other than an increase in the number of cohabitation days prior to mating, no effects on other reproductive parameters were observed in F1 generation.

- Local tolerance

The local tolerance of RO0503821 formulations was tested in the rabbit model with no significant findings. No evidence of local irritation was observed when the final formulation of RO0503821 was administered to rabbits by IV or SC route. Although for SC route minimal dermal inflammatory cell infiltrates were present in a few vehicle and vehicle-RO0503821 injection sites, no increased irritation due to RO0503821 was noted.

- Other toxicity studies

Antigenicity

RO0503821 has antigenic potential as observed in repeated-dose toxicity studies. Development of antibodies towards RO0503821 was evident in rats and dogs by both IV and SC. Antibodies were neutralising of the pharmacodynamic effect of RO0503821 and resulted in anaemia.

Immunotoxicity

No specific studies have been performed. However, no systemic hypersensitivity or allergic reactions were observed in toxicology studies in animals.

Studies on impurities

Data from the comparability toxicity and the local tolerance studies show that the levels of impurities in the final clinical formulation were lower than in the preliminary formulation.

Ecotoxicity/environmental risk assessment

Considering maximal dosage, estimated amounts and use pattern as well as ecotoxicological properties of RO0503821, no exposure levels of concern to the environment are to be expected.

Discussion on the non-clinical aspects

Pharmacology
RO0503821 is a stimulator of erythroid progenitor cells in the bone marrow. *In vitro*, RO0503821 has been shown to have a lower affinity to erythropoetin-receptor compared to epoetin beta. Frequently, PEG-conjugated biomolecules exhibit physiochemical properties that are different from those of the parent molecules and often affect binding affinities to the receptors. In relation to *in vivo* studies, RO0503821 was found to be a more potent stimulator of erythropoiesis than epoetin beta both in magnitude and duration of response. This improved response in mouse models may be due to the enhanced stability of pegylated proteins and prolonged exposure conferred by the addition of a PEG moiety to the protein molecule.

Studies on erythropoiesis in a non-rodent model (beagle dogs) also clearly indicated a longer-lasting stimulation of erythropoiesis by RO0503821 compared to epoetin beta. The dosing regime used for erythropoetin in the non-rodent species was different from those used in rodent species. Significant differences in the pharmacokinetics of epoetins have been observed in various species and an adaptation of dosing regimens was necessary. Pharmacokinetics and -dynamics of epoetin beta after i.v. and s.c. application in dogs, rats, and mice are described in detail by Bleuel et al (2). Based on those data, the application to dogs of 500 U/kg, corresponding to 2.5 µg/kg, over a period of 6 days is expected to more than double erythropoiesis-indicating parameters.

Effects on cardiovascular and respiratory safety pharmacology parameters were also evaluated in beagle dogs administered RO0503821 intravenously. There were no notable effects on any of the cardiovascular or respiratory variables measured. No acute cardiovascular safety signals were observed in canine studies although the long-term effects on blood pressure were not fully evaluated. Functional effects of RO0503821 on central nervous system were also absent from the core battery test, however, neurological examinations included in the single-dose toxicity studies in rats and mice and in the 13-week SC toxicity study in rats showed no indications for effects on the CNS.

Only one gender has been tested in these pharmacological studies because of the absence of evidence for gender specific differences in erythropoiesis, erythropoetin metabolism, or erythropoietin receptor expression. Nevertheless, all pivotal safety studies were performed in both sexes.

Pharmacokinetics
Pegylation of erythropoetin seems to limit tissue penetration. Although RO0503821 is distributed widely in body, its distribution volume is only half of that of epoetin (erythropoetin) (0.05 L / kg in rats and 0.04 L / kg (median) in dogs). In addition elimination of RO0503821 is clearly retarded compared to that of epoetin. Thus, the observed difference in the relative potencies *in vitro* and *in vivo* may be largely dependent on the differences in elimination and distribution.

Distribution and elimination studies of PEG were carried out with doses about 1000 to 5000 times of that intended to be used clinically. For this reason it should be noted that animal data may not directly extrapolate to the human situation. In addition, although the highest concentrations of PEG were seen in liver and substantial amounts of its fragments in urine, metabolism and elimination remains unclear since analytical methods were not sensitive enough.

To assess whether a drug-drug interaction could be expected from the PEG moiety, a review of the literature in US and EU labels of drugs that employ pegylated proteins (Adagen, Oncaspar, Intron/Peg-Intron, Roferon-A/Pegasys, and Filgastrim) has been included. This review revealed no drug-drug interaction that could be related to the PEG moiety. Furthermore, PEG is also used as an excipient in many pharmaceutical products, including products for parenteral use.

In relation to drugs that bind or penetrate into RBCs (red blood cells), there is a theoretical potential for an interaction of erythropoiesis stimulating agents (ESAs) with these drugs. The increase in RBCs with ESAs treatment may lead to a decrease in unbound levels of these drugs and potentially decreased efficacy. There is a high probability that RO0503821 will be administrated in clinical

situations combined with other drugs currently used in the treatment of CKD. However no PK interactions are expected taking into account clinical data.

Toxicology
In repeated-dose toxicity studies, fixed dosing regimens caused serious pathogenesis and mortality associated with exaggerated pharmacological effects and uncontrolled polycythemia in affected animals. It is expected that this findings are unlikely to be encountered in clinical practice, where the dose will be adjusted on the basis of measured haemoglobin (Hb), which is regularly monitored to maintain the Hb level in the target range as specifically outlined in the SPC.

As expected when heterologous human protein is introduced to animal species, some rats and dogs developed anti-erythropoetin antibodies following administration of RO050382 in the repeated dose toxicity studies. The neutralizing capacity of these antibodies is reflected by the increasing rate of anemia over time. Therefore, toxicokinetic results should be interpreted with caution due to the likely interference of the antibodies with the ELISA used to measure the serum RO0503821 concentrations.

For epoetins there is a concern related to the expression of the erythropoietin receptors present on the surface of some tumours cells and the possibility that erythropoetins could stimulate the growth of any type of malignancy. In vitro cell proliferating studies following RO0503821 treatment have been presented, however the number of human tumour cell lines investigated was limited. This issue has been addressed in the SPC and the proposed indications do not include the treatment of symptomatic anaemia in cancer patients.

All effects observed in dams and pups could be explained by an exaggerated pharmacodynamic action of RO0503821. The developmental observations occurred in rats and rabbits are a consequence of the maternal polycythemia during early pregnancy. In the case of normal erythrocyte parameters there are no reproductive toxicological risks whatsoever. RO0503821 should not be identified as a selective developmental toxicant. Although no teratogenic effects have seen in rats and rabbits these class-related effects have been properly addressed in the SPC.

1.4 Clinical aspects

Introduction

The global clinical development program for RO0503821 includes 13 Phase I clinical pharmacology studies in 499 healthy volunteers and 40 CKD patients, and ten therapeutic studies comprising four Phase II and six Phase III studies in a total of 1789 patients with CKD, including patients on dialysis and not on dialysis.

GCP

It is stated by the Applicant that all studies in this clinical program were conducted in accordance with the principles of Good Clinical Practice, the principles of the Declaration of Helsinki and its amendments and the local laws and regulations of the countries in which the research was conducted.

Clinical Pharmacology

The clinical pharmacology program is included in 13 phase I studies. Eleven of these were performed on healthy volunteers (n=499) and two were conducted in 40 CKD patients, one in CKD patients not on dialysis and one in patients undergoing peritoneal dialysis. In addition, three population PK and PD analyses were performed, based on samples from over 500 CKD patients in the clinical program.

Three validated ELISA (sandwich enzyme-linked immunosorbent assay) were used in the clinical studies to determine the concentration of RO0503821 in human serum samples. The most recent optimised and used in the Phase III program was developed to reduce the non-specific background signal and to expand the assay range from 150-1500 pg/mL to 150-4000 pg/mL. A commercial assay was used to determine serum concentrations of endogenous erythropoietin at baseline prior to drug administration as well as to determine epoetin alfa and beta levels in patients treated with epoetin in

the Phase III study (Quantikine® IVD® Erythropoietin ELISA, R&D Systems Inc). Anti-erythropoietin antibodies were detected by using two validated ELISA, a sandwich type assay and a bridging-type assay.

Pharmacokinetic analyses of RO0503821 were carried out mainly using non-compartmental methods using WinNonlin Version 2. Statistical analyses in comparability bridging studies were done using a cross over three-way ANOVA. The other PK-studies were reported using descriptive characterisation only or one-way ANOVA with a factor drug-period.

Pharmacokinetics

- Absorption and distribution

In healthy volunteers
Single ascending doses of 0.1, 0.2, 0.4, 0.8, 1.6, 2.4 and 3.2 µg/kg were administered SC. In addition, two doses of 2.0 µg/kg were administered two weeks apart. Across the dose groups, RO0503821 had a median time to maximum concentration (t_{max}) ranging from 42-120 hours with a mean elimination half-life ($t_{1/2}$) ranging from 102-216 hours. The mean ratio of total clearance over absolute bioavailability (CL/F) ranged from 1.06-7.84 mL/h/kg. The concentration of RO0503821 in urine was assessed only in the 3.2 µg/kg dose group and none was detected. Statistical analysis for dose proportionality showed non-linearity in the PK of RO0503821 with area under the concentration-time curve (AUC_{last}) and maximum concentration (C_{max}) increasing more than proportionally with dose.

Single ascending doses of 0.4, 0.8, 1.6 and 3.2 µg/kg were administered IV to 38 healthy volunteers (blind, randomized, placebo controlled). Across the dose groups, RO0503821 had a mean $t_{1/2}$ range of 70-122 hours. Mean values for clearance (CL) ranged from 0.34-0.74 mL/h/kg. AUC_{last} and C_{max} increased more than proportionally with dose, indicating a non-linear PK.

A single ascending study using doses of 0.8, 1.6 and 3.2 µg/kg were administered to 72 healthy male volunteers, 36 of Japanese and 36 of Caucasian origin. The primary PK parameter, AUC_{last}, was comparable between Japanese and Caucasian subjects for all doses tested. The ratio Japanese/Caucasian of means for all doses combined was 1.10 (90% CI 0.86-1.42). There were no major differences between Japanese and Caucasian subjects for other PK parameters. Mean values of $t_{1/2}$ ranged from 60-105 hours. There was no evidence for deviation from dose proportionality for both AUC_{last} and C_{max} in the dose range tested.

Table 9: Summary of results from single dose studies

Subj. entered/completed (M/F)	HV/P (age: mean, range)	Treatment (µg/kg) / Route	Mean Pharmacokinetic Parameters (±SEM) Substrate Drug				
			C_{max} (ng/mL)	t_{max} (h)	AUC_{last} (ng*h/mL)	$t_{1/2}$ (h)	CL** (mL/h/kg)
72/68 (72M)	HV (30, 20-48) C and J	0.8 IV					
		C	17.3± 1.18	0.50	1030± 145	59.7± 10.9	0.82± 0.12
		J	29.2± 2.49	0.75	1350± 129	66.8± 10.6	0.62± 0.06
		1.6 IV					
		C	39.4± 2.33	0.37	2880± 370	79.3± 21.4	0.59± 0.11
		J	38.1± 2.67	0.25	2760± 468	66.2± 12.4	0.92± 0.30
		3.2 IV					
		C	75.8± 6.23	0.75	6910± 1094	105± 23.2	0.55± 0.16
		J	81.5± 7.91	0.39	6040± 765	75.8± 14.6	0.60± 0.07

*: median value; **: CL, following IV administration; CL/F, following SC administration

In a multiple ascending dose study a total of 61 subjects received three doses of 0.4, 0.8, 1.6 or 3.2 µg/kg RO0503821 IV 1x/3 weeks (days 1, 22 and 43). Across the dose groups, mean values for CL were small (range 0.37-0.62 mL/h/kg), and mean $t_{1/2}$ values ranged from 67–140 hours. Mean values for volume of distribution at steady-state (V_{ss}) were low and ranged from 3.0–5.4 L. AUC_{last} and C_{max} increased linearly with dose and statistical analysis (3-way analysis of variance, ANOVA) showed no evidence of a deviation from dose proportionality for these two parameters. Drug

accumulation values in serum (R_{acc}, ratios of AUC from day 43 to day 1) were small (≤ 1.4) and were independent of dose.

In a second multiple ascending dose study a total of 48 subjects received four doses of 0.4, 0.8, 1.6 or 3.2 µg/kg RO0503821 SC 1x/2 weeks (days 1, 15, 29 and 43). In all dose groups and for all dosing periods, the median value for t_{max} was 72 hours. Mean values for CL/F were small (range 1.34-4.18 mL/h/kg) and mean $t_{1/2}$ values ranged from 73-170 hours. C_{max} and AUC_{last} increased linearly with dose. Mean R_{acc} values varied between 1.4 and 2.2 and were independent of dose.

Table 10: Summary of results from multiple dose studies

# Subj. entered / completed (M/F)	HV/P (age: mean, range)	Treatment (µg/kg) / Route	Mean Pharmacokinetic Parameters (±SEM) Substrate Drug				
			C_{max} (ng/mL)	t_{max}* (h)	AUC_{last} (ng*h/mL)	$t_{1/2}$ (h)	CL** (mL/h/kg)
61/59 (56M/5F)	HV (33, 18-59)	0.4 IV					
		day 1	11.6± 0.86	0.38	735± 41.4	84.9± 10.1	0.53± 0.03
		day 22	11.0± 1.12	0.63	740± 65.1	80.5± 6.51	0.52± 0.04
		day 43	12.9± 0.74	0.29	1007± 69.3	135± 19.7	0.40± 0.03
		0.8 IV					
		day 1	27.3± 2.66	1.00	1787± 160	96.2± 6.05	0.44± 0.04
		day 22	23.6± 3.12	0.25	1890± 221	86.2± 3.07	0.41± 0.050.
		day 43	20.8± 3.89	0.08	1879± 200	140± 15.2	44± 0.06
		1.6 IV					
		day 1	43.0± 4.23	0.50	3067± 132	66.9± 5.80	0.50± 0.03
		day 22	42.3± 3.73	1.00	3278± 389	83.2± 7.80	0.49± 0.07
		day 43	45.3± 3.63	0.25	4350± 300	133± 9.26	0.37± 0.03
		3.2 IV					
		day 1	78.2± 5.70	0.50	6168± 865	70.4± 11.9	0.62± 0.13
		day 22	72.8± 10.6	0.50	6258± 894	77.6± 9.83	0.60± 0.14
		day 43	69.7± 5.56	0.27	7442± 861	98.6± 10.2	0.46± 0.05
48/46 (33M/15F)	HV (41, 20-60)	0.4 SC					
		day 1	1.21± 0.167	72.0	164± 20.4	87.8± 22.0	2.03± 0.28
		day 43	1.60± 0.174	72.0	349± 43.2	130± 14.6	1.34± 0.17
		0.8 SC					
		day 1	2.14± 0.25	72.0	314± 38.9	73.0± 12.0	1.97± 0.14
		day 43	2.68± 0.38	72.0	546± 66.6	135± 19.5	1.91± 0.28
		1.6 SC					
		day 1	4.74± 0.66	72.0	702± 76.8	75.7± 13.3	2.93± 0.89
		day 43	6.20± 1.17	72.0	1507± 316	137± 21.9	4.18± 2.77
		3.2 SC					
		day 1	10.7± 1.15	72.0	1747± 203	98.4± 9.16	1.85± 0.47
		day 43	15.4± 2.19	72.0	4485± 669	170± 20.4	1.50± 0.59

*: median value; **: CL, following IV administration; CL/F, following SC administration

Absolute bioavailability (F) of RO0503821 was assessed. A total of 47 subjects received one IV dose of 0.8 µg/kg and three SC doses of 0.8, 1.6 µg/kg and 3.2 µg/kg. Drug administration was done with a two-week interval between doses. Mean F ranged from 40% to 60%. A high degree of inter-subject variability was observed (coefficient of variation, CV, of 53%-92%). Statistical analysis showed no evidence for dose dependency of F. After the first dose, the mean values for C_{max} were 22.6, 3.94 and 7.65 ng/mL for the 0.8 µg/kg IV, 0.8 µg/kg SC and 1.6 µg/kg SC groups, respectively. The mean value for C_{max} for 3.2 µg/kg SC was 21.1 ng/mL after the last dose. The median value for t_{max} was 0.5 hours after IV doses and 72-108 hours after SC dosing. CL after IV administration was small with a mean value of 1.05± 0.28 mL/h/kg after the first dose.

SC administration to three different sites (abdomen, arm and thigh) was assessed in a total of 42 healthy subjects. After SC injection of 3.0 µg/kg, values for C_{max} (mean ± SEM) were 15.7 ± 0.87 ng/mL, 14.2 ± 0.9 ng/mL and 16.5 ± 0.87 ng/mL for the abdomen, arm and thigh, respectively. The median t_{max} was the same (96 hours) for the three sites of administration. The mean AUC_{last} was similar for the three sites of administration (means between 4088 and 4323 ng*h/mL). Mean t1/2 values were also similar for the three sites (160-164 hours).

In CKD patients

In a cross-over, open label, randomised study a total of 16 patients on peritoneal dialysis received two doses of RO0503821 (0.4 µg/kg IV and 0.8 µg/kg SC) with a six-week interval between doses. After IV injection, C_{max} was 9.05 ± 0.75 ng/mL (mean ± SEM) and V_{ss} was 66.5 ± 8.3 mL/kg (mean ± SEM). Due to low CL (mean of 0.49 ± 0.05 mL/h/kg), long $t_{1/2}$ values were determined in the post-distribution phase with a mean of 134 hours. Following SC administration, C_{max} was 4.60 ± 0.58 ng/mL (mean ± SEM) and the median t_{max} was 72 hours. Long $t_{1/2}$ values were determined in the post-distribution phase with a mean of 139 hours. Mean F was 62% (based on AUC_{last}).

In a second study a total of 24 CKD patients not on dialysis (stages 3-4) received a single dose of RO0503821 (0.8 µg/kg IV or 1.2 µg/kg SC) in a single dose, open label, randomised, parallel group study. After IV injection of 0.8 µg/kg, C_{max} was 16.0 ± 1.36 ng/mL (mean ± SEM) and V_{ss} was 57.6 ± 4.54 mL/kg (mean ± SEM). Due to low CL (mean of 0.93 mL/h/kg), long $t_{1/2}$ values were determined in the post-distribution phase with a mean of 77.4 hours. After SC injection of 1.2 µg/kg, C_{max} was 3.19 ± 0.72 ng/mL (mean ± SEM) and median t_{max} was 94.5 hours. Due to low CL (mean of 1.67 mL/h/kg), long $t_{1/2}$ values were determined in the post-distribution phase with a mean of 142 hours. Mean F was 54%.

Table 11: PK results from CKD patients

Subj. entered/completed (M/F)	HV/P (age: mean, range)	Treatment (µg/kg) / Route	Mean Pharmacokinetic Parameters (±SEM) Substrate Drug				
			C_{max} (ng/mL)	t_{max} (h)	AUC_{last} (ng*h/mL)	$t_{1/2}$ (h)	CL** (mL/h/kg)
16/16 (14M/2F)	CKD P on dialysis (59, 37-80)	0.4 IV	9.05± 0.75	2.00	1028± 272	134± 19.0	0.49± 0.05
		0.8 SC	4.60± 0.58	72.0	1106± 266	139± 20.0	0.90± 0.13
24/24 (10M/14F)	CKD P not on dialysis (55, 28-79)	0.8 IV	16.0± 1.36	0.25	949± 264	77.4± 19.1	0.93± 0.27
		1.2 SC	3.19± 0.72	94.5	771± 235	142± 26.0	1.67± 0.54

*: median value;**: CL, following IV administration; CL/F, following SC administration

Population PK/PD analyses

Three population analyses were performed to describe the PK and PD following administration of RO0503821. The covariates gender, race and peritoneal dialysis (status) had no effect on the population PK parameters of RO0503821. Clearance and volume of distribution increased with body weight but these effects were not considered to be clinically relevant.

- Elimination

No data on RO0503821 metabolism is available. An exploratory analysis assessed the effect of dialysis on serum concentrations of RO0503821. In this study, 61 patients were enrolled, 42 of which were on haemodialysis and 19 on peritoneal dialysis. The effect of time (i.e., before dialysis and after dialysis) on the concentration of RO0503821 was not statistically significant ($p = 0.46$). These results indicate that haemodialysis has no effect on RO0503821 serum concentrations in patients.

- Dose proportionality and time dependencies

In healthy volunteers, following multiple administrations of RO0503821 IV and SC, Cmax and AUC were linear with respect to dose for the range from 0.4 to 3.2 µg/kg. In CKD patients clearance and volume of distribution did not seemingly depend on dose or weight. There was no accumulation of RO0503821 in once every four weeks' dosing. The mean ratio of accumulation was 1.03. After administration every 2 weeks, the mean ratio of accumulation was 1.12.

- Intra- and inter-individual variability

High variability in PK of RO0503821 was observed in most of the Phase I studies (from bioequivalence studies data, see Table 12). Low systemic exposure was detected in approximately 7% of healthy volunteers. In this small proportion of healthy subjects with very low or no systemic exposure to RO0503821, a low or no PD response (reticulocyte count) was observed in the

corresponding time period post-drug administration. In Phase III studies, 2-4% of the patients had low systemic exposure on one occasion but these low concentrations of RO0503821 in serum were not predictive of a lack of Hb response.

Table 12: Variability (expressed as CV %) of the bioavailability of RO0503821 after SC injections

Pharmacokinetic Parameter	C_{max}	AUC_{last}
No. of subjects	42	42
Inter-subject variability with SC injection into the abdomen[a]	35%	33%
Inter-subject variability with SC injection into the arm[a]	41%	38%
Inter-subject variability with SC injection into the thigh[a]	34%	35%
Intra-subject variability	33%	27%

[a]: CV% from SDx100/mean; b: residual variability in the ANOVA

- Special populations

No specific clinical pharmacology studies were performed in special populations except the studies in renally impaired patients, which constitute the target population. RO0503821 alfa has not been specifically studies in hepatic impaired patients.

Gender effect on pharmacokinetics of RO0503821 was investigated in four studies. The comparisons have been carried out in patients with CKD and in adult healthy men and women. There were no apparent differences between males and females in the pharmacokinetics of RO0503821.

One study in healthy volunteers was performed to compare the PK in Japanese and Caucasian subjects. The results indicated similarity in the PK of RO0503821 between Japanese and Caucasian healthy subjects.

The effect of body weight on the PK of RO0503821 was assessed using data from population pharmacokinetic analyses. There was a statistically significant increase of clearance (CL) and volume of distribution (Vd) of RO0503821 with body weight.

No specific pharmacokinetic studies were conducted in elderly. The PK of RO0503821 was compared in adult and elderly patients (>65 years old) using data from population analysis. The results showed that PK parameters are comparable in subjects aged 65 years or more and younger subjects (18 to 65 years).

No specific studies were performed in children.

- Pharmacokinetic interaction studies

Pharmacokinetic interactions have not been investigated in clinical trials. The effect of other drugs on the PK and PD of RO0503821 was explored using a population analysis approach. There was no indication of an effect of concomitant medications on the PK of RO0503821.

Pharmacodynamics

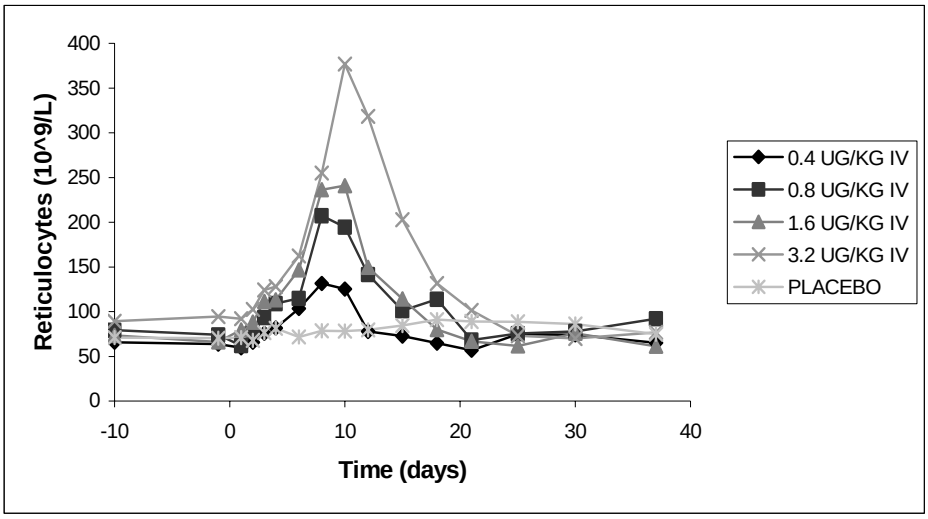
No clinical studies investigating the mechanism of action have been conducted.

- Primary and Secondary pharmacology

The pharmacodynamic profile of RO0503821 after IV and SC administration was evaluated in healthy volunteers and in CKD patients in all clinical pharmacology studies. The PD markers used in the clinical pharmacology studies were reticulocyte counts (primary PD marker), red blood cells count (RBC), haemoglobin (Hb), haematocrit (Hct) and iron related parameters such as serum iron, serum ferritin, soluble transferrin receptor and transferrin saturation (TSAT).

Reticulocyte counts:
Following single dose administrations of RO0503821 SC or IV in healthy volunteers, the reticulocyte response was characterized by a rapid increase in reticulocyte counts that peaked 8-10 days after dosing followed by a decline and return to values near baseline 20-30 days post-dose (see Figure 1). Dosing at 0.4 µg/kg was the lowest IV dose that induced a reticulocyte response. Treatment in CKD patients induced similar pattern of reticulocyte response regardless of dialysis. The peak in reticulocyte counts was approximately 2 days earlier following IV dosing than following SC dosing, in agreement with the peak in systemic exposure that also occurred earlier (2-3 days) after IV dosing. For both SC and IV dosing, the relationship between reticulocyte response and dose is almost linear. At the highest dose level tested (3.2 µg/kg); the maximum observed reticulocyte response was 251% after SC dosing and 334% after IV dosing.

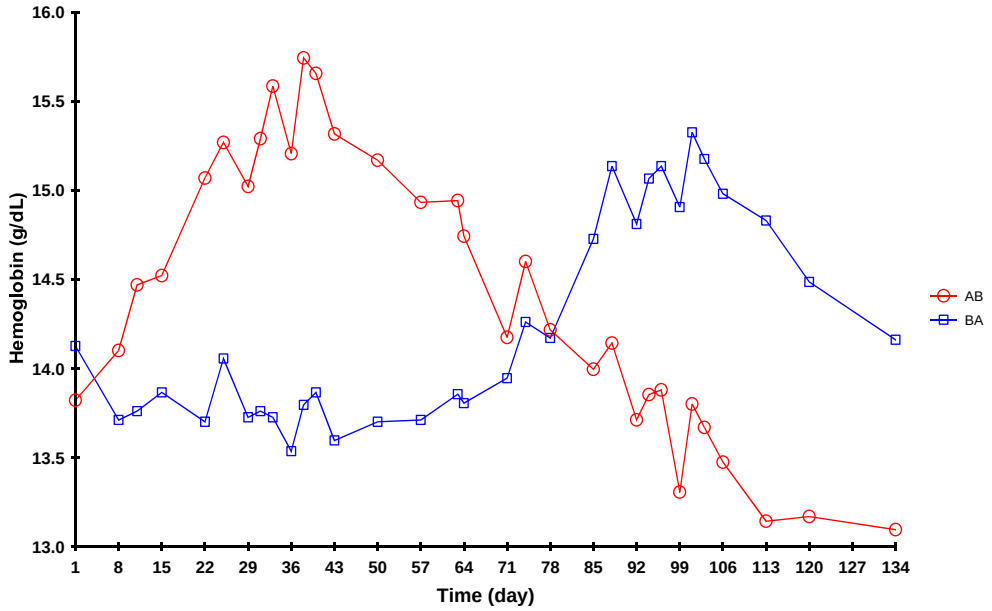
Figure 1: Mean Reticulocyte Counts (10^9/L) after Single IV Injections of RO0503821 in Healthy Subjects



After repeated administrations of RO0503821 1x/2 weeks, a cyclical increase in reticulocyte count was seen. The reticulocyte count response diminished over time after both SC and IV dosing. In contrast, the reticulocyte count response remained constant over time after IV dosing 1x/3 weeks.

Haemoglobin:
After single dose administrations of RO0503821 to healthy volunteers (1.6 IV and 3.2 µg/kg SC), there was an increase in Hb levels compared with placebo (see Figure 2). In CKD patients, a single administration of RO0503821 (0.4 µg/kg IV and 1.2 µg/kg SC) induced an increase Hb (defined as an increase > 0.4 g/dL from baseline) observable after 7 to 15 days. After multiple dose administrations to healthy volunteers, a pronounced cumulative increase in levels of Hb over time was observed in all studies. In the doses of 0.4, 0.8, 1.6 and 3.2 µg/kg, a general dose-dependent increase in Hb level was observed, although the curves of mean Hb values over time for doses of 1.6 and 3.2 µg/kg tended to overlap.

Figure 2: Mean Haemoglobin Levels (g/dL) After Two IV Treatment Sequences with RO0503821 and Placebo in Healthy Subjects



Treatments: A = RO0503821 3.2 µg/kg IV 1x/2 weeks, B = placebo;
N = 39; data not corrected for baseline values;
Days of dosing were days 1, 15, 29 (first treatment), 64, 78 and 92 (second treatment).

Iron-Related Parameters: Serum Iron, Serum Ferritin, Soluble Transferrin Receptor and Transferrin Saturation

After single and multiple administrations of RO0503821, serum iron and serum ferritin decreased, while soluble transferrin receptor increased in response to the decrease in serum iron. TSAT, calculated as the ratio of serum iron to transferrin, first decreased and then increased with a peak 2-3 weeks after dosing.

- **Population pharmacodynamics**

In the PD model, the covariates gender, age, body weight, race, transferrin saturation, ferritin, albumin, platelets, dialysis and dialysis adequacy measurement had no effect on the PD parameters of RO0503821. The apparent RO0503821 serum concentration needed for half-maximal effect on production rate of Hb (PD parameter SC_{50}) increased with C-reactive protein and previous epoetin dose. High variability was seen in PD parameters, especially in SC_{50} (with a CV% of 559%). Within-patient variability of haemoglobin is about half of the standard deviation of haemoglobin values (the median values in SC dosing 0.45 in once every 2 weeks' dosing and 0.50 every 4 weeks' dosing).

- Discussion on clinical pharmacology

The PK properties of RO0503821 in human have been characterised in healthy adult volunteers and in CKD patients. Non-linearity was observed after single ascending doses, with higher increases in AUC than expected at higher doses. In addition high variability in PK parameters is also seen, with intra and inter-subject variability expressed as AUC coefficient of variation around 30-40%. In some volunteers (approximately 7%), a very low systemic exposure was detected after SC dosing. This high interindividual variability might explain the discrepancies seen in PK studies with regard to the non-linearity of the product. Following the pharmacodynamics studies, inter-and intra-individual variability in haemoglobin and red blood cell count levels has also been observed. This variability can be explained taking into account the amount of iron available and the accelerated rate of removal of erythrocytes (due to increased viscosity).

The high variability in PD parameters, especially SC_{50} (with a CV% of 559%), suggests strongly monitoring of haemoglobin in treatment of anaemia with RO0503821. Data support the clinical practice to individually monitor haemoglobin and individual adjustment of RO0503821 dose based on

measured Hb concentrations. In addition, population PK studies show that body weight significantly correlates with volume of distribution and clearance. Dose correction with body weight at the initiation of treatment with RO0503821 is not necessary when the BMI < 28 kg/m2 however it may be necessary in more severe obesity.

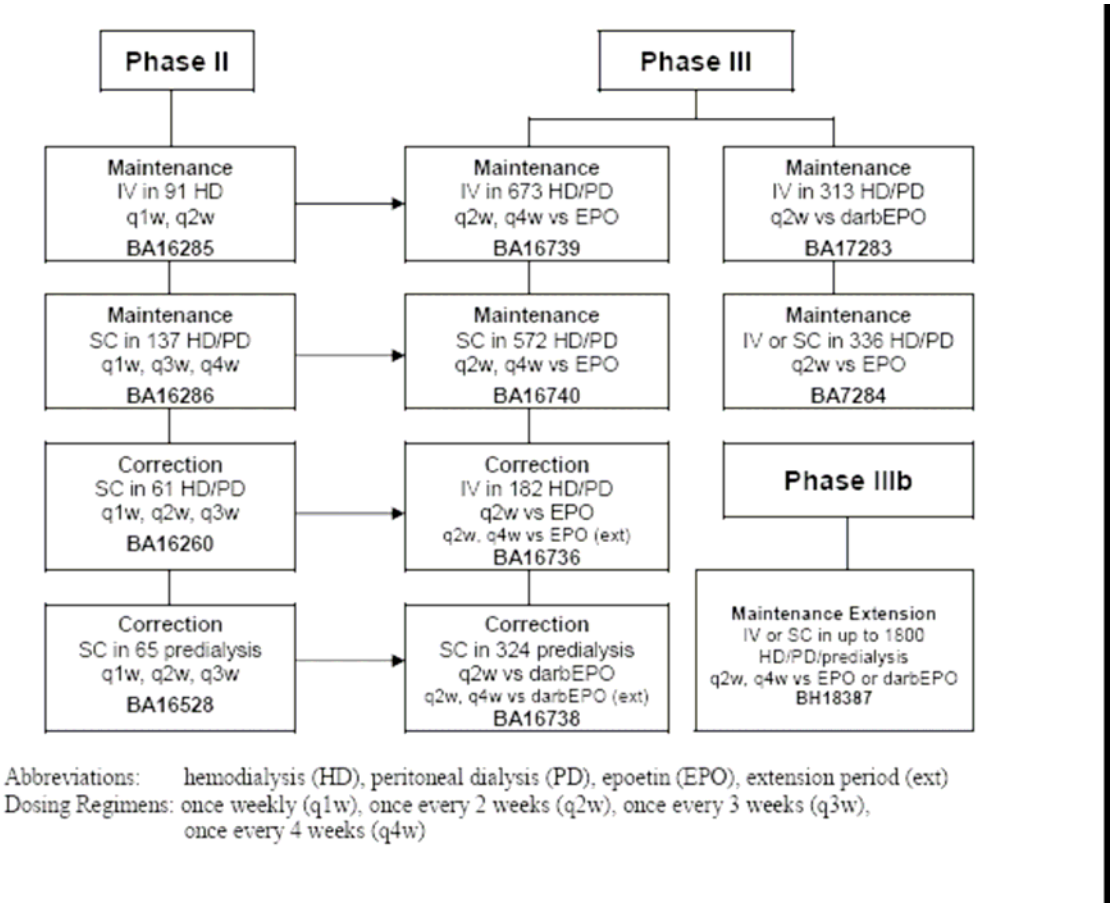
No studies have been performed in patients with hepatic impairment. It should be taken into account that a) the main route of elimination of RO0503821 has not been addressed, b) the liver is thought to be the principal route of elimination of other erythropoiesis stimulating agent (r-Huerythropoetin and darbepoetin alfa) and c) patients with severe liver disease were not included in phase III studies. An adequate statement with the relevant information has been incorporated in section 4.2 of the SPC.

Clinical efficacy

Eleven multicentre clinical trials (4 dose-finding, 6 pivotal studies and 1 long term treatment) were conducted to establish the efficacy, safety and dose response of RO0503821 in the treatment of anaemia associated with chronic renal failure (see Figure 3). A total of 2737 patients with CKD including patients on dialysis and not on dialysis were included in the 4 phase II and the six pivotal trials: 1789 received RO0503821 and 948 were treated with active comparator (epoetin alfa, epoetin beta or darbepoetin).

The Phase II and Phase III studies are divided in *correction studies* for treatment of anaemia associated with CKD in patients not treated with an ESA and *maintenance studies* for the treatment of anaemia associated with CKD in patients converting from an ESA to treatment with RO0503821.

Figure 3: Overview of the Phase II/ Phase III Clinical Development Program in Anaemia associated with CKD



Abbreviations: hemodialysis (HD), peritoneal dialysis (PD), epoetin (EPO), extension period (ext)
Dosing Regimens: once weekly (q1w), once every 2 weeks (q2w), once every 3 weeks (q3w), once every 4 weeks (q4w)

- Dose response studies

The Phase II dose finding program consisted of four open-labelled, randomised, multicentre studies which included a total of 354 patients. The starting dose was based on reticulocyte response determined in clinical pharmacology studies. In maintenance studies, three conversion factors for

switching from an ESA to RO0503821 were tested. Conversion factors were chosen based on the doses that produce response in RO0503821 studies and in epoetin (alfa or beta) studies.

The primary efficacy parameter of all phase II studies was the analysis of the regression slopes of the haemoglobin (Hb) values over time between baseline and the end of initial treatment period (EOIT, defined as the last observed value before a dose change or blood transfusion). No changes of dose were allowed before six weeks.

Two correction studies (BA16260 and BA16258) were in SC administration with starting doses of 0.15, 0.30, 0.45 and 0.60 µg/kg/week. Statistically significant dose effect was confirmed in the analyses of the Hb increases over 6 weeks and the dose selected was 0.6 µg/kg/2week. Different schedules, 1x/week, 1x/2 weeks, or 1x/3 weeks were tested for each dose level and no differences were observed. A high variability in the response was shown, with a very high dispersion of Hb responses for any tested dose. No dose finding IV studies were performed. Based on previous kinetic data, a lower starting dose of 0.4 µg/kg/2 weeks by IV administration was chosen for phase III trials.

Maintenance studies examined several conversion factors and different schedules for converting from IV epoetin alfa (study BA16285) or from SC epoetin alfa or beta (study BA16286) to RO0503821 in dialysis patients. Data from BA16285 study showed that the smallest median changes from baseline in Hb concentrations were seen using the conversions 0.4 µg/150 IU 1x/week and 0.6 µg/150 IU 1x/2 weeks. In study BA16286 the 0.8 µg/150 IU conversion factor in SC administration resulted in the most stable Hb concentrations. Dosing schedule did not appear to have an effect on the stability of Hb concentrations.

In spite of these data, in phase III trials the same conversion categories were used for both SC and IV routes of administration and the conversion regimen was simplified to a conversion based on three fixed categories of RO050382 dose (30, 50 or 90µg/week).

- Main studies

Six Phase III pivotal studies are provided to support the efficacy of RO0503821 in anaemia associated with chronic kidney disease (CKD). Two correction studies were performed in epo naive patients with CKD on dialysis (BA16736) or not on dialysis (BA16738). Four maintenance studies (BA16739, BA16740, BA17283 and BA17284) were in patients on dialysis who have been receiving ESAs. All studies were randomized, open-label, multicentre, comparative active-controlled, parallel group studies.

METHODS

In correction studies, after two weeks screening period, namely to ensure that only patients with stable Hb concentrations and adequate iron status, patients were randomized. In study ***BA16736*** patients on dialysis received RO0503821 1x/2 weeks IV vs. epoetin alfa or beta (3x/week) during 24 weeks correction (dose-titration) period. In study ***BA16738*** patients not on dialysis were treated with RO0503821 1x/2 weeks SC vs. darbepoetin alfa (1x/week) during correction (18 week) and evaluation period (10 week). At the end of these period patients were categorized as responders or non-responders. Responders in the RO0503821 groups were re-randomized to receive RO0503821 either 1x/2 weeks or 1x/4 week for the extension period. Responders in the reference group remained on their reference drug for the extension period. Non-responders in the RO0503821 groups had to be withdrawn. Non-responders in the reference group were withdrawn in study ***BA16738.***

Four maintenance studies had the same basic design: a 4-week screening/baseline period to ensure that patients maintained their previous dose and regimen of ESAs; a 28-week dose titration period used for RO0503821 dose titration and stabilization of Hb concentration; and an 8-week evaluation period. With the exception of study ***BA17284*** *(*prefilled syringes), the studies also included a 16-week long-term safety follow-up period.

Study Participants

The inclusion/exclusion criteria were generally similar among the studies within the program. Small differences were due primarily to the different study designs (correction or maintenance), and the different stages of CKD that were studied (studies in dialysis patients or studies in patients not on dialysis).

All studies were in adult patients ≥ 18 years of age with anaemia associated with CKD. Haemoglobin entry criteria and adequate iron status were defined as follows:

- The Phase III correction studies (BA16736 and BA16738) required mean screening/baseline Hb concentrations of 8 to 11 g/dL. A serum ferritin levels ≥ 100 µg/L or TSAT ≥ 20% (or hypochromic red cells < 10%).
- The Phase III maintenance studies (BA16739, BA16740, BA17283, and BA17284) required mean screening/baseline Hb concentrations of 10.5 to 13 g/dL, assessed at week -1 / week 1. In addition, Hb concentrations had to be stable (defined as an absolute difference ≤ 1 g/dL between the mean Hb values determined at weeks -4 and -3 and the mean Hb values determined at weeks -2 and -1). Serum ferritin levels ≥ 100 µg/L or TSAT ≥ 20% (or hypochromic red cells < 10%), assessed at week -3.

Treatments

1. Dosing

Correction studies

The starting doses for the correction studies are shown in Table 13. A 50% dose adjustments at monthly intervals was recommended in case of inadequate Hb response to RO0503821. Target Hb during these periods was defined as an Hb concentration ≥ 11g/dL and an increase in Hb from baseline ≥ 1.0g/dL.

Table 13: Starting Doses in Correction Studies

Study	Drug	Starting Dose and Regimen	Route
BA16736	RO0503821* epoetin alfa or beta	0.4 µg/kg/2 weeks 3x/week, according to approved labeling	IV IV
BA16738	RO0503821* darbepoetin alfa	0.6 µg/kg/2 weeks weekly, according to approved labeling	SC SC

Patients in the reference group received SC darbepoetin alfa either weekly or 1x/2 weeks (extension period) according to approved labeling.

* All patients received RO0503821 1x/2 weeks at the beginning of the correction period and were randomized to receive RO0503821 either 1x/2 weeks or 1x/4 weeks during the extension period.

Maintenance studies

Conversion categories were established based on phase II data: ESA doses at study entry were compared to RO0503821 doses under stable condition of Hb maintenance to develop categories of equivalent ESA and RO0503821 doses. Dose adjustments for Phase III were based on current practice with other ESAs as well as Phase II data, with adjustments according to setting and dosing schedule (see Table 14). Because of individual variability in Hb, 25% dose adjustments were recommended every 4 weeks. Hb levels were kept within ± 1 g/dL of the individual patient's baseline and within the range of 10 to 13.5 g/dL throughout the dose titration/evaluation period. In all studies, dose interruption was recommended when Hb concentrations exceeded 14 g/dL. Hb was assessed weekly in the clinical studies.

Table 14: Starting Doses in Maintenance Studies

Study	Previous Drug & Dose	Starting RO0503821 Dose and Regimen	Route
BA16739	< 8000 IU/wk epo	30 µg/wk as: 60 µg/2 wks or 120 µg /4 wks	IV
	8000 – 16000 IU/wk epo	50 µg/wk as: 100 µg/2 wks or 200 µg/4 wks	IV
	> 16000 IU/wk epo	90 µg/wk as: 180 µg/2 wks or 360 µg/4 wks	IV
Patients in the reference arm continued their IV epoetin alfa or beta dose at the same route, dose, and dosing interval (1, 2, or 3x/week).			
BA16740	< 8000 IU/wk epo	30 µg/wk as: 60 µg/2 wks or 120 µg/4 wks	SC
	8000 – 16000 IU/wk epo	50 µg/wk as: 100 µg/2 wks or 200 µg/4 wks	SC
	> 16000 IU/wk epo	90 µg/wk as: 180 µg/2 wks or 360 µg/4 wks	SC
Patients in the reference arm continued their SC epoetin alfa or beta dose at the same route, dose, and dosing interval (1, 2, or 3x/week).			
BA17283	< 40 µg/wk darbepo	30 µg/wk as: 60 µg/2 wks	IV
	< 80 µg/2 wks darbepo	30 µg/wk as: 60 µg/2 wks	
	40 – 80 µg/wk darbepo	50 µg/wk as: 100 µg/2 wks	IV
	80 – 160 µg/2 wks darbepo	50 µg/wk as: 100 µg/2 wks	
	> 80 µg/wk darbepo	90 µg/wk as: 180 µg/2 wks	IV
	> 160 µg/2 wks darbepo	90 µg/wk as: 180 µg/2 wks	
Patients in the reference arm continued their IV darbepoetin alfa dose at the same route, dose, and dosing interval (1x/wk or 1x/2 wks).			
BA17284	< 8000 IU/wk epo	30 µg/wk as: 60 µg/2 wks	IV or SC prefilled syringes
	8000 – 16000 IU/wk epo	50 µg/wk as: 100 µg/2 wks	IV or SC prefilled syringes
	> 16000 IU/wk epo	90 µg/wk as: 180 µg/2 wks	IV or SC prefilled syringes
Patients in the reference arm continued their SC or IV epoetin alfa or beta dose at the same route, dose, and dosing interval (1, 2, or 3x/week).			

2. Iron Supplementation

Most patients received iron supplementation according to centre practice and their individual study protocols. In study BA16736, iron was given IV during the entire study period. In study BA16738, supplemental iron was administered during the screening period and during all treatment periods when serum ferritin < 100 µg/L, TSAT < 20% or percentage of hypochromic RBCs ≥ 10%. Maintenance studies were to initiate or intensify iron supplementation in case of iron deficiency during the study. In all Phase III studies, in order to avoid iron toxicity, iron supplementation was to be temporarily discontinued in patients with serum ferritin > 800 µg/L or TSAT > 50% until serum ferritin ≤ 800 µg/L and TSAT ≤ 50%.

Outcomes/endpoints

The primary efficacy parameter in correction studies was the Hb response rate. This was defined as an increase in Hb ≥ 1.0g/dL from baseline and a Hb concentration ≥ 11g/dL without RBC transfusion during the correction period in study BA16736 (the first 24 weeks after first dose, through week 25), and during the correction and evaluation periods in study BA16738 (the first 28 weeks after first dose, through week 29). The average baseline Hb value was calculated using all values recorded between the first study day (before dose was given) and the previous 20 days. Study BA16738 had as second primary efficacy parameter - the change in Hb concentration between the baseline and evaluation periods. Secondary endpoints for correction included Hb values and their changes from baseline over time, time to target Hb response, and the proportion of patients requiring transfusions.

The primary efficacy parameter for the Phase III maintenance studies was the change in Hb concentration (g/dL) between the baseline and evaluation period (an 8-week period following a 28-week dose-titration period). The baseline period was defined as all assessments between the first study day and the previous 30 days (including Hb assessments on the day of first dose). During the baseline period when more than one Hb measurement was taken, a time-adjusted average baseline Hb value was calculated. The average Hb level for each individual during the evaluation period was estimated using the same method as for baseline and subtracting the baseline value from the evaluation period value gave the final endpoint. Secondary endpoints included the number of patients maintaining

average Hb concentration during the evaluation period within ±1 g/dL of their average baseline Hb concentration and the proportion of patients requiring RBC transfusions.

Statistical methods and sample size

Studies BA16736 and BA16738 were intended to demonstrate that the response rate was at least 60% in the RO0503821 group. A two-sided 95% confidence interval (CI) based on the exact method of Clopper and Pearson was calculated: a lower limit (of the CI) above 60% would allow the conclusion that RO0503821 administered every 2 weeks would result in the correction of anaemia. For the purposes of the sample size calculation, it was assumed that the observed response rate would be at least 75%. The reference group was included in study BA16736 only to show that the results observed in the RO0503821 group were comparable to those seen with an approved compound (IV epoetin alfa or beta) but no formal comparison was done. In addition, BA16738 and the rest of maintenance studies intended to demonstrate that the RO0503821 group was non-inferior to the reference group, assuming a non-inferiority limit of –0.75 g/dl, a power of 90%, and a significance level of 5%. A 95% confidence level for single comparison (studies BA16736, BA16738, BA17283 and BA17284) or 97.5% (studies BA16739 and BA16740) were used for in order to adjust for the multiplicity resulting from the independent comparisons of two RO0503821 groups with one reference group.

Analysis Population

Three different patient populations were defined for the assessment of efficacy: intent-to-treat (ITT), eligible (E), and per protocol (PP) populations. The ITT population was defined for all studies as all patients randomised. The E population was a subset of the ITT population, excluding patients with pre-defined study entry criteria violations likely to impact efficacy. The PP population was a subset of the E population excluding patients with selected, pre-specified, on-study deviations likely to impact outcome or its assessment, i.e., inadequate iron status, blood transfusion for blood loss, fewer than five Hb assessments or missing drug administrations during the evaluation period.

The ITT population was the primary analysis population in the correction studies. In the maintenance studies, which had the primary aim of assessing non-inferiority of treatment in patients converting from an approved ESA to RO0503821, the PP population was the primary analysis population. The safety population, defined as all patients who had received at least one dose of study medication and a safety follow-up, whether withdrawn prematurely or not, was used to present summaries of RBC transfusions and dose changes.

Efficacy data missing at the end of the evaluation period were handled using the last observation carried forward method in ITT and E analyses. Values missing between recorded assessments were automatically interpolated by the trapezoidal rule. If a blood transfusion occurred during the evaluation period, the Hb values measured in the following three weeks were replaced by the Hb value measured immediately before the transfusion to correct for the increase caused by the transfusion.

RESULTS

A) CORRECTION STUDIES

Recruitment

A total of 506 patients were enrolled into the Phase III correction studies, of which 505 were randomised. One patient in study BA16736 was not randomised, but participated in the epoetin arm of the study. The data from this patient are excluded from the efficacy analyses. One patient in study BA16738 was randomized into the RO0503821 group but was withdrawn before receiving the first dose of study medication. The data from this patient were, nevertheless, included in the efficacy analyses. The number of patients entering and completing the correction period of studies BA16736 and BA16738 is presented in Table 15.

Table 15: Disposition of Patients in the Phase III Correction Studies

Correction Studies	Randomized	Completed the Correction Period
BA16736		
RO0503821 1x/2 wk IV	135	124
Reference (epoetin alfa or beta)	46	41*
BA16738		
RO0503821 1x/2 wk SC	162	145
Reference (darbepoetin alfa)	162	153

* Includes the un-randomized patient

Approximately 59% (297/506) of the enrolled patients were randomized to receive RO0503821 and 41% (208/506) to receive reference medication. Patients in the reference arm of study BA16736 received epoetin alfa or beta administered 1x/week to 3x/week (n= 46). Patients in the reference arm of study BA16738 received darbepoetin alfa administered 1x/week to 1x/2 weeks (n= 162). RO0503821 was administered 1x/2 weeks to 297 patients.

Baseline data

Treatment groups within each of the studies were generally well balanced with respect to demographic characteristics. Baseline demographic and disease characteristics are presented in Tables 16 and 17.

Table 16: Demographic Data of Phase III Correction Studies (ITT Population)

	BA16736		***BA16738***	
	RO0503821 1*/2 weeks (N =135)	Reference (N =46)	RO0503821 1*/2 weeks (N =162)	Reference (N =162)
Gender				
Female	53 (39.3%)	14 (30.4%)	92 (56.8%)	82 (50.6%)
Male	82 (60.7%)	32 (69.6%)	70 (43.2%)	80 (49.4%)
Race				
Black	17 (12.6%)	7 (15.2%)	35 (21.6%)	19 (11.7%)
Caucasian	106 (78.5%)	33 (71.7%)	113 (69.8%)	131 (80.9%)
Oriental	8 (5.9%)	3 (6.5%)	7 (4.3%)	9 (5.6%)
Other	4 (3.0%)	3 (6.5%)	7 (4.3%)	3 (1.9%)
Age [Years]				
N	135	46	162	162
Mean	54.7	53.4	63.9	66.9
Std. Dev.	14.43	15.19	14.11	12.80
Median	54.0	54.5	67.0	69.0
Min-Max	18 - 89	18 - 80	20 - 90	26 - 89
Age Category [Years]				
<65	99 (73.3%)	35 (76.1%)	70 (43.2%)	62 (38.3%)
65-74	23 (17.0%)	6 (13.0%)	44 (27.2%)	48 (29.6%)
>=75	13 (9.6%)	5 (10.9%)	48 (29.6%)	52 (32.1%)
Body Weight [kg]				
Mean	67.9	73.9	76.8	80.5
Std. Dev.	14.10	15.62	16.22	19.48
Median	66.0	71.9	74.5	75.8
Min-Max	43 -106	42 -105	45 -130	42 -159
Body Weight Category [kg]				
<65	65 (48.1%)	16 (34.8%)	35 (21.6%)	35 (21.6%)
65-<80	41 (30.4%)	13 (28.3%)	65 (40.1%)	57 (35.2%)
>=80	29 (21.5%)	17 (37.0%)	62 (38.3%)	70 (43.2%)

Table 17: Disease and Treatment Status at Baseline in the Phase III Correction Studies (ITT Population)

	BA16736		*BA16738*	
	RO0503821 1*/2 weeks (N =135)	Reference (N =46)	RO0503821 1*/2 weeks (N =162)	Reference (N =162)
Primary Cause(s) of Renal Failure				
Diabetes	28 (20.7%)	13 (28.3%)	73 (45.1%)	79 (48.8%)
Glomerulonephritis	33 (24.4%)	13 (28.3%)	15 (9.3%)	19 (11.7%)
Secondary Glomerulonephritis / Vasculitis	5 (3.7%)	1 (2.2%)	2 (1.2%)	3 (1.9%)
Interstitial nephritis / Pyelonephritis	12 (8.9%)	5 (10.9%)	9 (5.6%)	10 (6.2%)
Hypertension / Large vessel disease	28 (20.7%)	12 (26.1%)	65 (40.1%)	72 (44.4%)
Polycistic kidney disease/ (adult type, dominant)	16 (11.9%)	1 (2.2%)	7 (4.3%)	9 (5.6%)
Other hereditary / Congenital diseases	4 (3.0%)		2 (1.2%)	2 (1.2%)
Neoplasms / Tumors				1 (0.6%)
Other	5 (3.7%)	1 (2.2%)	14 (8.6%)	11 (6.8%)
Undefined etiology	17 (12.6%)	5 (10.9%)	12 (7.4%)	5 (3.1%)
Study Day of First Dialysis				
N	135	46		
Mean	-775	-760		
Std. Dev.	1412.6	1415.9		
Median	-166	-113		
Min-Max	-8359 - 11	-7716 - -19		
Mode of Current Dialysis				
Hemodialysis	132 (97.8%)	46 (100.0%)		
Peritoneal Dialysis	3 (2.2%)			
Not yet on Dialysis			162 (100.0%)	162 (100.0%)
Vascular Access Type (Hemodialysis, n)				
Arteriovenous fistula	106 (80.3%)	35 (76.1%)		
Arteriovenous graft	9 (6.8%)	4 (8.7%)		
Indwelling (tunneled) catheter	17 (12.9%)	7 (15.2%)		
Peritoneal Dialysis Type (n				
CAPD	3 (100.0%)			

Most patients received iron supplementation according to centre practice and their individual study protocols. The most commonly used iron supplements in both studies were iron sucrose and ferrous sulphate. Use of these supplements was well balanced between treatment groups within each study. There were no clinically relevant differences in iron parameters or Hb concentrations between treatment groups within any of the studies. Mean Hb at baseline was approximately 9.4 g/dL in each of the BA16736 treatment groups and 10.2 g/dL in each of the BA16738 treatment groups.

Numbers analysed

The percentage of patients in the PP population was 77% to 89% in each study. The percentage of patients in study BA16736 who were included in the PP population was not well balanced between treatment groups. This was primarily due to a lower percentage of patients withdrawn for missing administrations before week 24 in the RO0503821 group (2%) compared with reference group (11%).

Outcomes and estimation

Primary variable:

A summary of the responders in the two correction studies is shown in Table 18. In study BA16736A a total of 181 patients were randomised and 165 completed the correction period. In study BA16738 324 patients were randomised and 298 completed the correction/evaluation period. The lower level of the CI interval was well above 60% with statistically significant p-values ($p < 0.0001$), confirming that RO0503821 resulted in the correction of anaemia.

Table 18: Summary of Responders in the Phase III Correction Studies (ITT Population)

Study	Treatment	Total Number	Number of Responder	Response Rate (%)	Clopper-Pearson 95% CI Lower Limit	Clopper-Pearson 95% CI Upper Limit	p-value*
BA16736	RO0503821 1*/2 weeks	135	126	93.33	87.72	96.91	<.0001
	Reference	46	42	91.30	79.21	97.58	<.0001
BA16738	RO0503821 1*/2 weeks	162	158	97.53	93.80	99.32	<.0001
	Reference	162	156	96.30	92.11	98.63	<.0001

Study BA16738 had as second primary efficacy parameter the change in average Hb concentration between baseline and the evaluation periods. An analysis of covariance (ANCOVA) comparing the RO0503821 dosing regimen to the darbepoetin alfa group showed that the adjusted mean change from baseline Hb in the RO0503821 group (2.12 g/dL) was non-inferior to that of the darbepoetin alfa group (1.95 g/dL). The lower limit of the CI was greater than -0.75, with p-values < 0.0001, supporting the non-inferiority of the RO0503821 treatment group to the darbepoetin alfa group. Overall, results for the PP and eligible populations were similar to those in the ITT population.

Secondary Efficacy Results:

Haemoglobin levels

In both studies, median Hb concentrations reached levels ≥ 11 g/dL during the correction period in all treatment groups. The increase in Hb concentrations was more gradual in the RO0503821 treatment arms showing a longer time to reach the target concentration.

The proportion of patients with maximum Hb values > 13 g/dL during correction period was evaluated in both studies. A summary showing is provided in Table 19. The percentage of patients reaching maximum Hb values >13 g/dL was higher during the first 4 months in the reference group compared with the RO0503821 group in both studies. A similar pattern was seen in the proportion of patients with maximum Hb values > 14 g/dL by month.

Table 19: Summary of Hb Values > 13 g/dL by Month in the Phase III Correction Studies (ITT Population)

		Patients with max Hb value			
		<=13 g/dL		> 13 g/dL	
Treatment	Month	N	%	N	%
RO0503821 1*/2 weeks **BA16736**	1	133	98.52	2	1.48
	2	124	92.54	10	7.46
	3	106	80.30	26	19.70
	4	85	65.89	44	34.11
	5	72	57.60	53	42.40
	6	75	60.48	49	39.52
Epoetin Reference **BA16736**	1	45	97.83	1	2.17
	2	37	82.22	8	17.78
	3	31	68.89	14	31.11
	4	28	65.12	15	34.88
	5	26	61.90	16	38.10
	6	34	82.93	7	17.07

		Patients with max Hb value			
		<=13 g/dL		> 13 g/dL	
Treatment	Month	N	%	N	%
RO0503821 1*/2 weeks **BA16738**	1	158	98.14	3	1.86
	2	140	88.61	18	11.39
	3	108	68.35	50	31.65
	4	81	52.60	73	47.40
	5	81	52.94	72	47.06
	6	97	63.82	55	36.18
	7	111	75.51	36	24.49
Darbepoetin Reference **BA16738**	1	149	92.55	12	7.45
	2	105	66.04	54	33.96
	3	71	44.94	87	55.06
	4	73	46.20	85	53.80
	5	86	54.43	72	45.57
	6	113	71.97	44	28.03
	7	129	83.77	25	16.23

Other secondary parameters: time to target Hb response, RBC transfusions, dose changes
In study BA16736 the median time to reach the Hb target concentration was 57 days in the RO0503821 group and 31 days in the epoetin group. In study BA16738 the median time to response was 43 days in the RO0503821 group compared with 29 days in the darbepoetin alfa group. In both studies, the difference was statistically significant ($p < 0.0001$). With regard to RBC transfusions, the percentage of patients in the safety population who required blood transfusions during the correction period was 5% of patients in the RO0503821 group vs. 4% of patients in the reference group in study BA16736, and 3% of patients in the RO0503821 group vs. 7% of patients in the reference group in study BA16738. The majority of patients in both studies in all treatment arms had dose adjustments. The median number of dose changes per patient throughout the entire study period in the RO0503821 1x/2 weeks groups for both correction studies was between 3 and 5, with the majority of patients having between 1 and 5 and 3 and 7 dose changes (interquartile ranges). The number of median dose changes was slightly higher in the darbepoetin/epoetin groups.

Comparison of Results in Subpopulations
The demographic characteristics of age, weight, gender, and race did not affect the response rates in the reference or RO0503821 treatment groups. Responder analyses of CKD aetiology and co-morbid diabetes subgroups showed no evidence of drug-disease interactions.

B) MAINTENANCE STUDIES

Recruitment

A total of 1894 patients were randomised into the Phase III maintenance studies. Overall, approximately 61% (1153/1894) of the enrolled patients were randomised to receive RO0503821, and 39% (741/1894) were randomised to receive a reference medication. A slightly higher percentage of patients received RO0503821 than reference drug because studies BA16739 and BA16740 were randomised 1:1:1 (RO0503821 1x/2 weeks: RO0503821 1x/4 weeks: reference drug). The other 2 studies were randomised in a 1:1 ratio (RO0503821: reference drug). The number of patients to enter and complete the studies is presented in Table 20.

Table 20: Disposition of Patients in the Phase III Maintenance Studies

Maintenance Studies	Randomized	Completed the Titration Period	Completed the Evaluation Period	Completed the Safety Follow-up
BA16739				
RO0503821 1x/2 wk	223	197	190	169
RO0503821 1x/4 wk	224	188	183	168
Reference	226	205	199	180
BA16740				
RO0503821 1x/2 wk	190	164	161	154
RO0503821 1x/4 wk	191	170	166	148
Reference	191	181	175	159
BA17283				
RO0503821 1x/2 wk	157	139	130	118
Reference	156	143	136	131
BA17284				
RO0503821 1x/2 wk	168	146	132	N/A
Reference	168	160	150	N/A

Baseline data

Demographic characteristics were generally similar among treatment groups within each of the studies. The geographic distribution of the study sites had a large impact on demographic characteristics such as race distribution, disease aetiology, and dialysis type. However, differences in baseline characteristics did not significantly impact the efficacy of study medication, as shown in the subgroup analyses. Baseline demographic and disease characteristics are presented in Tables 21 and 22 (see below).

The majority of patients received previous or previous/concomitant iron treatment at baseline; this percentage was similar among the treatment groups within the following studies: 77% to 81% in study BA16739, 81% to 84% in study BA16740, and 83% to 85% in study BA17283. In study BA17284, 72% of patients in the RO0503821 group and 63% of patients in the reference group received previous or previous/concomitant iron at baseline. The most commonly administered iron supplements were iron sucrose (in 31% to 49% of patients in each treatment group across the maintenance studies) and ferrous gluconate (in 20% to 39% of patients in each treatment group across the maintenance studies).

There were no clinically relevant differences in iron parameters or Hb concentrations between treatment groups within any of the Phase III studies. To enter the studies, patients were required to have mean baseline Hb concentrations between 10.5 and 13 g/dL, ferritin ≥ 100 µg/L or TSAT ≥ 20% in all Phase III studies. Mean Hb at baseline was similar in all treatment groups of the Phase III maintenance studies, 11.7 to 12.0 g/dL. Baseline median TSAT levels were within acceptable range and were comparable in all treatment groups across the studies: median values ranged from 27% to 31%, overall. Baseline median ferritin levels in each treatment group within each study were also within an acceptable range.

Table 21: Summary of Demographic Data, Phase III Maintenance Studies (ITT Population)

	BA16739			*BA16740*			*BA17283*		*BA17284*	
	RO0503821 1*/2 weeks (N =223)	RO0503821 1*/4 weeks (N =224)	Reference (N =226)	RO0503821 1*/2 weeks (N =190)	RO0503821 1*/4 weeks (N =191)	Reference (N =191)	RO0503821 1*/2 weeks (N =157)	Reference (N =156)	RO0503821 1*/2 weeks (N =168)	Reference (N =168)
Gender										
Female	90 (40.4%)	98 (43.8%)	92 (40.7%)	82 (43.2%)	74 (38.7%)	81 (42.4%)	57 (36.3%)	75 (48.1%)	64 (38.1%)	55 (32.7%)
Male	133 (59.6%)	126 (56.3%)	134 (59.3%)	108 (56.8%)	117 (61.3%)	110 (57.6%)	100 (63.7%)	81 (51.9%)	104 (61.9%)	113 (67.3%)
Race										
Black	74 (33.2%)	82 (36.6%)	82 (36.3%)	13 (6.8%)	15 (7.9%)	17 (8.9%)	5 (3.2%)	4 (2.6%)	49 (29.2%)	57 (33.9%)
Caucasian	140 (62.8%)	131 (58.5%)	133 (58.8%)	155 (81.6%)	156 (81.7%)	148 (77.5%)	143 (91.1%)	144 (92.3%)	104 (61.9%)	87 (51.8%)
Oriental	9 (4.0%)	7 (3.1%)	11 (4.9%)	15 (7.9%)	16 (8.4%)	19 (9.9%)	4 (2.5%)	5 (3.2%)	10 (6.0%)	13 (7.7%)
Other		4 (1.8%)		7 (3.7%)	4 (2.1%)	7 (3.7%)	5 (3.2%)	3 (1.9%)	5 (3.0%)	11 (6.5%)
Age [Years]										
N	223	224	226	190	191	191	157	156	168	168
Mean	59.0	59.0	58.6	60.5	62.3	60.4	62.4	61.8	59.8	60.1
Std. Dev.	15.20	14.99	15.13	15.40	15.36	14.70	16.17	14.74	14.38	13.85
Median	61.0	60.0	60.5	63.0	65.0	61.0	65.0	64.0	60.0	60.0
Min-Max	24 - 88	23 - 89	23 - 89	25 - 84	20 - 92	24 - 88	23 - 88	26 - 87	19 - 87	22 - 92
Age Category [Years]										
<65	134 (60.1%)	134 (59.8%)	141 (62.4%)	103 (54.2%)	94 (49.2%)	107 (56.0%)	76 (48.4%)	79 (50.6%)	104 (61.9%)	104 (61.9%)
65-74	47 (21.1%)	52 (23.2%)	50 (22.1%)	46 (24.2%)	49 (25.7%)	43 (22.5%)	39 (24.8%)	41 (26.3%)	37 (22.0%)	34 (20.2%)
>=75	42 (18.8%)	38 (17.0%)	35 (15.5%)	41 (21.6%)	48 (25.1%)	41 (21.5%)	42 (26.8%)	36 (23.1%)	27 (16.1%)	30 (17.9%)
Body Weight [kg]										
Mean	77.2	77.6	80.6	70.8	69.0	69.3	68.9	70.3	75.5	77.4
Std. Dev.	19.70	18.84	22.03	15.66	14.89	15.75	16.69	16.50	19.73	19.71
Median	73.7	75.4	75.7	70.1	68.5	68.2	66.9	69.0	72.0	73.4
Min-Max	36 -132	37 -132	47 -164	40 -129	38 -115	38 -123	37 -147	34 -126	38 -128	39 -135
Body Weight Category [kg]										
<65	70 (31.4%)	55 (24.6%)	52 (23.0%)	75 (39.5%)	71 (37.2%)	75 (39.3%)	70 (44.6%)	64 (41.0%)	56 (33.3%)	45 (26.8%)
65-<80	68 (30.5%)	83 (37.1%)	81 (35.8%)	74 (38.9%)	80 (41.9%)	73 (38.2%)	57 (36.3%)	56 (35.9%)	49 (29.2%)	56 (33.3%)
>=80	85 (38.1%)	86 (38.4%)	93 (41.2%)	41 (21.6%)	40 (20.9%)	43 (22.5%)	30 (19.1%)	36 (23.1%)	62 (36.9%)	66 (39.3%)
No Value									1 (0.6%)	1 (0.6%)

Table 22: Disease and Treatment Status at Baseline in the Phase III Maintenance Studies (ITT Population)

	BA16739			BA16740			BA17283		BA17284	
	RO0503821 1*/2 weeks (N =223)	RO0503821 1*/4 weeks (N =224)	Reference (N =226)	RO0503821 1*/2 weeks (N =190)	RO0503821 1*/4 weeks (N =191)	Reference (N =191)	RO0503821 1*/2 weeks (N =157)	Reference (N =156)	RO0503821 1*/2 weeks (N =168)	Reference (N =168)
Primary Cause(s) of Renal Failure										
Diabetes	91 (40.8%)	77 (34.4%)	100 (44.2%)	48 (25.3%)	44 (23.0%)	50 (26.2%)	40 (25.5%)	35 (22.4%)	60 (35.7%)	61 (36.3%)
Glomerulonephritis	37 (16.6%)	36 (16.1%)	30 (13.3%)	43 (22.6%)	42 (22.0%)	35 (18.3%)	39 (24.8%)	33 (21.2%)	39 (23.2%)	28 (16.7%)
Secondary Glomerulonephritis / Vasculitis	3 (1.3%)	6 (2.7%)	6 (2.7%)	3 (1.6%)	8 (4.2%)	7 (3.7%)	9 (5.7%)	10 (6.4%)	5 (3.0%)	2 (1.2%)
Interstitial nephritis / Pyelonephritis	13 (5.8%)	9 (4.0%)	12 (5.3%)	23 (12.1%)	29 (15.2%)	30 (15.7%)	13 (8.3%)	25 (16.0%)	7 (4.2%)	13 (7.7%)
Hypertension / Large vessel disease	99 (44.4%)	104 (46.4%)	107 (47.3%)	53 (27.9%)	52 (27.2%)	54 (28.3%)	37 (23.6%)	30 (19.2%)	73 (43.5%)	81 (48.2%)
Polycistic kidney disease/ (adult type, dominant)	8 (3.6%)	10 (4.5%)	10 (4.4%)	11 (5.8%)	8 (4.2%)	16 (8.4%)	7 (4.5%)	8 (5.1%)	7 (4.2%)	7 (4.2%)
Other hereditary / Congenital diseases	3 (1.3%)	8 (3.6%)	4 (1.8%)	5 (2.6%)		3 (1.6%)	8 (5.1%)	1 (0.6%)	5 (3.0%)	6 (3.6%)

Neoplasms / Tumors	2 (0.9%)	5 (2.2%)	5 (2.2%)	2 (1.1%)	2 (1.0%)	4 (2.1%)	2 (1.3%)	1 (0.6%)	4 (2.4%)	3 (1.8%)
Other	9 (4.0%)	12 (5.4%)	10 (4.4%)	1 (0.5%)	5 (2.6%)	1 (0.5%)	13 (8.3%)	16 (10.3%)	7 (4.2%)	7 (4.2%)
Undefined etiology	13 (5.8%)	14 (6.3%)	9 (4.0%)	24 (12.6%)	20 (10.5%)	18 (9.4%)	10 (6.4%)	14 (9.0%)	8 (4.8%)	7 (4.2%)
Study Day of First Dialysis										
N	223	224	226	190	191	191	157	156	168	168
Mean	-1651	-1777	-1620	-1529	-1616	-1629	-2067	-2207	-1769	-1757
Std. Dev.	1913.9	1886.5	1592.5	1576.5	1648.9	1625.9	2225.4	2243.3	2102.0	2050.6
Median	-1018	-1199	-1088	-1006	-1211	-1019	-1254	-1319	-1071	-1018
Min-Max	-12681 - -118	-13473 - -136	-9734 - -125	-10053 - -161	-9310 - -154	-8598 - -101	-12332 - -156	-10519 - -141	-13481 - -126	-13301 - -145
Mode of Current Dialysis										
Hemodialysis	223 (100.0%)	224 (100.0%)	226 (100.0%)	176 (92.6%)	177 (92.7%)	171 (89.5%)	157 (100.0%)	155 (99.4%)	159 (94.6%)	158 (94.0%)
Peritoneal Dialysis				14 (7.4%)	14 (7.3%)	20 (10.5%)		1 (0.6%)	9 (5.4%)	10 (6.0%)
Vascular Access Type (Hemodialysis, n)										
Arteriovenous fistula	134 (60.1%)	120 (53.6%)	135 (59.7%)	154 (87.5%)	146 (82.5%)	149 (87.1%)	132 (84.1%)	117 (75.5%)	112 (70.4%)	107 (67.7%)
Arteriovenous fistula / indwelling (tunneled) catheter	1 (0.4%)	1 (0.4%)	3 (1.3%)	1 (0.6%)		1 (0.6%)			1 (0.6%)	
Arteriovenous graft	66 (29.6%)	78 (34.8%)	60 (26.5%)	12 (6.8%)	16 (9.0%)	11 (6.4%)	18 (11.5%)	26 (16.8%)	29 (18.2%)	38 (24.1%)
Arteriovenous graft / indwelling (tunneled) catheter	1 (0.4%)		1 (0.4%)						1 (0.6%)	
Indwelling (tunneled) catheter	20 (9.0%)	25 (11.2%)	27 (11.9%)	9 (5.1%)	15 (8.5%)	10 (5.8%)	7 (4.5%)	12 (7.7%)	16 (10.1%)	13 (8.2%)
Not specified / missing	1 (0.4%)									
Vascular Access Type (Hemodialysis, n)										
Arteriovenous fistula	134 (60.1%)	120 (53.6%)	135 (59.7%)	154 (87.5%)	146 (82.5%)	149 (87.1%)	132 (84.1%)	117 (75.5%)	112 (70.4%)	107 (67.7%)
Arteriovenous fistula / indwelling (tunneled) catheter	1 (0.4%)	1 (0.4%)	3 (1.3%)	1 (0.6%)		1 (0.6%)			1 (0.6%)	
Arteriovenous graft	66 (29.6%)	78 (34.8%)	60 (26.5%)	12 (6.8%)	16 (9.0%)	11 (6.4%)	18 (11.5%)	26 (16.8%)	29 (18.2%)	38 (24.1%)
Arteriovenous graft / indwelling (tunneled) catheter	1 (0.4%)		1 (0.4%)						1 (0.6%)	
Indwelling (tunneled) catheter	20 (9.0%)	25 (11.2%)	27 (11.9%)	9 (5.1%)	15 (8.5%)	10 (5.8%)	7 (4.5%)	12 (7.7%)	16 (10.1%)	13 (8.2%)
Not specified / missing	1 (0.4%)									
Peritoneal Dialysis Type (n)										
APD								1 (100.0%)		
CAPD				6 (42.9%)	7 (50.0%)	7 (35.0%)			1 (11.1%)	3 (30.0%)
CCPD				7 (50.0%)	6 (42.9%)	10 (50.0%)			6 (66.7%)	7 (70.0%)
NIPD				1 (7.1%)	1 (7.1%)	3 (15.0%)				
Other									2 (22.2%)	

Numbers analysed

The percentages of patients included in the ITT, safety, and eligible populations in each of the studies were well balanced across treatment groups. The percentage of patients in the PP population was from 73% to 87% in every study.

In study ***BA16739***, a slightly higher percentage of patients in the RO0503821 1x/4 week group were excluded from the PP population for the following reasons:

- Less than 5 Hb values or no valid values during the evaluation period (16% compared with 12% in the RO0503821 1x/2 week group and 10% in the reference group).
- Inadequate iron status during the evaluation period, or no valid value (14% compared with 12% in the RO0503821 1x/2 week group and 10% in the reference group).

In study ***BA16740,*** a slightly higher percentage of patients in the RO0503821 1x/4 week and 1x/2 week groups were excluded from the PP population for the following reasons:

- Less than 5 Hb values or no valid values during the evaluation period (13% to 14% compared with 8% in the reference group).
- Inadequate iron status during the evaluation period, or no valid value (12% to 15% compared with 8% in the reference group).

Outcomes and estimation in maintenance studies

Primary variable

The change in Hb concentration between baseline and the evaluation period was comparable between the RO0503821 treatment group(s) and the reference group within each of the four studies, mostly close to zero (no change) (see Table 23). Differences in RO0503821 dosing regimen had no discernible effect on changes in mean Hb concentrations from baseline to the evaluation period. The lower limits of the CI were greater than -0.75, confirming the non-inferiority of RO0503821 to epoetin alfa, epoetin beta and darbepoetin alfa. All p-values were statistically significant ($p < 0.0001$). The results were consistent in the four data sets analysed (PP, ITT, eligible, and complete observations populations).

Table 23: Summary of Change in Average Haemoglobin [g/dL] Between Baseline and Evaluation Period in the Phase III Maintenance Studies (PP Population)

Study	Treatment	N	Mean	Std	Minimum	Median	Maximum
BA16739	RO0503821 1*/2 weeks	188	-0.10	1.06	-3.14	-0.07	2.67
	RO0503821 1*/4 weeks	172	0.01	0.96	-3.49	0.02	2.34
	Reference	180	-0.10	0.92	-2.64	0.00	2.14
BA16740	RO0503821 1*/2 weeks	154	-0.00	0.96	-3.16	0.06	2.53
	RO0503821 1*/4 weeks	153	-0.11	0.97	-3.43	-0.21	2.89
	Reference	167	-0.12	1.04	-4.45	0.01	2.90
BA17283	RO0503821 1*/2 weeks	123	0.05	0.96	-2.69	0.10	2.57
	Reference	126	-0.10	0.92	-2.88	-0.08	1.87
BA17284	RO0503821 1*/2 weeks	123	0.14	0.93	-1.96	-0.25	2.19
	Reference	133	-0.01	1.03	-2.89	-0.05	4.21

Secondary Efficacy Results:

Haemoglobin levels

During the evaluation period, between a 66% and 76% of patients maintained an average Hb concentrations within ±1 g/dL of their average baseline Hb concentrations. The percentages of patients who maintained Hb concentrations within this range were similar between the RO0503821 treatment

group(s) and the epoetin or darbepoetin alfa reference groups in each study. Results in the PP population were similar to those in the ITT population.

The proportion of patients with hemoglobin values above 13 g/dL and ≤ 14 g/dL and hemoglobin values above 14 g/dL has also been evaluated (see Table 24). The percentage of patients with one or more Hb values > 14 g/dL in each treatment group was highest during the titration periods. A higher percentage of patients with one or more Hb values > 14 g/dL occurred in the RO0503821 groups than in the reference groups.

Table 24: Summary of Proportion of Patients with a hemoglobin values above 13 g/dL and ≤ 14 g/dL and hemoglobin values above 14 g/dL by Study Period, (ITT Population)

		RO0503821 1*/2 weeks (N = 223)		RO0503821 1*/4 weeks (N = 224)		Reference (N = 226)	
		N	(%)	N	(%)	N	(%)
BA16739							
Titration Period	Valid Hb Value(s)	223	(100.0%)	220	(100.0%)	225	(100.0%)
	Hb Value > 13 g/dL and <= 14 g/dL	70	(31.4%)	83	(37.7%)	90	(40.0%)
	Hb Value > 14 g/dL	107	(48.0%)	47	(21.4%)	44	(19.6%)
Evaluation Period	Valid Hb Value(s)	196	(100.0%)	188	(100.0%)	205	(100.0%)
	Hb Value > 13 g/dL and <= 14 g/dL	53	(27.0%)	41	(21.8%)	45	(22.0%)
	Hb Value > 14 g/dL	21	(10.7%)	19	(10.1%)	12	(5.9%)
Safety Follow-Up Period	Valid Hb Value(s)	187	(100.0%)	181	(100.0%)	196	(100.0%)
	Hb Value > 13 g/dL and <= 14 g/dL	52	(27.8%)	47	(26.0%)	52	(26.5%)
	Hb Value > 14 g/dL	32	(17.1%)	16	(8.8%)	25	(12.8%)
BA16740							
Titration Period	Valid Hb Value(s)	190	(100.0%)	190	(100.0%)	189	(100.0%)
	Hb Value > 13 g/dL and <= 14 g/dL	58	(30.5%)	57	(30.0%)	56	(29.6%)
	Hb Value > 14 g/dL	59	(31.1%)	33	(17.4%)	29	(15.3%)
Evaluation Period	Valid Hb Value(s)	164	(100.0%)	168	(100.0%)	176	(100.0%)
	Hb Value > 13 g/dL and <= 14 g/dL	32	(19.5%)	26	(15.5%)	38	(21.6%)
	Hb Value > 14 g/dL	12	(7.3%)	7	(4.2%)	13	(7.4%)
Safety Follow-Up Period	Valid Hb Value(s)	161	(100.0%)	160	(100.0%)	172	(100.0%)
	Hb Value > 13 g/dL and <= 14 g/dL	37	(23.0%)	35	(21.9%)	25	(14.5%)
	Hb Value > 14 g/dL	15	(9.3%)	17	(10.6%)	18	(10.5%)
BA17283							
Titration Period	Valid Hb Value(s)	153	(100.0%)			155	(100.0%)
	Hb Value > 13 g/dL and <= 14 g/dL	46	(30.1%)			56	(36.1%)
	Hb Value > 14 g/dL	65	(42.5%)			28	(18.1%)
Evaluation Period	Valid Hb Value(s)	139	(100.0%)			142	(100.0%)
	Hb Value > 13 g/dL and <= 14 g/dL	37	(26.6%)			27	(19.0%)
	Hb Value > 14 g/dL	22	(15.8%)			9	(6.3%)
Safety Follow-Up Period	Valid Hb Value(s)	129	(100.0%)			134	(100.0%)
	Hb Value > 13 g/dL and <= 14 g/dL	38	(29.5%)			33	(24.6%)
	Hb Value > 14 g/dL	16	(12.4%)			10	(7.5%)
BA17284							
Titration Period	Valid Hb Value(s)	167	(100.0%)			168	(100.0%)
	Hb Value > 13 g/dL and <= 14 g/dL	57	(34.1%)			60	(35.7%)
	Hb Value > 14 g/dL	57	(34.1%)			27	(16.1%)
Evaluation Period	Valid Hb Value(s)	143	(100.0%)			158	(100.0%)
	Hb Value > 13 g/dL and <= 14 g/dL	39	(27.3%)			39	(24.7%)
	Hb Value > 14 g/dL	20	(14.0%)			12	(7.6%)

Other secondary parameters: RBC transfusions and dose changes

The percentage of patients requiring blood transfusions during the titration and evaluation periods was similar between treatment groups within each of the maintenance studies. Overall, 6% to 12% of patients in the RO0503821 groups required transfusions compared with 8% to 11% in the reference groups. A similar percentage of patients in the 1x/4 weeks RO0503821 treatment groups compared with patients in the 1x/2 weeks RO0503821 groups or the reference groups had blood transfusions: 7% compared with 10% and 8%, respectively, in study BA16739; and 11% compared with 6% and 10%, respectively, in study BA16740. Dose adjustments were required by almost all patients. In the safety population, the median number of dose changes per-patient throughout the entire study period was similar between the RO0503821 and the reference arm (5 to 8).

Comparison of Results in Subpopulations

Average Hb by treatment period as well as Hb changes from baseline to the evaluation period were analyzed in subgroups of interest, namely: age, weight, gender, race, ethnicity, geographic region, etiology of CKD, diabetes status, previous ESA, iron status, dialysis type, and route of drug administration. Overall, the demographic characteristics did not affect the change in average Hb between baseline and the evaluation period in the reference or RO0503821 treatment groups.

- Clinical studies in special populations

No specific studies in special population have been submitted

- Supportive studies

Two long-term, open-labelled, multicentre studies including patients from the Phase II and Phase III are ongoing. Both studies are being performed in order to collect long-term safety follow-up information for 2 years.

- Discussion on clinical efficacy

Six Phase III pivotal studies support the efficacy of RO0503821 in anemia associated with chronic kidney disease (CKD). All studies were randomized, open-label, multimember, comparative active-controlled and parallel group studies. Blinding would have been desirable due to the existence of concurrent decisions (transfusion, iron supplements, dose adjustments…), which could confound the efficacy assessment. The decision of not blinding the study was based on ethical considerations (need for placebo injections) together with the fact that the Hb response is an objective parameter. Deviations from the protocol were observed equally in all treatment groups and no effect was documented in the Phase III studies.

Response rates in Hb levels were good in the RO0503821 group at the end of the correction period and comparable to reference treatments. However, in CKD patients not currently treated with an ESA the increase in Hb concentrations was slower in the RO0503821 group than reference treatments. The median time to reach target Hb was 57 and 43 days after RO0503821 treatment against to 31 and 29 days after epoetin and darbepoetin treatment. Even though these data are not relevant for the management of this clinical situation, this information is considered useful for the prescribers therefore and has been adequately incorporated in the SPC section 5.1.

Dose adjustments are current practice with other ESAs and they were considered acceptable. Taking into account all available safety information in the clinical program and the recently published studies including other ESAs (CHOIR (3) and CREATE (4)) which have shown an increased risk of death and other serious CV events associated to the target of Hb levels grater than 12-13 g/dL, the upper limit of Hb has been lowered to 12 g/dL instead of 14 g/dL (as originally proposed). This is also relevant for patients who received RO0503821, some of whom showed higher Hb levels than reference treatment. In correction studies BA16736 and BA16738, the proportion of patients with Hb values >13 g/dL at month 6 was 39.5% and 36.1 for RO0503821 vs. 17.0% and 28.0% for control treatments. In maintenance studies a greater proportion of patients who received RO0503821 (1/2week schedule) had Hb values >14 g/dL during evaluation period than those treated with comparator: 10.7% vs. 5.9% (BA16739), 15.8% vs. 6.3% (BA17283) and 34.1 vs. 16.1% (BA17284). Hb target levels of 12 g/dL are stated in section 4.2 of the SPC, both for correction and maintenance settings, and the dose adjustment rules have been modified accordingly.

In the maintenance studies phase, the regimen of RO0503821 1/2weeks has not shown any additional benefit over the 1/4weeks in terms of efficacy. Section 4.2 of the SPC indicates that RO0503821 should be administered monthly for patients currently treated with an ESA.

Clinical safety

Safety data from 28 clinical trials performed with RO0503821 SC and IV during the clinical development were collected. The overall safety population was pooled from the four dose-finding Phase II and six pivotal Phase III studies. Supportive safety data from 13 clinical pharmacology studies, one Phase I/II and two Phase II studies in oncology patients, two Phase IIIb extension studies in CKD patients on dialysis and not on dialysis, and 13 studies included in a Japanese development program, were also included. These safety data were not pooled with the data from the therapeutic Phase II and Phase III trials in CKD patients due to the different nature of these studies.

- Patient exposure

A total of 2737 CKD patients were included in the overall safety population; of these 1789 received treatment with RO0503821 and 948 a reference comparator. Most of the patients in each treatment group participated in maintenance studies (76% and 78%, respectively). Also, the majority of patients were on haemodialysis (2254 patients; 82%); a total of 95 patients were on peritoneal dialysis and 388 (14%) were not on dialysis. A summary of the safety population and the overall extent of exposure are shown in Tables 25 and 26.

Table 25: Overview of Safety Population (Overall Safety Population)

	RO0503821 (N = 1789)	Reference (N = 948)
Study Design		
Correction	422 (23.6%)	208 (21.9%)
Maintenance	1367 (76.4%)	740 (78.1%)
Route of Study Drug Administration		
IV	930 (52.0%)	541 (57.1%)
SC	859 (48.0%)	407 (42.9%)
Mode of Current Dialysis		
Hemodialysis	1499 (83.8%)	755 (79.6%)
Peritoneal Dialysis	64 (3.6%)	31 (3.3%)
Not yet on Dialysis	226 (12.6%)	162 (17.1%)
Schedule of Study Drug Administration		
<=1*/ week	136 (7.6%)	923 (97.4%)
1*/2 weeks	1112 (62.2%)	25 (2.6%)
1*/3 weeks	88 (4.9%)	
1*/4 weeks	453 (25.3%)	

Table 26: Summary of Equivalent Weekly Dose of Trial Medication (Overall Safety Population)

Treatment	N	Mean	Std	Median	Maximum
RO0503821 (ug/Week)					
6 Months (day 176-182)	1422	38.53	40.81	30.00	
12 Months (day 358-364)	1011	43.39	66.98	30.00	1282.25
18 Months (day 540-546)	108	36.55	41.27	21.73	250.00
24 Months (day 722-728)	95	32.70	38.37	20.89	208.35
29 Months (day 876-882)	35	45.32	58.18	23.50	268.00
Epoetin Reference IU/Week)					
6 Months (day 176-182)	584	10846.5	12597.8	7500.0	150000.0
12 Months (day 358-364)	356	11018.5	10487.3	8000.0	66000.0
Darbepoetin Reference (ug/Week)					
6 Months (day 176-182)	300	28.41	29.07	20.00	212.86
12 Months (day 358-364)	157	39.50	44.82	30.00	280.00

- Adverse events

The overall AE profile was similar between RO0503821 and reference groups (see Table 27). The average number of AEs per patient was approximately 5 AEs per patient in each group. A higher proportion of patients withdrew from treatment in the RO0503821 group than in the reference group. The difference was mainly attributable to withdrawals for non-safety reasons.

Table 27: Adverse Event Experience (Phase II/III Safety Population)

	RO0503821 (N = 1789)		Reference (N = 948)	
Adverse Events				
Any AE	1589	(88.8%)	862	(90.9%)
Serious AEs	660	(36.9%)	383	(40.4%)
Severe AEs	563	(31.5%)	301	(31.8%)
AEs leading to withdrawal	45	(2.5%)	17	(1.8%)
AEs related to TT	108	(6.0%)	33	(3.5%)
Serious AEs related to TT	16	(0.9%)	8	(0.8%)
Severe AEs related to TT	21	(1.2%)	10	(1.1%)
Withdrawals and Patient Deaths				
Withdrawals incl. Deaths	399	(22.3%)	146	(15.4%)
Deaths	126	(7.0%)	58	(6.1%)

Multiple occurrences of the same adverse event in one individual counted only once.
CRTN/Pt. No. 30329/1104 (Phase II BA16286) died on study day 116, but is not identified as premature withdrawal by investigator.
TT = trial treatment

The most common AEs in both groups included hypertension, diarrhoea, headache, and upper respiratory tract; hypertension being the most frequent treatment-related AE with 1% of patients in both treatment groups (see Table *28*). Adverse events that occurred in at least 2% of patients and at a higher frequency in the RO0503821 group compared with the reference group were procedural hypotension (8.2% vs. 5.6%), gastrointestinal haemorrhage (2.0% vs. 0.7%), and tachycardia (2.1% vs. 1.0%).

The majority of AEs reported were mild or moderate in intensity. Severe AEs (including life-threatening AEs) occurred with similar frequency in the RO0503821 (31.5%) and reference groups (31.8%). All individual AEs judged to be severe by the investigator occurred at a frequency of ≤ 1% in each group, with the exception of myocardial infarction, which occurred at a frequency of 1.6% and 1.5% in the RO0503821 and reference groups, respectively.

The distribution of AEs across most body systems was similar between treatment groups. The most commonly affected body systems were infections and infestations (51% RO0503821, 54% reference); injury, poisoning and procedural complications (40% both RO0503821 and reference groups), and gastrointestinal disorders (37% both groups). Although the overall incidence was low, in several body systems it was slightly higher in the RO0503821 group than in the reference group. These included: vascular disorders (2% vs. 1%), general disorders and administration site conditions (1.2% vs. 0.1%), blood and lymphatic system disorders (0.6% vs. 0.1%), and skin disorders (0.5% vs. 0.1%). Related cardiac disorders were reported by four patients in the RO0503821 group and none in the reference group.

Table 28: Summary of Most Frequent Adverse Events (≥ 5%) (Overall Safety Population)

Adverse Event	RO0503821 N = 1789 No. (%)	Reference N = 948 No. (%)
HYPERTENSION	239 (13)	131 (14)
DIARRHOEA	189 (11)	106 (11)
NASOPHARYNGITIS	194 (11)	93 (10)
HEADACHE	167 (9)	85 (9)
UPPER RESPIRATORY TRACT INFECTION	154 (9)	76 (8)
MUSCLE SPASMS	135 (8)	70 (7)
PROCEDURAL HYPOTENSION	147 (8)	53 (6)
FLUID OVERLOAD	120 (7)	62 (7)
COUGH	110 (6)	51 (5)
VOMITING	98 (5)	60 (6)
URINARY TRACT INFECTION	93 (5)	55 (6)
BACK PAIN	100 (6)	47 (5)
PAIN IN EXTREMITY	92 (5)	55 (6)
ARTERIOVENOUS FISTULA THROMBOSIS	89 (5)	50 (5)
HYPOTENSION	96 (5)	38 (4)
CONSTIPATION	80 (4)	50 (5)
ARTERIOVENOUS FISTULA SITE COMPLICATION	81 (5)	48 (5)

ARTERIOVENOUS GRAFT	79 (4)	49 (5)
THROMBOSIS OEDEMA PERIPHERAL	48 (3)	48 (5)

An update pooled safety data from the completed Phase II and Phase III studies and safety data for the 1302 patients participating in the ongoing extension studies BH18387 and ML19382 were provided up to a clinical **cut-off of September 1, 2006** (referred to as September 1 extended population). The overall number of patients in the September 1 extended population is the same however, the extent of exposure has increased because of the longer treatment duration in the ongoing extension studies (see Table 29).The incidence of related SAEs in the September 1 extended population was similar between groups (1.1% RO0503821; 1.2% reference).

Table 29: Overview of RO0503821 Safety as of September 1, 2006

	MAA Population		September 1 Extended Population	
	MIRCERA (N=1789) No. (%)	Reference (N=948) No. (%)	MIRCERA (N=1789) No. (%)	Reference (N=948) No. (%)
Patient Exposure Years (PEYs)	1531.98	777.98	2400.31	1320.38
All Adverse Events (AEs)	1589 (89)	862 (91)	1646 (92)	902 (95)
Severe AEs	563 (31)	301 (32)	771 (43)	440 (46)
Related AEs	108 (6)	33 (3)	128 (7)	49 (5)
Serious AEs (SAEs)	660 (37)	383 (40)	836 (47)	509 (54)
AEs leading to withdrawal	45 (3)	17 (2)	53 (3)	23 (2)

- Serious adverse event/deaths/other significant events

In the overall safety population, the frequency of serious adverse (fatal and non-fatal) events (SAEs) in the RO0503821 group was 37% compared with 40% in the reference group (see Table 30). The most frequently reported SAEs, (>1.5% in either treatment group) were those expected in a CKD population and included pneumonia, sepsis, MI, congestive heart failure (CHF), arteriovenous fistula thrombosis, and fluid overload. These occurred with similar frequencies in the two treatment groups, it did not differ by ≥ 2%. The majority of SAEs were considered unrelated to study treatment and there were no events associated with high Hb levels in both treatment groups. The percentage of patients with SAEs that were considered by the investigator as related to study treatment was similar between groups (16 patients, 0.9% RO0503821; 8 patients, 0.8% reference).

The only serious AE that occurred more frequently in the RO0503821 group compared with the reference group was **GI haemorrhage** and it is associated with low Hb concentrations. When all serious hemorrhagic events were analysed together, the frequency was similar between the RO0503821 group (5%) and the reference group (4%). Among the different haemorrhage categories upper GI haemorrhage was reported more often in the RO0503821 group than in the reference group (2.1% vs. 0.9%).

Related SAEs in the nervous system occurred more frequently in the RO0503821 group (6 patients) than in the reference group (1 patient). In the September 1, 2006 extended population the number of patients who experienced related SAEs in the nervous system was maintained slightly higher in RO0503821 group: 7 patients (0.4%) vs. 2 patients (0.2%). However, all related SAEs in the nervous system occurred in single patients, and these events, cerebrovascular in nature, are not uncommon for a CKD population. All other related SAEs occurred in ≤ 1 patient per group. There were two reports in patients, one in each treatment arm, of treatment-related SAE of hypersensitivity reactions. No anti-erythropoietin or anti-RO0503821 antibodies were detected in any of the cases.

Table 30: Most Frequent (≥ 0.5% in either Treatment Group) Serious Adverse Events (Overall Safety Population)

Body System/ Adverse Event	RO0503821 N = 1789 No. (%)	Reference N = 948 No. (%)
ALL BODY SYSTEMS		
Total Pts with at Least one AE	660 (37)	383 (40)
Total Number of AEs	1280	772
INFECTIONS AND INFESTATIONS		
PNEUMONIA	43 (2.4)	29 (3.1)
SEPSIS	25 (1.4)	16 (1.7)
CELLULITIS	17 (1.0)	10 (1.1)
GANGRENE	9 (0.5)	10 (1.1)
URINARY TRACT INFECTION	9 (0.5)	6 (0.6)
GASTROENTERITIS	9 (0.5)	3 (0.3)
STAPHYLOCOCCAL BACTERAEMIA	7 (0.4)	5 (0.5)
ARTERIOVENOUS GRAFT SITE INFECTION	6 (0.3)	5 (0.5)
BACTERAEMIA	3 (0.2)	6 (0.6)
STAPHYLOCOCCAL INFECTION	1 (0.1)	5 (0.5)
CARDIAC DISORDERS		
MYOCARDIAL INFARCTION	30 (1.7)	18 (1.9)
CARDIAC FAILURE CONGESTIVE	25 (1.4)	16 (1.7)
CARDIAC ARREST	22 (1.2)	14 (1.5)
ATRIAL FIBRILLATION	17 (1.0)	4 (0.4)
ANGINA PECTORIS	13 (0.7)	7 (0.7)
ACUTE MYOCARDIAL INFARCTION	14 (0.8)	4 (0.4)
CARDIO-RESPIRATORY ARREST	11 (0.6)	5 (0.5)
CORONARY ARTERY DISEASE	8 (0.4)	8 (0.8)
ACUTE CORONARY SYNDROME	4 (0.2)	6 (0.6)
ANGINA UNSTABLE	9 (0.5)	1 (0.1)
INJURY, POISONING AND PROCEDURAL COMPLICATIONS		
ARTERIOVENOUS FISTULA THROMBOSIS	23 (1.3)	16 (1.7)
ARTERIOVENOUS GRAFT THROMBOSIS	15 (0.8)	14 (1.5)
ARTERIOVENOUS FISTULA SITE HAEMORRHAGE	5 (0.3)	5 (0.5)
HIP FRACTURE	4 (0.2)	5 (0.5)
GASTROINTESTINAL DISORDERS		
GASTROINTESTINAL HAEMORRHAGE	21 (1.2)	2 (0.2)

Deaths

In the overall safety population, the proportion of patients who died was 7% (n=126) in the RO0503821 group and 6% (n=58) in the reference group. When the long-term extensions of the Phase II studies were excluded (because the period of observation and risk of death was prolonged for up to two years and there was no comparator for this time), the incidence of death was similar: 102 (5.7%) in the RO0503821 group vs. 58 (6.1%) in the reference group). In relation to deaths in the phase II long-term extensions, of the 166 patients who continued treatment in these extensions, 24 (14%) died, with the causes of death similar to those seen in the overall population (excluding the extensions).

The causes of death were varied and none of the events was uncommon for a CKD population. The most common cause was cardiac arrest (0.8% of patients in each group) followed by myocardial infarction (MI), cardiorespiratory arrest, chronic renal failure, and sepsis/septic shock. Two deaths were considered related to study treatment, one patient died of cardiac arrest and another died of sepsis. Both patients had significant underlying risk factors for vascular events and haemorrhage and Hb values prior to death were within the target range. All other deaths were considered by the investigators to be unrelated to treatment.

Eight fatal events (‘sudden death’) occurred in the RO0503821 group and none in the reference arm. All eight cases had significant underlying risk factors for vascular events and haemorrhage. There was no trend with respect to Hb values preceding the events (three events were associated with Hb decreases and five with Hb increases). All eight cases were considered by the investigator to be unrelated to study treatment. Deaths due to septic shock were also higher in the RO0503821 group (5 cases) than in the reference group (0 cases).

The number of deaths in the September 1 extended population was higher in both the RO0503821 and reference groups [182 (10.2%) and 103 (10.9%), respectively] compared with the number of deaths reported in the MAA [126 (7%) and 58, (6%), respectively] (see Table 31). As in the MAA, the most common cause of death was cardiac arrest, which occurred at a similar rate in both treatment groups (1.2% in RO0503821 group vs. 1.3% in reference). Other common causes of death in both groups were myocardial infarction, cardiorespiratory arrest, chronic renal failure, and sepsis.

Table 31: RO0503821 Safety as of September 1, 2006

	MAA Population		September 1 Extended Population	
	MIRCERA (N=1789) No. (%)	Reference (N=948) No. (%)	MIRCERA (N=1789) No. (%)	Reference (N=948) No. (%)
All Deaths	126 (7.0)	58 (6.1)	182 (10.2)	103 (10.9)
Sudden Deaths*	9 (0.5)	0 (0)	14 (0.8)	5 (0.5)
Cardiac Arrest Deaths*	35 (2.0)	12 (1.3)	51 (2.9)	25 (2.6)
Cardiac Deaths*	67 (3.7)	27 (2.8)	95 (5.3)	51 (5.4)
Myocardial Infarction Deaths*	15 (0.8)	6 (0.6)	22 (1.2)	11 (1.2)
Ischemic Stroke Deaths*	7 (0.4)	3 (0.3)	9 (0.5)	5 (0.5)
Withdrawals	399 (22)	146 (15)	560 (31)	260 (27)

* Aggregated preferred terms

Deaths associated with high Hb values

A total of 15 of 126 AEs leading to death (11.9%) occurred in association with Hb values >13 g/dL, which is lower than the prevalence of Hb values > 13 g/dL (17.8%) in the population. In the Hb > 14 g/dL category, there was a total of 6 of 126 AEs leading to death (4.7%) which was slightly higher than the prevalence of Hb values = 14 g/dL in the population (3.7%).

With the increased period of observation in both the RO0503821 and pooled reference treated patients (extended population, with cut-off date of September 1, 2006), a relative shift in the number and proportion of AEs leading to death associated with Hb values ≥ 13 g/dL has been recorded (see Table 32). In the RO0503821 group a total of 20 of 182 AEs leading to death (11% of all fatal events) occurred in association with Hb values ≥ 13 g/dL; 6 of these were in the ≥ 14 g/dL category. In the reference group, a total of 6 of 103 AEs leading to death (6%) occurred in association with Hb values ≥ 13; 1 of these was in the ≥ 14 g/dL category. With this increased period of observation, the distribution of AEs leading to death across the five Hb categories was also similar in the RO0503821 and reference groups: 38%, 30%, 21%, 8%, and 3% in the RO0503821 group and 40%, 34%, 19%, 5%, and 1% in the reference group.

Table 32 Summary of AE leading to Death and Hb values by Categories for the Last Preceding 4 weeks Hb Levels (Clinical Cut-off September 1 2006)

	MIRCERA (N = 1789) N fatal AE = 182					Reference (N = 948) N fatal AE = 103				
Hemoglobin Level (g/dL)	<11 No. (%)	11-<12 No. (%)	12-<13 No. (%)	13-<14 No. (%)	≥ 14 No. (%)	<11 No. (%)	11-<12 No. (%)	12-<13 No. (%)	13-<14 No. (%)	≥ 14 No. (%)
Safety Parameter										
Total Number of AEs Leading to Death per last Hb (g/dl)	69 (38)	55 (30)	38 (21)	14 (8)	6 (3)	42 (40)	35 (34)	20 (19)	5 (5)	1 (1)
Number of Hb values	19525 (27)	20189 (28)	19910 (27)	10253 (14)	2754 (4)	8985 (22)	13220 (33)	12369 (31)	4937 (12)	1091 (3)

When grouped by body system, AEs leading to death that were associated with Hb values ≥ 13 g/dL in the RO0503821 group included 6 cardiac disorders, 4 nervous system disorders, 3 renal disorders, 3 general disorders, 2 infections and infestations, 1 gastrointestinal disorder, and 1 neoplasm. Of these, new AEs leading to death in the ≥ 13 category of Hb included five in the RO0503821 group: myocardial infarction, cardiac failure, sepsis, bronchopneumonia, and lung adenocarcinoma; in none of these patients was the Hb ≥ 14 g/dL. None of the new fatal events in the RO0503821 group was considered drug related by the investigators. In the reference group, six new AEs leading to death in the ≥ 13 category of Hb occurred: 1 cardiac disorder (cardiopulmonary failure), 2 nervous system disorders (cerebrovascular accident, ischaemic cerebral infarction), 2 general disorders (sudden death, death), and 1 vascular disorder (aortic dissection). The ischaemic cerebral infarction occurred at an Hb level of 15.1 g/dL; the other five new AEs leading to death in the reference group occurred at an Hb

level of >13-<14 g/dL. One of the six new events reported in the reference group (sudden death in patient 1309) was reported as drug related.

- Laboratory findings

In general, iron parameters (iron, ferritin, and TSAT) were well maintained in both the RO0503821 and reference groups. Median values were similar between the two groups and changed little over time (see Table 33).

Table 33: Summary of Iron, Ferritin, and TSAT Data (Overall Safety Population)

Treatment	Month of Treatment	N	Mean	Std	Minimum	Q1	Median	Q3	Maximum
IRON									
RO0503821	Baseline	1784	13.11	5.09	0.54	9.85	12.26	15.30	79.18
	12 Months (day 336-369)	865	13.66	5.65	2.15	9.85	12.90	16.40	55.00
Reference	Baseline	941	13.53	5.85	3.94	10.00	12.35	15.75	80.50
	12 Months (day 336-369)	515	12.84	7.79	2.15	8.95	11.65	15.00	132.60
FERRITIN									
RO0503821	Baseline	1782	480.61	355.62	12.00	228.33	401.67	642.50	3831.00
	12 Months (day 336-369)	858	574.75	403.43	11.00	310.40	486.00	750.00	4746.00
Reference	Baseline	941	463.17	344.83	18.00	203.00	406.00	626.00	2945.50
	12 Months (day 336-369)	513	556.72	432.02	12.00	283.00	502.00	737.00	5160.00
TSAT									
RO0503821	Baseline	1762	30.38	11.85	1.00	22.90	28.00	35.17	160.00
	12 Months (day 336-369)	851	33.02	17.74	7.47	23.00	30.00	39.00	233.71
Reference	Baseline	932	30.79	13.23	9.85	22.32	28.50	36.47	160.50
	12 Months (day 336-369)	510	32.72	41.82	6.30	21.00	28.00	36.00	904.10

The mean and median values for **platelets** in the pooled overall safety population during 12 months of treatment were lower in the RO0503821 group than in the reference group. In the RO0503821 group, the mean and median levels of platelets fell immediately at the start of treatment, but then remained stable. The magnitude of the decrease is approximately 7% occurring for all platelet counts, with the result that most patients stay within the normal range. However, a greater proportion of patients in the RO0503821 group have markedly low levels (5% vs. 2%), defined as a platelet count below $100*10^9$/L and a decrease of at least 30% from baseline (see Table *34*).

Table 34: Summary of Marked Laboratory Abnormalities (Overall Safety Population)

Parameter Abnormality	Value	RO0503821 N = 1789	Reference N = 948
HEMATOLOGY			
PLATELETS (10**9/L) - HIGH	n	1774	938
	single not last	2 (<1%)	7 (<1%)
	last or replicated	2 (<1%)	6 (<1%)
	any	4 (<1%)	13 (1%)
PLATELETS (10**9/L) - LOW	single not last	44 (2%)	10 (1%)
	last or replicated	39 (2%)	9 (<1%)
	any	83 (5%)	19 (2%)
RBC (10**12/L) - HIGH	n	618	229
	single not last	3 (<1%)	1 (<1%)
	last or replicated	2 (<1%)	0
	any	5 (<1%)	1 (<1%)
RBC (10**12/L) - LOW	single not last	56 (9%)	22 (10%)
	last or replicated	164 (27%)	105 (46%)
	any	220 (36%)	127 (55%)
WBC (10**9/L) - HIGH	n	1772	938
	single not last	7 (<1%)	11 (1%)
	last or replicated	11 (<1%)	3 (<1%)

	any	18 (1%)	14 (1%)
WBC (10**9/L) - LOW	single not last	31 (2%)	13 (1%)
	last or replicated	10 (<1%)	2 (<1%)
	any	41 (2%)	15 (2%)

The mean and median values for WBCs were all within the standard reference range and were similar throughout the study and between treatment groups. The same was true for the following parameters: AST, ALT, albumin, ALP, and fasting glucose (non-diabetic patients only). Mean and median values for electrolytes (potassium and phosphate) were similar throughout the study and between treatment groups. Laboratory abnormalities in the RO0503821 and reference groups were generally similar. The most common were low RBCs, high phosphate, and high potassium. A similar proportion of patients in the RO0503821 and reference groups had high phosphate (39% and 36%, respectively) and high potassium (15% in each group), while the proportion of patients with markedly low RBCs was higher in the reference group (55%) than in the RO0503821 group (36%).

- Vital Signs, Physical Findings and Other Observations Related to Safety

In the overall safety population, the mean and median values of diastolic and systolic blood pressure were generally stable over time and similar between treatment groups. Median diastolic blood pressure was 78 mm Hg in both groups at baseline, and 77 mm Hg after one year. Similarly, median systolic blood pressure was 140 mm Hg and 141 mm Hg at baseline in the RO0503821 and reference groups, respectively, and 140 mm Hg in both groups after one year. Similar results were seen for the correction and maintenance study populations.

Summary statistics over time for ECG intervals, HR, PR, RR, QRS, QT, QTcB, QTcF, and QTcR showed similar mean values at each time point between treatment groups. The percentage of patients with QTcR changes from baseline ≥60 msec was greater in the reference group (5.7%) than in the RO0503821 group (3.8%). The percentage of patients with QTcR intervals >480 msec was similar in the RO0503821 group (11.8%) and the reference group (11.0%). Overall the data suggest that there is no clinical evidence of QTc prolongation or associated clinical events.

- Safety in special populations

When AEs were examined by baseline characteristics of gender, age, race, ethnicity, body weight, diabetes, and cardiovascular disease, no safety concerns were identified with RO0503821 treatment in patients with CKD, as compared with ESA reference compounds. Limitations of this analysis to consider include multiplicity of analyses, data from separate randomized trials and small numbers of patients.

No data are available in subjects with hepatic impairment.

No data are available in paediatric population.

- Immunological events

To date, no patients treated with RO0503821 had newly developed detectable anti-RO0503821 or anti-erythropoietin antibodies in any of the clinical trials.

- Safety related to drug-drug interactions and other interactions

No formal drug-drug interaction studies with RO0503821 were conducted during the clinical development.

- Discontinuation due to adverse events

The percentage of patients prematurely withdrawn from the studies was higher in the RO0503821 group (22%) compared with the reference group (15%). This difference was primarily due to the higher percentage of withdrawals for non-safety reasons in the RO0503821 treatment group (14%)

compared with the reference group (9%). The most common reason was kidney transplantation (101 patients, 6% in the RO0503821 group and 34 patients, 4% in the reference group). Another imbalance was observed in the number of patients who 'refused treatment' (47 vs. 13 patients). The reasons for withdrawal included patients experiencing an AE at the time of withdrawal (16 patients for RO0503821, 2 patients for reference) and most had anaemia (Hb <11g/dL). Withdrawals for insufficient therapeutic response were rare in both groups (14 and 3 patients for the RO0503821 and reference groups respectively).

The majority of withdrawals for safety reasons were deaths, the incidence of which was not markedly different across groups. The proportion of patients experienced AEs which led to withdrawal from the study in RO0503821 and reference groups was 45 (2.5%) and 17 (1.8%) respectively. There was no discernible pattern in the types of AEs leading to withdrawal and the incidence of each of these AEs was low (≤ 1%) in both treatment groups. The number of these events considered related to study treatment were 10 in the RO0503821 group and 2 in the reference group. The most frequent AEs leading to study withdrawal in both treatment groups were cardiac disorders, renal and urinary disorders, and blood and lymphatic system disorders; the percentage of patients with AEs in these body systems was similar between groups. Nervous system AEs and neoplasms led to withdrawal more often in the RO0503821 group (6 vs. 0 patients and 5 vs. 1 patient, respectively).

- Post marketing experience

Currently, RO0503821 is not commercially available in any part of the world

- Discussion on clinical safety

The clinical safety program for RO0503821 has not detected any specific safety signal from animal or human data, apart from those already known to be associated with epoetins. No differences regarding to the safety profile or quality of life were identified among patients with a slower time to Hb response under treatment with RO0503821, however a statement addressing this peculiarity of RO0503821 compared to other epoetins has been incorporated in section 5.1 of the SPC.

The overall AE profile was similar between RO0503821 and reference groups. The most common AEs in both groups included hypertension, diarrhoea, headache, and upper respiratory tract, being hypertension the most frequent treatment-related AE. The incidence of related vascular disorders is identical between both arms of treatments (2%), however **hypertension** is reported more frequently in RO0503821 group (27/1789, 2% vs. 14/948, 1%). AEs of special interest were nine reports of **pulmonary embolism** (1 out of the 9 reports was reported as "embolism") and none in the reference group. Both hypertension and vascular access thrombosis have been addressed properly in the relevant sections of the SPC and follow up measures have been implemented in the Risk Minimization Plan. Collection of detailed information on pulmonary embolism will be performed via a dedicated questionnaire sent after receipt of an Adverse Drug Reaction report in the post-authorisation period. Furthermore, the AE of pulmonary embolism has been added in the RMP as "Important Missing Information".

The only serious AE that occurred more frequently in the RO0503821 group compared with the reference group was **GI haemorrhage.** These observations suggest that cautiousness is appropriate when considering administration to patients who are bleeding or who have a history of bleeding. An adequate statement has been included in SPC and this safety concern will also be addressed in the context of a randomized post-authorization safety study. In addition, in order to provide additional information on the risk of GI haemorrhage, the applicant will collect detailed information via a dedicated questionnaire sent after receipt of a report of a serious Adverse Drug Reaction in the post-authorisation period. The submitted PSUR will contain this information. Furthermore, the AE of GI haemorrhage has been added in the updated RMP as "Important Missing Information".

The mean and median values for **platelets** were lower in the RO0503821 group than in the reference group. The reason for the decrease in platelets count remains unknown. In principle, a low platelet count might signal a risk for bleeding (impaired haemostasis) or for thrombosis (increased

adhesiveness of platelets). The SPC has been modified to appropriately address this concern. Moreover, a FUM has been agreed to provide new experimental data on platelets via an ex vivo study on blood drawn from healthy volunteers and CKD patients. Potential undesirable effects of RO0503821 on platelet function will be investigated compared to a conventional epoetin. The study is planned to begin in Jan-2007 and the report will be submitted for review by the regulatory authorities.

Related to Anti-Erythropoietin Antibody-Mediated Pure Red Cell Aplasia (**AEAB-mediated PRCA**), an educational and monitoring programme including a Questionnaire will be implemented. It will be aimed to increase awareness of the importance of spontaneous reporting in general and especially for AEAB-mediated PRCA and to emphasize the need to report and document case reports of unexplained loss of effect.

According to the analyses from the Updated Pooled Safety Data from Phase II, Phase III, and Ongoing Extension Studies, no new or unexpected safety issues have been identified. Although the rate of mortality has increased in the September 1 extended population compared to previous data (MAA population), the proportion of patients who died remains similar between both treatment groups (10.2% in RO0503821 group vs. 10.9% in reference). However, some previously detected safety concerns persist, i.e. related **cardiac disorder**s are reported by five patients in the RO0503821 group and none in the reference group. The incidence of related vascular disorders is identical between both arms of treatments (2%), although hypertension is reported more frequently in RO0503821 group. This safety concern has been addressed in the RMP via the requirement of a post-marketing randomized clinical trial. The primary objective of the study is to determine whether RO0503821 is non-inferior to other ESAs in the time to a composite endpoint of death or non fatal cardiovascular events (MI and stroke).

Recently published studies have shown an increased cardiovascular risk associated with high Hb levels (REF: CHOIR, CREATE). These findings have recently led to new recommendations from different regulatory authorities and are under discussion at an EU level. A more conservative approach targeting maximum Hb levels of **12 g/dL** has been agreed to minimize the potential cardiovascular risk associated to the use of RO0503821.

Supplementary iron therapy is recommended for all patients with serum ferritin values below 100 microgram/l or with transferrin saturation below 20%. To ensure effective erythropoiesis, iron status has to be evaluated for all patients prior to and during treatment.

RO0503821, like other ESAs, is a growth factor that primarily stimulates red blood cell production. Erythropoietin receptors may be expressed on the surface of a variety of tumour cells. As with all growth factors, there is a concern that ESAs could stimulate the growth of any type of malignancy. Two controlled clinical studies in which epoetins were administered to patients with various cancers including head and neck and breast, have shown an unexplained excess mortality. In addition, an increased mortality rate associated to RO0503821 therapy has been observed in a study in patients with Non-Small Cell Lung Cancer (NSCLC). This observation has not been confirmed in other oncology studies with RO0503821. In patients with CKD no increase in mortality has been found associated to the use of RO0503821 as compared to other epos under controlled conditions. The current application does not include the use of RO0503821 in the oncology setting; however, these findings highlight the need of reinforcing the risk minimisation measures aimed to avoid the use of RO0503821 outside the approved indication. The following warning has been included in section 4.4 of the SPC: “RO0503821 is not approved for the treatment of anaemia in patients with cancer”. Moreover, the Applicant will conduct a RCT in CKD as a post-marketing commitment in which overall mortality will form part of the primary endpoint.

The safety and efficacy of RO0503821 therapy has not been established in patients with severe liver disease therefore caution should be used in these patients. A warning addressing hepatic impaired patients has been included in section 4.2 of SPC.

RO0503821 is not recommended for use in children and adolescents below 18 years due to a lack of safety and efficacy data. This has been address accordingly in section 4.2 of the SPC.

There are no data from the use of RO0503821 in pregnant woman. Animal studies do not indicate direct harmful effects with respect to pregnancy, embryonal/foetal development, parturition or postnatal development but indicate a class-related reversible reduction in foetal weight. It is unknown whether RO0503821 is excreted in human breast milk. One animal study has shown excretion of RO0503821 in maternal milk. A decision on whether to continue or discontinue breast-feeding or to continue or discontinue therapy with RO0503821 should be made taking into account the benefit of breast-feeding to the child and the benefit of RO0503821 therapy to the woman. Caution should be exercised when prescribing to pregnant or lactating women. This risk has been clearly indicated in section 4.6 of the SPC.

1.5 Pharmacovigilance

Detailed description of the Pharmacovigilance system

The CHMP considered that the Pharmacovigilance system as described by the applicant fulfils the legislative requirements.

Risk Management Plan

The MAA submitted a risk management plan, which included a risk minimisation plan.

Table 35: Summary of the risk management plan

Safety issue	Proposed pharmacovigilance activities	Proposed risk minimisation activities
Vascular access thrombosis	Routine Pharmacovigilance	Routine risk minimization measures via labelling: Listed as an ADR in SPC Section 4.8 Risk minimization measures in Section 4.2 “Posology and method of administration”: To restrict haemoglobin levels To provide refined dose adjustment rules to ensure appropriate haemoglobin
Hypertension incl. hypertensive encephalopathy	Routine Pharmacovigilance	Routine risk minimization measures via labelling: Contraindication Section 4.3: - Uncontrolled hypertension Warnings and Precautions Section 4.4: - Blood pressure monitoring and treatment Listed as an ADR in SPC Section 4.8
Decrease in Platelet count	Routine Pharmacovigilance Additional Pharmacovigilance: Questionnaire Study	Routine risk minimization measures via labelling: Listed as an ADR in SPC Section 4.8 Additional risk minimization measures: None
AEAB-mediated PRCA	Routine Pharmacovigilance Additional Pharmacovigilance: Monitoring programme with dedicated database Questionnaire	Routine risk minimization measures via labelling: Warnings and Precautions Section 4.4 Additional risk minimization measures: Educational material Free anti-erythropoietin antibody

	Randomized post-authorisation safety study	testing
GI bleeding	Routine Pharmacovigilance Additional Pharmacovigilance: Questionnaire Randomized post-authorisation safety study	Warnings and Precautions Section 4.4
Thromboembolic events including pulmonary events	Routine Pharmacovigilance Additional Pharmacovigilance: Questionnaire for ADRs of pulmonary embolism from postmarketing experience Randomized post-authorisation safety study	Yes (Vascular access thrombosis listed as ADR in SPC section 4.8)
Chronic inflammatory disease	Routine pharmacovigilance Additional pharmacovigilance : gathering safety information on conditions of chronic inflammatory diseases by including these patients in post approval studies	None
Pregnancy, lactation	Routine pharmacovigilance	Section 4.6 “Pregnancy and lactation”
Paediatric	Routine pharmacovigilance Additional pharmacovigilance: Paediatric development programme	Section 4.2 Posology and methods of administration / Paediatric use.
Avoidance of off-label use		Section 4.1 Indication Section 4.4 Warnings and precautions

The CHMP, having considered the data submitted in the application is of the opinion that the following risk minimisation activities are necessary for the safe and effective use of the medicinal product: See as detailed in Section 2.3.

1.6 Overall conclusions, risk/benefit assessment and recommendation

Quality

During the evaluation of RO0503821 two major objections were identified concerning the demonstration of product heterogeneity and formal validation program respectively. Satisfactory responses have been provided to resolve them. Other minor concerns have been adequately addressed, however, several commitments are made by the applicant and several follow-up measures are defined to provide further information post-approval. In conclusion, all quality issues are resolved.

Non-clinical pharmacology and toxicology

RO0503821 is a stimulator of erythroid progenitor cells in the bone marrow. In contrast to epoetin beta, RO0503821 showed a reduced specific activity *in vitro* and a different activity at the receptor level characterized by a slower association and faster dissociation. In relation to *in vivo* studies,

RO0503821 was found to be a more potent stimulator of erythropoiesis than epoetin beta (normocythemic mouse model and beagle dogs). Both the magnitude and the duration of the erythropoietic responses elicited by RO0503821 were substantially increased over that of epoetin beta. Erythropoietic effects were identical regardless the route of administration (subcutaneous or intravenous). The improved response to pegylated conjugates in the normal mouse model may be due to the enhanced stability of pegylated proteins and prolonged exposure conferred by the addition of a PEG moiety to the protein molecule. In addition, less immunogenic response were found with RO0503821 over that of the unmodified protein.

Repeated dose toxicity studies resulted in several histopathological findings including vascular congestion, haemorrhage, thrombosis, necrosis, and/or inflammation in various organs and tissues including brain, heart, kidney, liver, spleen, stomach, and thymus. These findings were associated with the polycythemic condition due to the exaggerated pharmacological effects which were manifested as clinical observations, haematologically and at histopathological level. There were no increases in platelet counts. The coagulation parameters Prothrombin Time (PT) and Activated Partial Thrombin Time (APTT) increased when RBC counts increased following treatment.

Some rats and dogs developed anti-erythropoetin antibodies and at the same time developed resistance in responding to the administration of RO0503821 resulting in anaemia. Resistance to RO0503821 in animal studies is considered a reflection of the presence of neutralizing antibodies to RO0503821 and endogenous erythropoetin in these animals.

When RO0503821 was administered subcutaneously to rats and rabbits during gestation, no evidence of a direct embryotoxic, foetotoxic, or teratogenic potential was identified. The adverse effects observed were a reduction in fetal weights and also in rats a correlated retarded growth which occurred at doses causing exaggerated pharmacodynamic effects in dams. RO0503821 was secreted into milk in a study in rats.

Efficacy

Six Phase III pivotal studies are provided to support the efficacy of methoxy polyethylene glycol-epoetin beta in anaemia associated with chronic kidney disease (CKD). Two correction studies were performed in epo naive patients with CKD on dialysis (BA16736) or not on dialysis (BA16738). Four Maintenance studies (BA16739, BA16740, BA17283 and BA17284) were in patients on dialysis who have been receiving ESAs. All studies were randomized, open-label, multicentre, comparative active-controlled, parallel group studies. Pivotal studies were not blinded. Three comparators (epoetin alfa, epoetin beta or darbepoetin alfa) were used and administered both IV and SC routes at approved posology regimens.

The main efficacy end point in naive patients was the Hb response rate, defined as an increase in Hb $\geq$ 1.0g/dL from baseline and a Hb concentration $\geq$ 11g/dL without RBC transfusion during the correction period (24 weeks after first dose) in study BA16736, and during correction plus evaluation period (28 first weeks) in study BA16738. This study had a co-primary endpoint, namely the change in Hb concentration (g/dL) between the baseline and evaluation periods. In maintenance (switching) studies, the main efficacy endpoint was the change in Hb concentration (g/dL) between the baseline and the evaluation period that follows the 28-week dose-titration period.

Data from correction studies show that the Hb response rates in the RO0503821 group at the end of the correction period were high: 93.3% and 97.5% in the studies BA16736 and BA16738, respectively and comparable to reference treatment in both studies (91.3% and 96.3%, respectively). Non-inferiority of RO0503821 treatment compared to darbepoetin was demonstrated. Although both treatment groups are comparable in correcting Hb levels in CKD patients not currently treated with an ESA, the increase in Hb concentrations is slower in the RO0503821 group than reference treatments. Since treatment will continue over many months and years, this slight delay is not considered clinically relevant for the patients.

The results from maintenance studies show that the change in Hb concentrations between baseline and the evaluation period was comparable between the RO0503821 treatment group(s) and the reference group within each of the four maintenance studies and most were close to zero (no change). The differences between the RO0503821 and reference groups in mean changes from baseline ranged from -0.022 to 0.18 g/dL (based on analysis of covariance). Non-inferiority was demonstrated in all studies and was irrespective of dosing regimen (1x/2 weeks or 1x/4 weeks) and route of administration (IV or SC).

In conclusion, RO0503821 shows a non-inferior efficacy as compared to other epoetins to correct and maintain Hb levels in CKD patients with a monthly administration.

Safety

Safety data from 28 clinical trials performed with RO0503821 SC and IV during the clinical development have been collected. The overall safety population is pooled from the four dose-finding Phase II and six pivotal Phase III studies. The most frequently reported treatment-related AE was hypertension, which was reported in 2% of patients in RO0503821 group and 1% in reference group. The rest of individual treatment-related AEs occurred in < 1% of patients in each group with no difference between groups in the occurrence of any single treatment-related AE. The most frequent AE reported in both treatment groups were hypertension, diarrhoea, nasopharyngitis, headache, upper respiratory infection, muscle spasms and procedural hypotension. The incidence of AEs considered by the investigator as related to treatment was 7.0% in the RO0503821 group and 5% in the reference group. In several body systems, the frequency was slightly higher in the RO0503821 group than in the reference group. These included: general disorders and administration site conditions (1.3% vs. 0.4%), blood and lymphatic system disorders (0.7% vs. 0.3%), and skin and subcutaneous tissue disorders (0.5% vs. 0.1%). Related cardiac disorders were more frequently reported in RO0503821 group (5 patients vs. 0).

A total of 836 subjects treated with RO0503821 (47%) reported a serious adverse event compared with 509 patients (54%) in the reference group. Serious upper GI haemorrhage was reported more frequently in the RO0503821 group (3% vs. 2%); when all serious hemorrhagic events were pooled, the frequency was similar across groups (7% vs. 6%). Pulmonary embolism was reported by 9 patients in the RO0503821 group and none in the reference group. None of the pulmonary embolism cases were considered related to RO0503821 as assessed by the investigator. The rate of early discontinuation due to adverse events, regardless of relation to the study medication, was 3% in patients treated with RO0503821 and 2% in patients who received reference treatment.

In patients with CKD no increase in mortality has been found associated to the use of RO0503821 as compared to other epos under controlled conditions.

From the safety database all the adverse reactions reported in clinical trials <and post-marketing> have been included in the Summary of Product Characteristics.

Having considered the safety concerns in the risk management plan, the CHMP considered that the proposed activities described in section 3.5 adequately addressed these.

- User consultation

The Applicant performed a readability testing (“user consultation”) and a satisfactory report has been provided.

Risk-benefit assessment

Anaemia is a common complication in patients with chronic renal failure. Its correction may relieve symptoms and improve the quality of life. Exogenous replacement of erythropoetin by the recombinant hormone is a well-accepted therapy for the treatment of anaemia in patients with chronic kidney disease (CKD). In terms of efficacy, the benefits of RO0503821 have been demonstrated in six

randomized, open-label, multicentre, comparative active-controlled and parallel group Phase III pivotal studies. RO0503821 corrects and maintains Hb levels in patients with CKD and is comparable to other epoetins. In terms of safety, the main adverse events of RO0503821 were those already known to be associated to epoetins and a CDK population. The most common adverse events included hypertension, headache and vascular access thrombosis. Hypertension was reported more frequently after RO0503821 treatment and there were nine reports of pulmonary embolism not observed after reference epoetins. A conservative dosing schedule aiming Hb target level of 12 g/dL has been implemented to minimise cardiovascular risk. Jointly to the proposed risk minimization measures (see Table 35), the MAH has committed to perform a robust post-marketing study aimed to rule out an increased risk of vascular events associated to the use of RO0503821.

A risk management plan was submitted. The CHMP, having considered the data submitted, was of the opinion that:

- Pharmacovigilance activities in addition to the use of routine pharmacovigilance were needed to investigate further some of the safety concerns.

- The following additional risk minimisation activities were required; see as detailed in section 2.3.

Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered by consensus that the risk-benefit balance of MIRCERA in the treatment of anaemia associated with chronic kidney disease (CKD) was favourable and therefore recommended the granting of the marketing authorisation.