SCIENTIFIC DISCUSSION

This module reflects the initial scientific discussion for the approval of Aranesp. This scientific discussion has been updated until 1 September 2003. For scientific information on procedures after this date please refer to module 8B.

1. Introduction

Patients with chronic renal failure (CRF) develop uremic anaemia as one of the most obvious signs of the disease. This symptom is caused by impeded renal production of erythropoietin (EPO). EPO controls red blood cell counts (RBC) production by promoting survival, proliferation and differentiation of erythroid progenitors in the bone marrow. Effective management of anaemia in chronic renal failure (CRF) has a major impact on quality of life and may influence survival. Supplementation with recombinant human erythropoietin (r-HuEPO) is currently the standard treatment for anaemia in those patients. It is regarded as an effective drug, which has an established safety record.

Recombinant human erythropoietin is currently available as a treatment for anaemia in end stage renal disease. Administration 2 to 3 times weekly is required in the majority of subjects. The aim of inventing this new molecular entity of darbepoetin alfa was to obtain a therapeutic with a longer biological half-life compared to r-HuEPO, allowing a reduction of the frequency of injections necessary to maintain a desired level of systemic haemoglobin and haematocrit. The chronic nature of CRF (unless a subject receives a kidney transplant) means that treatment may continue for a long part of the subject's life and multiple weekly injections of r-HuEPO can have a major impact on subjects and care givers. Research has indicated that the sialic acid containing carbohydrate of erythropoietin determines its serum half-live. Darbepoetin alfa is novel erythropoeisis stimulating protein with increased sialic acid content compared to recombinant human erythropoietin and therefore exhibiting different pharmacokinetic properties.

2. Part II: Chemical, pharmaceutical and biological aspects

Composition

The finished product of Aranesp is a ready-to-use aqueous formulation of darbepoetin alfa. The formulation contains polysorbate 80 as a protective agent. During the development of darbepoetin alfa, the level of excipients was selected to prevent adsorption and minimise aggregation induced by shear and agitation stress.

The formulation is presented in pre-filled syringes (PFS) and vials. The doses for the PFS range from 10 µg to 300 µg in volumes of 0.3 to 0.6 ml. The doses for the vials range from 15 µg to 60 µg in 1 ml withdrawable volume. The vial container closure system consists of a 3-ml borosilicate type I glass with rubber stoppers and aluminium flip-off seals. The pre-filled syringes consist of a borosilicate type 1 barrel (1ml) with a rubber plunger stopper and a staked-in-place needle.

Clinical studies were performed with the albumin formulation. The polysorbate and albumin formulations have been compared by analysing quality-indicating parameters, preclinical studies and a clinical bioequivalence study in healthy subjects.

Active substance

Darbepoetin alfa (also known by its laboratory code NESP) is a novel erythropoeisis stimulating protein produced in Chinese hamster ovary (CHO) cells by recombinant DNA technology. The final processed form is a 165 amino acid protein.

A description of the expression vector and encoding gene construct used for generation of the cell line and preparation of the master cell bank (MCB) and working cell bank (WCB) are provided in the

dossier. The genetic stability and integrity were analysed using southern and northern blot techniques and copy number analysis. The cell banks and end-of-production (EOP) cells were tested for the presence of viral contaminants (including testing for retroviruses/reverse transcriptase activity) and for microbial contaminants (sterility and mycoplasma).

Cell Culture

The active substance is produced using roller bottle technology. Darbepoetin alfa is constitutively expressed from adherent CHO cells into cell culture production medium. The cell conditioned media is collected, clarified, and concentrated for further processing. Prior to the inoculation of the production roller bottles, cells are serially cultivated and expanded in adherent and suspension cultures. Assurance is given that raw materials from animal origin, used during the expansion of the cell culture, comply with the requirements of the TSE Note for Guidance. Bioburden testing, mycoplasma, and adventitious viral testing are performed for each cell culture lot. In process controls during the cell culture phase includes a determination of the cell density, viability, pH, pCO_2, pO_2, glucose, total protein, and darbepoetin alfa concentration.

Purification

Purification of darbepoetin alfa is performed by a sequence of chromatographic and filtration steps for the generation of filtered purified bulk product. In process controls and specifications are adequate to control product quality and consistency of the purification process. In process rejection limits have been set for the most critical parameters. The purification process has been validated and maximum lifetimes for the chromatography columns have been established.

Characterisation

Darbepoetin alfa has been characterised using physico-chemical and biological assays. State-of-the-art analytical procedures have been used to elucidate the protein sequence and the structural elements of the N- and O-linked oligosaccharides. N-terminal sequencing of overlapping peptides prepared by RP-HPLC from a tryptic and GluC digest respectively confirmed the native positions of the internal disulfide bridges, especially in terms of correct folding, and the sites for N-linked- and O-linked oligosaccharide chains. A RP-HPLC method was developed to separate differently glycosylated darbepoetin alfa forms. Darbepoetin alfa biological activity has been characterised using a number of assays based on its erythropoietic activity.

Appropriate specifications have been set for the testing of the active substance at release and at the end of the shelf life. All methods used have been described and validated.

Other ingredients

All excipients and immediate packaging materials meet the requirements of the Ph. Eur.

Product development and finished product

The manufacturing process for the final product consists of a dilution of the filtered purified bulk (active substance) to the desired concentration with buffer, sterile filtration and sterile filling into vials or syringes. Final product is manufactured at Amgen Puerto Rico, Juncos, PR. Packaging, labelling and final release will be done by Amgen Europe B.V., Breda, The Netherlands.

The manufacturing process for the final product has been validated and is controlled by in process controls and product release specifications. The quality of the final product is ensured by a combination of physico-chemical, biological, and immunological methods. All methods used for routine control have been described and validated.

Stability of the product

Based on the real time stability results after storage at 2 – 8 °C, a shelf life of 18 months is acceptable for both the vials and pre-filled syringes. The shelf life has been extended to 24 months at 2 – 8 °C via a variation application.

3. Part III: Toxico-pharmacological aspects

The objectives of the preclinical investigation were **i)** to demonstrate qualitative biological equivalence and specificity of darbepoetin-alfa with its predecessor epoetin-alfa (r-HuEPO) *in vitro* and *in vivo*, **ii)** to demonstrate higher biological activity of darbepoetin alfa and to explain this by the changes attained in the pharmacokinetic profile, and **iii)** to demonstrate relative non-toxicity of this new preparation in comparison to the former variety. **iv)** Additionally, as two formulations of darbepoetin alfa were under development for clinical use, one containing human serum albumin (HSA) and one containing polysorbate 80, but HSA-free, a series of biophysical assays *in vitro*, a pharmaceutical bioactivity study *in vitro*, and an *in vivo* preclinical study had to be conducted in order to demonstrate comparability of the albumin and polysorbate 80 formulations.

Pharmacodynamics

- *In vitro* studies

Darbepoetin alfa was shown to bind to the Hu-EPO receptor expressed on a human cell line and to exert a specific bioactivity for the differentiation and/or proliferation of different stages of primary cultures of mouse bone marrow progenitor cells, and also of a pluripotent murine bone marrow cell line (32D) *in vitro*. Desialylation increased its bioactivity by approximately 15-fold in the murine cell test system. These results support the notion that the affinity of darbepoetin alfa for the EPO receptor and its biological activity *in vitro* coincide with its grade of sialylation. This was attributed to steric and charge effects of the additional carbohydrate structure. Conversely, it can be concluded that affinity and bioactivity of darbepoetin alfa are not adversely influenced by the exchange of five amino acids of the peptide compared to the natural form. It is believed that desialylation is not a prerequisite for biological activity *in vivo* since intact darbepoetin alfa has been shown to bind to and activate the EPO receptor *in vitro*.

The specificity of tissue binding was tested by comparing the binding of darbepoetin alfa and r-HuEPO to different human tissues *ex vivo*, which might express EPO receptors or related receptors of the haematopoietic type. The only positive tissue identified to bind control-EPO as well as darbepoetin alfa, was bone marrow. This experiment indicated that there is no enhanced binding of darbepoetin alfa to human tissues other than bone marrow and any cross-reactivity with related cytokine receptors is very unlikely to occur. However, as these forms are cleared more rapidly systemically, their potential for in vivo biological effect is reduced.

- *In vivo* studies

In mice, darbepoetin alfa was generally about 4- to 5-fold more active than r-HuEPO in stimulating erythropoiesis (haematocrit, haemoglobin level and red cell counts) when injected 3-times weekly, and even about 8-times more active when comparing once weekly injections. Also, a faster initial rate of haematocrit rise was observed with darbepoetin alfa if compared to r-HuEPO after dosing once per week. A lower frequency of administration of equal weekly amounts of darbepoetin alfa always produced a lower response.

- General and safety pharmacology programme

In safety pharmacology studies, there was no immediate, unspecific influence of darbepoetin alfa on any important physiological functions of the body.

Local toxicity at the injection sites did not differ in treated and control groups of rats, rabbits, and dogs.

No formal interaction studies have been performed. The company agreed to include a statement in the SPC regarding potential interaction with cyclosporin A, since this component is known to bind to RBCs and may be used in the submitted indication.

Pharmacokinetics

- Absorption and distribution

Extensive preclinical pharmacokinetic studies have characterized the basic pharmacokinetic properties of darbepoetin alfa following single and multiple dose administration, both intravenously and subcutaneously, in Sprague-Dawley rats and Beagle dogs. These species are both responsive to the biological effects of darbepoetin alfa and are the primary species used in the toxicology studies. These analyses allow assessment of pharmacokinetic parameters, following multiple dose administration of darbepoetin alfa, with regard to dose-linearity, time-linearity, accumulation potential, effect of sex and effect of antibodies. A single intravenous dose, dose-ranging study was also performed in mice, which is the species used for the pharmacodynamic studies.

In mice, darbepoetin alfa IV pharmacokinetics appeared to be dose linear over the range 1 to 100 µg/kg, as assessed by compartmental modelling. In rats, all pharmacokinetic parameters were linear with dose, with the exception of absorption kinetics. Following SC dosing, bioavailability was approximately 50% and C_{max} occurred at 12-24 hrs post-dose. Upon multiple dosing of darbepoetin alfa to rats by either the intravenous or subcutaneous routes (up to 6 months), the pharmacokinetics of darbepoetin alfa were dose-linear and time-independent. Accumulation was low. There was no effect of gender, antibody formation, dose or route on the primary pharmacokinetic parameters. In radiolabelled studies, darbepoetin alfa stayed mainly in the vascular compartment and tissue penetration was generally low.

In dogs, serum levels following IV dosing were consistent with a three compartment model. The elimination half-lives increased with increasing dose, thus the elimination pathways appeared to be saturable. Following a single s.c. administration, T_{max} was 24 hours and bioavailability varied with the dose between 65 to 78%. C_{max} was about one quarter of the value obtained with i.v. administration. The pharmakokinetic parameters were not affected by sex. In a repeat dose s.c. study, over the dose range 1 to 10 µg/kg, darbepoetin alfa pharmacokinetics were generally predictable from the single dose scenario.

Both the formulations containing HSA or polysorbate 80 could be demonstrated to be biologically equivalent in pharmaceutical test systems *in vitro* and in a preclinical *in vivo* study. Neither the formulation nor the concentration of the darbepoetin alfa solution administered had any influence on the major non-compartmental pharmacokinetic parameters in dogs (AUC, C_{max}, CL/F).

- Metabolism and excretion

Sialic acid content (as indicated by IEF) is the critical correlate between the darbepoetin alfa structural composition and its *in vivo* pharmacokinetic profile. The underlying clearance mechanism of darbepoetin alfa was demonstrated to be desialylation by tissue and blood sialidases and ensuing hepatic removal via a galactose receptor mediated uptake. The first-pass extraction ratio of desialylated darbepoetin alfa was calculated to approximate 85%. The insignificant role of renal clearance was demonstrated in rats after uni- and bilateral nephrectomy. In a radiolabelled study, 57% and 24% of the radiolabel was excreted in urine and faeces, respectively within 168 hours. There were no apparent differences in either distribution or excretion between males and females.

Toxicology

Single and repeat dose toxicity

A full set of conventional toxicity tests in two species has been performed with darbepoetin alfa. Single and repeated dose toxicity tests were conducted in rats and dogs (6 studies in rat, 2 single and 4 repeat dose studies and 6 studies in dog, 2 single and 4 repeat dose studies). Measurement of the serum kinetics of darbepoetin alfa after diverse s.c. and i.v. doses were made in parallel to these studies. The pharmacological activity of darbepoetin alfa, due to its prolonged circulation in the body and the high doses administered, resulted in the production of extreme polycythemia and associated actions, including widespread extramedullary haemopoiesis. Decreases in serum iron and increases in iron-binding capacity are consistent with incorporation of iron into haemoglobin during erythropoiesis. Extramedullary haematopoiesis was paralleled by splenic hypertrophy, the appearance of immature erythrocytes and a decrease of platelets. By contrast, increased potassium levels also indicated an increased rate of erythrocyte destruction by splenic activity. Tissue hypoxia, secondary to polycythemia, was manifested in the livers of dogs receiving medium and high doses as centrilobular necrosis/degeneration and, after very high doses, also fibrotic changes in the heart and kidneys and myelofibrosis in the bone marrow became evident. All these signs can be regarded as typical for high doses of erythropoietin, and are also known for r-HuEPO's. The specific erythrogenic stimulation has been maintained for months, and has largely still been reversible, even after the short recovery period of one month after a 6-month treatment time.

Reproductive toxicity

Reproductive toxicity studies have been performed in rats and rabbits: fertility studies in male and female rats, embryotoxic studies in rat and rabbit and a pre/postnatal study in rats.

No clinically relevant evidence of harmful effects with respect to pregnancy, embryonal/foetal development, parturition or postnatal development was observed. Any adverse effects observed were typically associated with an increase in haematocrit and red blood cell counts. In a fertility study in rats, the number of live foetuses was reduced in the treated groups and post implantation losses were increased dose-dependently. Foetal or pup weights were generally reduced in the treated groups of the other reproductive studies but no definitive risks could be identified for low dose administration of darbepoetin alfa to pregnant animals.

Genotoxicity

Darbepoetin alfa was tested for genotoxic activity in a bacterial reverse mutation assay, in a mammalian cell gene mutation assay (HPRT assay using CHO cells) and in an *in vivo* bone marrow micronucleus test in male mice. As expected, the compound did not exert any genotoxic activity in these test systems.

Carcinogenicity

Carcinogenicity studies were omitted for plausible reasons.

Local tolerance

Local tolerance results were obtained from studies in rats (6 months, i.v.) and dogs (4 weeks, s.c.). A clinical formulation (free of human serum albumin and containing Polysorbate 80) was shown not cause any local reaction in GLP compliant tests when injected to rabbits as a single dose intravenously or intraarterially, perivenously, or subcutaneously injection.

Immunogenicity

Immunogenicity was determined in pharmacodynamic and repeated toxicity studies as well as in clinical studies. The abilities of darbepoetin alfa and r-HuEPO to induce IgG-antibody formation were

mostly indistinguishable from each other, when administered at equal weekly doses by the same route and frequency. Antigenicity was clearly dose-dependent, positively correlated with the frequency of administration, and at least twice as high after subcutaneous than after intraperitoneal injection, but practically absent after intravenous administration. Neutralising activity did not affect the kinetics of darbepoetin alfa. The erythrogenic response was not visibly affected during dosing, nor was the antibody titre related to the changes in haematocrit in subsequent recovery periods. The incidence of seroconversion declined during the recovery periods, but did not fully disappear. The pathological changes seen in both the rats and dogs did not indicate immunologically mediated tissue damage, i.e. there were no features of immune complex formation.

In human clinical studies, in over 1500 darbepoetin alfa treated patients followed for a mean of 12 months (with over 150 treated for 2 years), no antibodies were detected.

4. Part IV: Clinical aspects

Clinical pharmacology

Pharmacodynamics

Two exploratory dose- and schedule-finding studies (NESP-**960245** and NESP-**960246**) investigated the effects of darbepoetin alfa for correction of anaemia in HD and PD patients by IV and SC administration respectively. The results of both studies showed a clear dose-related effect of darbepoetin alfa by IV as well as by SC route and independent of the schedule (once or three times weekly). At two of the selected dose levels, 60 to 70% of patients produced an optimal rate of rise (ROR) in Hb, which was the primary endpoint, within the first 4 weeks(1g/dL). Based on the results of these studies it was concluded that 0.45 to 0.75µg/kg of darbepoetin alfa administered once weekly IV or SC is the most appropriate starting dose for treatment of anaemia in CRF patients.

Pharmacokinetics

- General:

The clinical program included studies aimed at establishing the pharmacokinetic profile of darbepoetin alfa in adult and paediatric CRF patients undergoing either HD or PD. In 5 studies, determination of darbepoetin alfa pharmacokinetic properties was the primary endpoint. Three of these studies were conducted in adult CRF patients and included single-dose IV/SC in PD patients (NESP **960224**), multidose IV in HD patients (NESP **970235**) and multidose SC in HD/PD patients (NESP **980194**).

Pharmacokinetics after a single dose was dose-linear, and there was no clinically relevant influence of demographic variables (sex, race, age, body weight) on single-dose pharmacokinetic parameters. In a comparative study (NESP-960224), darbepoetin alfa had a three-fold longer half-life than r-HuEPO after IV administration. Comparison with historical data at equivalent doses, suggested that the half-life after SC administration was two to three-fold longer than r-HuEPO, and bioavailability was very similar.

Following repeated IV and SC dosing, darbepoetin alfa pharmacokinetics remained time-linear and estimates for multiple-dose parameters were very similar to the single-dose parameters. Accumulation was minimal when investigated in over 700 patients for both IV and SC administration for up to 1 year. Regardless of dose, schedule, or route, the dose-normalised trough serum concentrations did not increase over time.

- Studies in special populations

Initial data from a single-dose IV/SC pharmacokinetic study in paediatric CRF patients with or without dialysis treatment (NESP **980212**) showed similar pharmacokinetic properties of darbepoetin alfa compared with adult CRF patients. However, on basis of the limited data of this clinical trial, the use of darbepoetin alfa for the treatment of children cannot be granted. The indication will therefore be

limited to the treatment of anaemia associated with chronic renal failure in adults and paediatric patients ≥ 11 years of age.

- Bioequivalence studies

A single-dose crossover study (NESP **990134**) was undertaken in healthy volunteers demonstrating that the HSA containing and HSA free darbepoetin alfa formulations are bio-equivalent following SC administration.

- Conclusion

The pharmacokinetic studies showed that darbepoetin alfa has a slower clearance and a significantly longer terminal half-life in CRF patients when compared to r-HuEPO. There was no evidence of accumulation of darbepoetin alfa over time. There are no special precautions for use based on the pharmacokinetic data, although darbepoetin alfa should be used with caution in patients with acute hepatic failure, as the liver is a likely route for drug elimination.

Two formulations of darbepoetin alfa were used for clinical use, one containing HSA and one without HSA. These two formulations are bio-equivalent and, therapeutically, can be regarded as interchangeable. The applicant will only be marketing the HSA free version.

Clinical efficacy

Dose-response studies and main clinical studies

Clinical studies have been performed for correction of anaemia in CRF patients (NESP-**980202** and NESP-**980211**) and for conversion from r-HuEPO to darbepoetin alfa in CRF patients (controlled studies NESP-**970200** and NESP-**980117**; non-controlled studies NESP-**980140** and NESP-**980160**).

Main Clinical studies

Correction of anaemia in CRF patients

The two multicentre randomised open-label trials explored the correction of anaemia in subjects on dialysis (NESP-980211) or pre-dialysis (NESP 980202). The aim of the studies was to correct anaemia and maintain haemoglobin concentration within a predefined target range for up to 20 and 24 weeks, respectively. Patients were randomised to receive darbepoetin alfa or r-HuEPO. The primary endpoint for both studies was defined as the proportion of subjects that achieved a Hb response (Hb increase of ≥ 1.0 g/dl from baseline and a Hb concentration of ≥ 11.0 g/dl during the initial 24 weeks of treatment). The selected starting dose was 0.45µg/kg once weekly for darbepoetin alfa as suggested by the exploratory dose finding studies. The starting dose for r-HuEPO was approximately equivalent to this dose in study NESP-980202 (50 U/kg twice weekly), and 40% higher than this dose in NESP-980211 (50 U/kg three times weekly).

In the first pivotal correction study (NESP-980202) r-HuEPO-naive patients were not yet on dialysis and study drugs were administered by the subcutaneous route. 129 patients received darbepoetin alfa, 37 r-HuEPO. The mean ROR in Hb in both treatment groups was within the European Best Practice Guideline recommendation (increase of 1 to 2 g/dl per month). A Hb response was achieved in 93% of patients in the darbepoetin alfa group and 92% in the rHuEPO group.. Subjects from this study had the option to extend dosing up to 104 weeks and were so eligible for long-term efficacy and safety.

In the second pivotal correction of anaemia study (NESP-980211, US trial) subjects were on dialysis (mainly HD) and received study drugs by SC or IV route. A total of 122 patients were randomised (darbepoetin alfa: 91; r-HuEPO: 31). The mean ROR in Hb in both treatment groups was within the European Best Practice Guideline recommendation. A Hb response was achieved in 72% of patients in the darbepoetin alfa group and 84% in the rHuEPO group. This reflects the 40% higher starting dose for r-HuEPO in this trial.

An open label setting was used for this trial since the primary and secondary efficacy endpoints were based on Hb response, and as haemoglobin is a standardised and objective measurement, the primary and secondary endpoint would not be affected by the open or blinded nature of the study.

Conversion phase

Controlled studies

Two pivotal phase 3 studies (NESP-970200 in Europe and Australia, duration 52 weeks, and NESP-980117 in United states and Canada, duration 28 weeks) evaluated the ability of darbepoetin alfa to maintain haemoglobin in the predefined target range, when CRF subjects on dialysis and stable on r-HuEPO therapy, were converted to darbepoetin alfa therapy. Patients in both studies changed to darbepoetin alfa therapy at an equivalent weekly dose but reduced frequency or remained on their r-HuEPO therapy. The dose of darbepoetin alfa for each patient was calculated in both studies using a formula on protein mass basis (200 U r-HuEPO = 1µg darbepoetin alfa). The route of administration remained the same as before conversion. There were three time periods of study

- the dose titration period (20 – 24 weeks),
- an evaluation period (8 weeks),
- and the maintenance period (20 weeks) (NESP-970200 only).

Both studies were "non-inferiority" studies, designed to show that darbepoetin alfa is not inferior to r-HuEPO for treating anaemia in CRF patients. The non-inferiority design was chosen since effective therapy with r-HuEPO is currently available for treatment of anaemia in patients with CRF European Best Practice Guidelines). Darbepoetin alfa would be considered non-inferior to r-HuEPO if the lower limit of the 2-sided 95% CI for the mean difference in change in haemoglobin between Darbepoetin alfa and r-HuEPO was above –0.5 g/dl (NESP 970200) and respectively –1.0 g/dl (NESP 980117). Beside the ROR of haemoglobin, several secondary parameter were prospectively defined to assess stability of haemoglobin measurements over the time.

Study **970200** is a multicentre, randomised, open-label, comparative study designed to demonstrate that IV or SC administration of darbepoetin alfa is comparable to r-HuEPO in preventing of anaemia in CRF patients receiving haemodialysis or peritoneal analysis. Patients were randomised on a 2:1 ratio (Darbepoetin alfa to r-HuEPO) and changed to darbepoetin alfa therapy at an equivalent weekly dose but reduced frequency or remained on their r-HuEPO therapy. 344 patients received darbepoetin alfa (39% IV, 61% SC), 175 patients received r-HuEPO (42% IV, 58% SC). Patients received the study drug for up to 52 weeks.

Study **980117** is a multicentre, randomised, double blind trial designed to demonstrate that IV darbepoetin alfa is not inferior to IV r-HuEPO for treating anaemia in clinically stable patients with CRF with chronic renal failure receiving haemodialysis and with stable r-HuEPO therapy. Patients were randomised on a 2:1 ratio (darbepoetin alfa to r-HuEPO) and changed to darbepoetin alfa therapy at an equivalent weekly dose but reduced frequency or remained on their r-HuEPO therapy. Due to an administrative error in the randomisation scheme, the treatment group allocation was reversed and patients were randomised in a 1:2 ratio (darbepoetin alfa to r-HuEPO). 169 patients were treated with darbepoetin alfa, 338 with r-HuEPO. The reversal in the randomisation process did not affect the statistical power of the treatment group comparison.

Both studies indicate that darbepoetin alfa was not inferior to r-HuEPO. The lower boundary of the 95% confidence interval for the difference in mean change in haemoglobin (darbepoetin alfa minus r-HuEPO) was far above the pre-specified clinical acceptable difference of –1.0 g/dl for NESP-980117 as well as above the more rigorous criteria of –0.5 g/dl for the NESP-970200. This applied equally to "per protocol" and "intention to treat" analysis sets and demonstrates that darbepoetin alfa is not inferior to r-HuEPO for maintaining subjects haemoglobin concentration within the predefined target range. The percentage of patients with "unstable" haemoglobin concentrations, percentage of haemoglobin levels within the target range and therapeutic range were similar in both treatment

groups. In both treatment groups and in both studies the median dose of study drug during the evaluation period was the same, and the change in median dose from baseline to evaluation was zero. This indicates that the dosing conversion formula was an appropriate starting point for subjects switching from r-HuEPO to darbepoetin alfa therapy. Nevertheless, because of the individual variability, and as with rHuEPO, titration to optimal therapeutic doses is expected for individual subjects. Overall 97% of darbepoetin alfa treated patients maintained their reduced dose frequency at the evaluation period.

Analyses of the primary efficacy endpoint in study 970200 by route of administration and dialysis modality (pre-defined in the protocol) demonstrated that darbepoetin alfa is comparable (not inferior) to r-HuEPO for maintaining haemoglobin in chronic renal failure patients.

Non-controlled studies

To investigate the long-term safety, a multicentre, open-label, single arm study (NESP-**980140**) was designed to evaluate safety and tolerability of chronic IV or SC darbepoetin alfa-administration for up to 52 weeks. The enrolled dialysis patients were on a stable dose of IV or SC r-HuEPO therapy. The subjects were changed to darbepoetin alfa at reduced dose frequency. The darbepoetin alfa starting dose was based on the previous r-HuEPO dose using the conversion formula. 703 patients received darbepoetin alfa for a mean duration of 45 weeks. The mean haemoglobin was maintained within the pre-defined target range and the median weekly dose was also maintained at the same level relative to baseline. 96 % of the subjects maintained their reduced dose frequency of darbepoetin alfa. These data demonstrate that darbepoetin alfa once weekly/once every two weeks by the IV or SC route, is safe and effective in maintaining Hb in patients with CRF undergoing dialysis.

A second multicentre, open-label, single arm safety study (NESP-**980160**) evaluated the long-term safety of darbepoetin alfa in patients who had completed one year of treatment on a previous darbepoetin alfa clinical trial. 552 patients were enrolled with a mean exposure to darbepoetin alfa for 77 weeks. Subjects on darbepoetin alfa IV or SC three-times weekly (from the NESP-**960245** and NESP-**960246** studies respectively) were switched to a reduced dose frequency, for all other subjects dose frequency as well as administration route remained unchanged. Efficacy was reported up to week 24 of treatment in this study (week 76 overall). The mean haemoglobin was maintained within the target range, the median weekly dose was maintained at the same level relative to baseline and 93% patients maintained their reduced dose frequency of darbepoetin alfa. More than 85% of subjects were within the pre-defined haemoglobin target level. The data from the interim analysis show that for patients who have already received 52 weeks of darbepoetin alfa treatment, the Hb concentration can be safely maintained at a stable level without changes in the average weekly darbepoetin alfa dose.

Comparability of the subcutaneous and intravenous route of administration

The phase 3 studies formally demonstrated the non-inferiority of darbepoetin alfa compared to r-HuEPO: the double-blind US trial (980117) was conducted in HD patients using IV administration, reflecting the main clinical practice in the US; the open-label EU/Australian trial (970200) was conducted in HD and PD patients with both SC and IV administration, again reflection clinical practice in these countries. In study 970200 the analysis of the 1° and 2° endpoints by route of administration and modality of dialysis was pre-specified in the statistical analysis plan.

The results formally demonstrated that for the study population as a whole, darbepoetin alfa is comparable to r-HuEPO in maintaining the Hb level. In accordance with the pre-specified analysis plan, darbepoetin alfa has been formally demonstrated to be comparable to r-HuEPO for the SC as well the IV route of administration. These results demonstrate that darbepoetin alfa is able to maintain Hb as effectively as r-HuEPO for up to 1 year of treatment, regardless of the route of administration (IV and SC) or dialysis modality (HD and PD).

Dose requirements not affected by route of administration

From the analysis of the data from studies 970200 and 980140 (EU/Aus safety study), the ratio of SC to IV r-HuEPO dose requirements was 0.81 and 0.74 after 6 to 12 months, representing a 19% to 26 %

dose efficiency with SC administration. This is similar to that reported in the literature. It has been suggested that the greater efficiency of the SC compared to IV r-HuEPO is due to the more favourable pharmacokinetic profile following SC administration. In contrast, the ratio of SC to IV darbepoetin alfa dose requirements was 1.08 and 1.12, suggesting on average, similar dose requirements are necessary when darbepoetin alfa is administered by either the IV or SC route. The SC and IV dose requirements for darbepoetin alfa were equivalent on a protein mass basis to the SC requirements for r-HuEPO.

Clinical safety

Patient exposure

Safety profile of darbepoetin alfa was evaluated in a total of 1578 CRF patients and compared with 591 patients with r-HuEPO therapy.

Safety data were analysed for the studies overall and separately for the 3 phases of treatment (correction, conversion and maintenance). Correction and conversion phase included data up to 20 weeks of treatment and the maintenance phase from week 21 to week 52 and from week 52 onwards.

Only one of the studies was a randomised double blind study (study 980117). The safety data from this study were therefore used as standard, on which to compare the combined safety data from all open label studies.

Adverse events and serious adverse events/deaths

In the overall comparison of safety, the proportion of **deaths** was very similar in the two groups (Darbepoetin alfa 7% and r-HuEPO 6%), although patients on darbepoetin alfa had a greater exposure to treatment due to enrolment on the long-term maintenance protocol.

When **all adverse events** were summarised a similar proportion of subjects on darbepoetin alfa (89%) and r-HuEPO (95%) experienced one or more events. Those events, considered by the investigator to be treatment related, were 25% in darbepoetin alfa and 14% in r-HuEPO. There was a similar proportion of treatment related adverse events in the darbepoetin alfa and r-HuEPO groups when summarised separately for the double-blind study, which supports the hypothesis that subjective assessment of treatment-relationship and severity of adverse events would be biased against darbepoetin alfa in the open-label studies.

Serious adverse events, and severe adverse events and those serious and severe adverse events considered by the investigator to be treatment-related were similar on darbepoetin alfa and r-HuEPO therapy.

Based on previous experience with r-HuEPO, six adverse events were prospectively defined to be of specific interest: hypertension, cerebrovascular disorder, myocardial infarction, convulsions, transient ischemic attack and vascular access thrombosis. The most common treatment-related adverse events were hypertension, vascular access thrombosis and injection-site pain (SC injection). Hypertension was reported at a similar incidence for darbepoetin alfa and r-HuEPO, and vascular access thrombosis occurred more frequently in r-HuEPO than darbepoetin alfa. The other adverse events of specific interest were reported at very low and similar incidence in both treatment groups. Analysis of odds ratios for events of specific interest occurring at haemoglobin concentration of > 12g/dl compared to 10 to 12 g/dl, indicated that there is no increased risk for any of these events when haemoglobin concentration was > 12g/dl. In addition, injection site discomfort was reported at a higher incidence with darbepoetin alfa than r-HuEPO; this adverse event was reported commonly (>1%, ≤10%).

No notable differences in the overall adverse event profiles between darbepoetin alfa and r-HuEPO were noted when data were analysed by route or frequency of administration, dose of study drug or modality of dialysis. When summarised by patient's age, incidence of deaths and of serious and severe adverse events increased similarly in both treatment groups with age. This reflects either the expected pattern of deaths and adverse events that would be anticipated with age than an increased risk

associated with darbepoetin alfa and r-HuEPO therapy. When summarised the overall incidence of adverse events by race, Black patients had a higher incidence of hypertension. This is based on the known co-morbidity in this population and does not reflect any treatment effect of darbepoetin alfa or r-HuEPO.

As hypertension is the most common adverse event with both darbepoetin alfa and r-HuEPO, and as most of deaths where due to cardiac events, control of blood pressure is an important consideration in all CRF patients at study. There were no overall changes in blood pressure or heart rate in study although there was considerable variability in individual blood pressure measurements.

The safety profile of darbepoetin alfa was also evaluated separately for the 3 different phases of treatment: correction, conversion and maintenance. In correction phase all studies were open-label and proportion of patients treated with r-HuEPO was too low for conclusive comparison, but the overall proportion of deaths and adverse events did not appear to differ between the groups.

In the conversion phase and in the maintenance phase there was also no difference in safety profile between the two treatment groups. There was no evidence in the maintenance phase that adverse event profile changed as treatment exposure increased to greater than 52 weeks.

Post Marketing experience: aggravated hypertension, cerebrovascular disorders, myocardial infarction, angina pectoris and skin reactions. A warning on possible cross reactivity of epoetin antibodies with different epoetins and to a number of adverse drug reactions is been also added in the SPC.

Laboratory findings

Due to nature of disease and its treatment, patients with CRF commonly experience marked changes in some laboratory parameters. Therefore modified reference ranges for laboratory parameters were defined. There were no consistent changes in individual parameters, that could be attributed to study drug.

Immunogenicity of darbepoetin alfa

Due to theoretical concern of antibody formation to darbepoetin alfa, serum was collected and assayed regularly in all studies for seroreactivity. A total of 1534 darbepoetin alfa subjects and 572 r-HuEPO subjects have been tested with exposure up to 20 weeks, up to 21 to 52 and greater than 52 weeks. No patient had a confirmed seroreactive assay in either treatment group. There was discussion as to the sensitivity and specificity of the current standard antibody assays. The company committed to investigate ways of improving their current assays or developing new assays for detecting potential darbepoetin alfa and r-HuEPO antibodies, and to provide updated antibody monitoring data from study 980160 as a post-marketing agreement.

Analysis of the safety database did not show any difference in incidence of ‘allergic” (potentially IgE mediated) adverse events among darbepoetin alfa-treated subjects compared with rHu-EPO-treated subjects, regardless of whether the subjects had a history of allergy or whether the study drug was administered SC or IV.

5. Overall conclusions and benefit/risk assessment

- **Quality**

The finished product is supplied as a sterile, colourless solution at various concentrations and volumes in Pre-filled Syringes and vials for single use.

The integrity of the active substance (darbepoetin alfa) during manufacturing process, storage and handling, as well as the impact of different formulation components was studied extensively using a variety of analytical techniques. The active substance is produced in Chinese hamster ovary (CHO) cells by recombinant DNA technology. The development genetics are adequately described. The cell

bank system used for production is adequately described. Testing on both the master and working cell banks has been performed according to the ICH guideline Q5D.

None of the excipients is of animal origin. Foetal bovine serum (FBS) was a constituent of the growth medium, both for the development of MCB/WCB and seeding phases in culture vessels. Scientific information was included in the original application, allowing the rapporteur to conclude that this reagent complies with the requirements of the TSE Note for Guidance. On 22 November 2000, the applicant submitted TSE certificates for two of the suppliers of this FBS. The certificate for the third supplier of FBS is pending. No other reagents on animal origin, covered by the scope of the TSE Note for Guidance are used in or during the manufacture of Aranesp®.

Product specifications, control methods and validation for the active substance, excipients and finished product are adequate. The storage period for both pharmaceutical forms (vials and syringes) is 18 months at 2-8°C.

Appropriate commitments were made by the company to provide, after authorisation, further information on various parts of the documentation.

- **Preclinical pharmacology and toxicology**

Preclinical data were presented in order to justify, whether darbepoetin alfa can be considered as an alternative therapy to r-HuEPO. Its erythrogenic, pharmacological action was dose-related and restricted to erythropoietic cells *in vitro* and *in vivo*. The effects were reversible on cessation of dosing. The toxicity profile of darbepoetin alfa was demonstrated to be widely identical to that of r-HuEPO, and adverse effects in the tests could be interpreted as being due to an exaggerated pharmacological effect (i.e. polycythemia, hematocrit > 60%), rather than related to the serum concentration of darbepoetin alfa. Serum reactivity against darbepoetin alfa has sometimes been seen in animals, but only after very high doses. It has not led to loss of the effect of injected darbepoetin alfa, nor did it appear to interfere with the normal control of erythropoiesis. The pharmacodynamic differences between darbepoetin alfa and r-HuEPO were principally shown to be in line with the theory about the role of sialylation grade of the glycan residue. In comparison to r-HuEPO, the pre-clinical findings with darbepoetin alfa did not raise any additional issues of concern for clinical safety.

- **Efficacy**

The pharmacokinetics of darbepoetin alfa was examined in adult CRF patients on dialysis by IV and SC route after single and multiple dosing at different dosing frequencies. The pharmacokinetic studies showed that darbepoetin alfa has a slower clearance and a significantly longer terminal half-life when compared to r-HuEPO. Multiple dose pharmacokinetic studies and darbepoetin alfa trough level concentrations confirmed that there is no accumulation of darbepoetin alfa over the time. The pharmacokinetic profile of darbepoetin alfa was similar in adult and paediatric CRF patients. However, due to the lack of clinical data in young paediatric patients (≤11 years), darbepoetin alfa should only be used in children ≥11 years of age until more data become available. No pharmacokinetic studies were specifically performed for elderly CRF subjects. However, elderly patients were adequately represented in the study population (42% of patients at study were ≥ 65 years of age). Analysis of more than 700 darbepoetin alfa trough level concentrations demonstrated that elderly CRF patients were not at higher risk of darbepoetin alfa accumulation than CRF subjects ≤ 65 years of age. The bioequivalence study in healthy subjects indicated that the HSA+ and HSA-formulations of darbepoetin alfa are bioequivalent.

Correction of anaemia was studied in CRF patients on dialysis and pre-dialysis by IV and SC route. A starting dose of 0.45µg/kg administered once weekly was effective for increasing the Hb. Rate of rise of haemoglobin (1 to 2 g/dl per month for clinical use of r-HuEPO) and the target haemoglobin range (11 to 13 g/dl) were in accordance to the recommendations in the European Best Practice Guidelines. Comparison with r-HuEPO as comparator drug suggested that effect of darbepoetin alfa was similar to that of r-HuEPO when equivalent doses were used. A total of 328 CRF subjects on darbepoetin alfa

and 68 CRF patients on r-HuEPO were investigated for correction of anaemia, a sample size, which is sufficient to verify the efficacy of darbepoetin alfa to correct anaemia in CRF subjects.

The conversion studies showed that darbepoetin alfa could maintain a patient's haemoglobin concentration as effectively as r-HuEPO with less frequent dosing. darbepoetin alfa dose can be calculated from previously administered r-HuEPO dose using a dose-conversion formula which equates the protein mass of the two molecules (200 U r-HuEPO = 1 µg darbepoetin alfa). Less frequent dosing does not lead to greater instability of haemoglobin concentration than with r-HuEPO. After correction of anaemia and conversion from r-HuEPO to darbepoetin alfa patients continued to be treated with darbepoetin alfa to maintain Hb concentration. Haemoglobin concentration was well controlled for up to more than 1 year. There was no evidence of any change in dose or frequency of darbepoetin alfa treatment indicating drug accumulation or diminution of the effect. In the conversion studies (including dose- and schedule-finding studies) darbepoetin alfa was evaluated in 1248 patients and compared with 525 r-HuEPO patients. This sample size is sufficient to verify the efficacy of darbepoetin alfa to maintain a subjects haemoglobin concentration within the predefined target range. The majority of patients then continued into the maintenance phase.

In accordance with the pre-specified analysis plan, darbepoetin alfa has been formally demonstrated to be comparable to r-HuEPO for the SC as well the IV route of administration. These results demonstrate that darbepoetin alfa is able to maintain Hb as effectively as r-HuEPO for up to 1 year treatment, regardless of the route of adminstration (IV and SC) or dialysis modality (HD and PD). From the analysis of the rHuEPO treated patients in studies 970200 and 980140 (EU/Aus safety study), the ratio of SC to IV r-HuEPO dose requirements was 0.81 and 0.74 after 6 to 12 months, representing a 19% to 26 % dose efficiency with SC administration (similar to that reported in the literature). In contrast, the ratio of SC to IV darbepoetin alfa dose requirements was 1.08 and 1.12, suggesting that on average, similar dose requirements are necessary when darbepoetin alfa is administered by either the IV or SC route. The SC and IV dose requirements for darbepoetin alfa were the same as the SC requirements for r-HuEPO.

- **Safety**

The safety profile of darbepoetin alfa was assessed in 1578 CRF patients treated with darbepoetin alfa and 591 treated with r-HuEPO. 847 of 1578 (54 %) received SC darbepoetin alfa. Adverse events were consistent across all studies and were similar in the two treatment groups – darbepoetin alfa and r-HuEPO. The majority of AE were due to underlying disease. Only hypertension, vascular access thrombosis and injection-site pain (SC route) were consistently reported as related to study drug.

The duration of treatment with darbepoetin alfa for safety evaluation exceeds the extent of population exposure to assess clinical safety (ICH-E1 guideline).

The clinical results showed that the safety profile of darbepoetin alfa is similar to that of r-HuEPO when administered by the IV or SC route. The Odds-ratio (95 % CI) between darbepoetin alfa and r-HuEPO for the incidence of adverse events showed that there is no evidence that adverse events occurred more frequently with IV darbepoetin alfa compared to r-HuEPO therapy. When administered by the SC route, the only event that occurred more frequently during darbepoetin alfa treatment was injection site pain.

The company committed to investigate ways of improving their current assays, or developing new assays, for detecting potential darbepoetin alfa and r-HuEPO antibodies, and to provide updated antibody monitoring data from study 980160 as a post-marketing agreement.

Benefit/risk assessment

Based on the CPMP review of data on quality, safety and efficacy, the CPMP considered by consensus that the benefit/risk profile of Aranesp was favourable in the following indications: Treatment of anaemia associated with chronic renal failure in adults and paediatric subjects ≥ 11 years of age.

6. Treatment of anaemia in adult cancer patients with solid tumours (non-haematological malignancies) receiving chemotherapy

6.1 Introduction

Patients with anaemia have a reduction in RBC mass and hgb, which translates in a decrease in the oxygen-carrying capacity of their blood. Normal hgb concentrations are between 12 and 18 g/dl, with slight variations due to methodological differences between laboratories. Anaemia is common in patients with cancer and has a multifactorial aetiology. It may be related to the malignancy itself and its extent, as well as to the type, duration and intensity of myelosuppressive chemotherapy. Moreover, most patients with cancer have been shown to have inappropriately low levels of circulating EPO for their degree of anaemia, reflecting a change in this homeostatic mechanism. The incidence of anaemia severe enough to result in blood transfusions may be as high as 60 % in certain tumour types. Anaemic patients with cancer may experience symptoms as fatigue, dizziness, shortness of breath, and cardiovascular symptoms such as palpitations and cardiac failure. Such clinical sequelae may decrease the quality of life of these patients. Furthermore, a potential relationship between the correction of anaemia and increased survival in patients receiving chemotherapy has recently been discussed.

Currently, therapeutic options for anaemia in cancer patients are RBC transfusions or r-HuEPO. Transfusion of RBCs can be associated with non-haemolytic and haemolytic transfusion reactions, iron overload in heavily transfused patients, or the transmission of infections. Safety and screening requirements in transfusion therapy have increased the logistics and cost of transfusion therapy thus restricting transfusions to cases of severe and/or symptomatic anaemia. r-HuEPO has provided an alternative to blood transfusions in the treatment of symptomatic anaemia which is still not severe enough to merit transfusions with current policies. However, a clear dose response relationship for rHuEPO has not been established, and 40% to 50% of patients show no hgb response at all or a delayed response.

6.2 Chemical, pharmaceutical and biological aspects

The highest pack size currently approved is 0.6 ml of a 500 µg/ml solution. The MAH has applied for a new pre-filled syringe pack size (1.0 ml of a 500 µg/ml solution) arguing that when a dose increase to 4.5 µg/kg/week is required, heavier patients may need 2 injections from the currently approved range of prefilled syringes to receive the correct dose.

The manufacturing process remains unchanged for the additional dosage strength and the new packsize is covered by the validation program provided in the original application. Batch analysis data for 3 lots of the additional presentation are provided and the results are within the approved specification. The container closure system remains unchanged. Additional stability studies are not required since full time stability data covering the shelf life of 24 months at 2-8°C for all dosage strengths have been previously evaluated. The additional dosage strength of 500 µg in 1ml prefilled syringe is thus approvable.

6.3 Toxico-pharmacological aspects

The MAH has undertaken a sequence of preclinical *in vitro* and *in vivo* studies to support the oncologic indication. Additional studies have been submitted to address outstanding pharmacodynamic questions.

Table 1 Summary of preclinical studies submitted.

Study Number	Study Title
01-0128	*In vitro* activity of *desialylated (DS) NESP and rHuEPO* on human erythroid progenitor cells
10 11 19	Limited *tissue in vitro binding study* of de-sialylated novel erythropoietin stimulating protein and erythropoietin to human tissue
10 12 80	Limited *tissue in vitro binding study* of *de-sialylated* novel erythropoietin stimulating protein and *de-sialylated erythropoietin* to human tissue
10 11 57	Pilot pharmacokinetic study to determine the effect of a partial hepatectomy on the clearance of NESP, rHuEPO, and ^{3}H-mannitol in male Sprague-Dawley rats following a single intravenous administration
01-025	Dose and dosing frequency of NESP in normal mice
PP 01-101	NESP relative to single dose chemotherapy
10 12 69	Pharmacokinetic and pharmacodynamic *multidose study* of NESP administered subcutaneously to female BDF-mice *following carboplatin and irradiation* exposure
01-026	Effect of NESP dose and scheduling in a murine model of *multicycle chemo- and radiotherapy*
10 11 85 and 10 11 85 TK Huntingdon 00-2676	4-week *toxicity and toxicokinetic* study of *NESP and G-CSF* in the rat via subcutaneous administration with a 4-week recovery

Pharmacodynamics related to the proposed indication

The anaemia caused by renal impairment is mainly due to limited production of endogenous EPO, whereas anaemia in cancer is multifactorial. The causes may include intrinsic factors such as proliferative defects in stem cells, multiple intrinsic causes such as chronic disorders, marrow infiltration, or nutritional deficiency. There may also exist extrinsic causes like mechanical erythrocyte destruction such as microangiopathic haemolytic anaemia; chemically induced anaemia e.g. by cancer chemotherapy); infection-induced anaemia; antibody-mediated processes; hypersplenism and occult or overt blood loss. Thus, the different disease processes and treatments may in turn affect the response to erythropoetins.

To determine the dose/frequency relationship of darbepoetin alfa and to provide a link to previous preclinical reports, detailed studies were undertaken to establish the interval between injections and the amount of darbepoetin alfa required to obtain a defined hgb response. In study 01-025, darbepoetin alfa was administered to mice across a range of doses and at frequencies ranging from continuous infusion to 1 injection every 4 weeks. It was established that the dose of darbepoetin alfa required to obtain a mean incremental rise in hgb of 1g/dl increased logarithmically as the interval between injections was increased. As previously established, the slower clearance of darbepoetin alfa relative to r-HuEPO allows less frequent injections to support a similar increment in hgb levels. These results indicate, however, that there may be a limit to the dosing interval. From these data, it is impossible to determine where that limitation may be in humans, as the lifespan of erythrocytes in mice is considerably shorter than in humans (40 days compared to the reported 120-days in normal humans). Moreover, the largely unknown and potentially highly variable red cell lifespan in patients with cancer adds another layer of complexity.

The influence of extrinsic or iatrogenic causes of anaemia such as chemotherapy was studied in another series of experiments as part of study PP 01-101. The administration of darbepoetin alfa at various times relative to a series of different chemotherapy agents was studied to gain insight into the suppression of erythropoiesis caused by a broad range of chemotherapy agents with disparate mechanisms of action. The effects on anaemia induction decreased in the following order: Carboplatin/irradiation > 5-FU > Cyclophosphamide > Busulfan. The magnitude of the increase in hgb was dependent on the number of days of pre-treatment, with a longer interval between darbepoetin alfa and chemotherapy administration leading to higher pre-treatment hgb levels. On the other hand,

there was no evidence of sensitisation of the erythroid compartment with darbepoetin alfa regardless of the timing of the administration. Further, it is speculated that the cohort of erythrocytes produced by the early darbepoetin alfa exposure was more resistant to the effect of treatment than the normal population of the red cells. This may be an effect of the age distribution of the circulating erythrocytes. In a second series of experiments in female mice (01-026), a model of multicycle combined chemo/radiotherapy was developed and used to examine the effects of darbepoetin alfa dosing as marrow damage accumulated over several cycles of therapy. While in earlier cycles expected pharmacological responses were observed, due to incremental cytotoxic damage of myeloid stem cells, the same doses failed to restore normal values in the latter two cycles. An increase in the doses of darbepoetin alfa during later cycles could be an alternative to overcome the problem of decreased bone marrow response.

A series of follow-up studies to the original *in vitro* binding study with intact darbepoetin alfa and rHuEPO (study report 100160 in the original MAA) were carried out to address issues raised in the initial MAA. Study 01-028 assessed the *in vitro* activity in erythroid progenitor assays of desialylated (DS) versions of rHuEPO and darbepoetin alfa. Both proteins possessed more *in vitro* BFU-E colony-stimulating activity than their fully sialylated counterparts and were also identical in terms of potency for colony induction. Thus, the altered primary structure of darbepoetin alfa has no effect on its binding affinity to its receptor or on its biological activity *in vitro*. Limited tissue *in-vitro* binding studies (studies 101119 and 101280) were conducted to prove that the desialylated molecules do not bind to extraneous receptors and would not be a concern for carcinogenic or proliferative effects in human tissues. The results with the partially and fully desialylated forms of darbepoetin alfa and rHuEPO were identical to the characteristics exhibited previously by intact darbepoetin alfa and rHuEPO and consistent with binding to the EPO receptor.

Pharmacokinetics

Two additional pharmacokinetic studies have been conducted in support of the oncology indication. Study 101157 investigated the role of the liver in the clearance of intact darbepoetin alfa via a partial hepatectomy study in rats and did not implicate the liver as a major clearance organ for intact darbepoetin alfa. Although desialylation and subsequent removal by the liver is thought to be the major clearance pathway for darbepoetin alfa, there is the possibility that the bone marrow may play a role in either the distribution or clearance of darbepoetin alfa. Study 101269 investigated the impact of multicycle chemo/radiation therapy on pharmacokinetic (PK) properties of darbepoetin alfa. The half-life of darbepoetin alfa was increased immediately following chemo/radiation therapy but tended towards normal values by week 4 of the cycle, suggesting an influence of the extent of myeloablation in the PK properties of darbepoetin alfa.

Toxicology

A 4-week toxicity and toxicokinetic study (101185) with a 4-week recovery period has been conducted in rats (n= 20 or 30 per group) to assess the toxicity and toxicokinetics of darbepoetin alfa and rHuG-CSF when administered in combination at sc doses as high as 100 µg/kg/week and 1000 µg/kg/day respectively for 4 weeks. Expected pharmacological effects were observed, erythropoiesis with darbepoetin alfa and granulopoiesis with rHuG-CSF. Respective ELISA tests showed a high, dose-dependent incidence of antibodies to rHuG-CSF and a low non dose-dependent incidence (13/120 animals) of antibodies to darbepoetin alfa. A cell-based test for darbepoetin alfa antibodies revealed 9 animals (between 1 and 3 animals per group) had neutralising antibodies to darbepoetin alfa with no dose-dependency observed. Eight of these 9 animals with neutralising antibodies had decreases in reticulocytes at week 4. Changes in bone remodelling and osseous proliferation (e.g. hypertrophic osteopathy, myelofibrosis, remodeling of endosteal and periosteal bone) were present in the skeleton of the extremities of many animals.

The toxicokinetics of rHuG-CSF and darbepoetin alfa administered in combination were also characterised in this study. For a given dose of darbepoetin alfa, high co-administration of rHuG-CSF had little impact on haematocrit. Differences in relative clearance and/or the volume of distribution after multiple administrations were observed and were probably secondary to antibody formation

against darbepoetin when given in this combination. Formation of anti-drug antibodies is a common finding when administering non-rodent proteins into a rodent model system. It was recognised that PK or immunogenic effects of this combination have not been reported in any patients treated with rHuG-CSF and darbepoetin alfa.

Conclusion

Overall, the pharmacokinetic data show that the intrinsic pharmacologic properties of darbepoetin alfa are comparable to those of r-HuEPO, but the increased sialic acid content allows less frequent dosing and superior performance to similarly administered r-HuEPO. New dosing paradigms can be developed to exploit the extended half-life of darbepoetin alfa, which lead to effective support of hgb in the anaemias of chronic disorders, single cycle multiagent chemotherapy and multiple cycle combination chemo/radiation therapy.

The toxicological findings in the darbepoetin alfa/GCSF combination study (101185) are not regarded to be of major concern given the relatively high doses administered, the extended treatment time with rHuG-CSF (1 month compared to 5-7 days in humans), a low or absent dose-response relationship, the apparent species-specific nature of the effects (absent in monkeys and humans), and the fact that the adverse event spectrum in humans does not greatly differ from the better known combination of rHuEPO with rHuG-CSF. Regarding the immunogenicity findings, the high doses administered and the longer treatment time with rHuG-CSF are acknowledged. Although anti-darbepoetin alfa antibodies have not been detected in clinical trials. The MAH has committed to further studying both the possible antigenicity of darbepoetin alpha and immunologically mediated events.

6.4 Clinical aspects

Four clinical studies, designed to investigate darbepoetin alfa in patients with solid tumours and lymphoproliferative malignancies (LPD) receiving platinum or non-platinum containing chemotherapy, were included in the submission. In addition, the pharmacokinetic profile of darbepoetin alfa in cancer patients, and the dose-response relationship of various dosing schedules and across various tumour types and chemotherapy regimens were addressed. The studies were conducted in Europe, USA, Costa Rica, Australia, and Canada in accordance with Good Clinical Practice. An overview of the clinical program is given below.

Table 2 Overview of the clinical Trials

Study No.	**Type of trial**			**Schedule** [a]	**Comparator**	**n** darbepoetin alfa/comp/Total
990146	Pharmac o kinetic	Chemotherapy	Cancer patients	QW	None	29
990114	Dose Response	Chemotherapy	LPD [b]	QW	Placebo	55/11/66
980290	Dose Response	Chemotherapy	Solid Tumors	QW and Q2W	**RHuEPO**	344/**85**/429
980291	Dose Response	Chemotherapy	Solid Tumors	Q3W	Placebo	198/51/249
980297	Phase III	Chemotherapy (Platinum Rx)	Lung cancer	QW	Placebo	156/158/314

[a] Schedule of administration: QW = once weekly; Q2W = every 2 weeks; Q3W = every 3 weeks

[b] LPD = Lymphoproliferative malignancies (eg multiple myeloma, Hodgkin's Disease and Non Hodgkin's Lymphoma)

Data from a phase 2 dose finding study (990111) conducted in patients with cancer not receiving chemotherapy was also submitted by the MAH, but was outside the scope of the indication and hence was not assessed in this procedure.

In the pivotal trial (980297) darbepoetin alfa was compared to placebo in patients receiving platinum-containing chemotherapy in lung cancer. The design was based on the assumption that the European standard of care is still RBC transfusions in patients with severe and/or symptomatic anemia. It is worth noting that the MAH sought scientific advice from the EMEA/CPMP (November 1999) with regards to the acceptability of hgb response as a primary endpoint for a pivotal registration study and from the FDA on the design of the pivotal trial. The CPMP advised the design of a non-inferiority trial with the active comparator rHuEPO and *hgb response* was regarded as an appropriate primary endpoint.

Supportive studies included two dose response studies evaluating weekly administration of darbepoetin alfa. An open-label randomized, rHuEPO controlled phase II trial (980290 part A) in patients with solid tumours receiving cytotoxic chemotherapy and a randomized, double blind, placebo controlled phase I/II trial (990114) in patients with lymphoproliferative disorders receiving cytotoxic chemotherapy. Additional data from two other dose-finding studies (980290 part B and 980291) at alternative schedules (every 2 week and every 3 week administration, respectively) were also included.

Clinical Pharmacology/Pharmacokinetics

The pharmacokinetics of darbepoetin alfa were assessed in one study specifically designed for this purpose (study 990146). Additional PK data were also obtained from a phase II dose-response study (study 980290).

Study 990146

The primary objective of this open-label study was to assess the pk profile of weekly darbepoetin alfa therapy administered subcutaneously or intravenously in subjects with non-myeloid malignancy receiving multicycle chemotherapy. Subjects were treated with weekly sc darbepoetin alfa during 3 cycles of chemotherapy. Darbepoetin alfa was administered at a starting dose of 2.25 μg/kg and was increased at week 7 to 4.5 μg/kg in those subjects who had an absolute hgb concentration ≤ 12.0 g/dl and who did not exhibit a hgb response (defined as an increase > 1.0 g/dl over baseline) during the initial 6 weeks of treatment. Pharmacokinetic samples, including weekly trough samples, were collected throughout the study. Intensive pharmacokinetic profiles over 168 hours were assessed during the first week in both the first and third cycles of chemotherapy. EPO (endogenous) and darbepoetin alfa serum concentrations were determined using an enzyme-linked immunosorbent assay (ELISA).

Results

Overall 29 patients (21 had solid tumours and 8 lymphoproliferative malignancies) were enrolled and received at least one dose of darbepoetin alfa.

The results indicate that darbepoetin alfa is slowly absorbed after sc administration, reaching mean peak concentrations approximately 91 hours (median = 94.5) hours after a single dose, with no apparent difference observed after multiple doses ofdarbepoetin alfa, although small expected increases (<2-fold) in darbepoetin alfa serum concentrations were observed as steady state was approached. Mean peak darbepoetin alfa serum concentrations after single (10.6 ng/ml) and multiple (11.3 ng/ml) doses slightly increased as expected, with no unexpected accumulation over the 7 to 9-week dosing period. In general, the weekly darbepoetin alfa trough serum concentrations within a cycle followed a similar pattern in each cycle, increasing to a peak in week 2 with a subsequent decline in weeks 3 and 4. Analysis of pre- darbepoetin alfa dose trough levels (c_{min}) revealed an approximately 3-fold increase of " darbepoetin alfa trough serum levels" during the first week of each chemotherapy cycle, suggesting possible confounding fluctuations in endogenous EPO serum concentrations due to either induction of endogenous EPO or suppression of the bone marrow by the chemotherapy administered. This hypothesis has been verified by analysing data from study 980291 and reanalysing data from 990146 with the new F12 monoclonal antibody ELISA, specific for endogenous EPO and which does not detect darbepoetin alfa. This analysis has confirmed that endogenous EPO accounts for approximately 30-40% of the trough sample composite signal. Taking

into account the normalisation of the pk data to correct for endogenous EPO, the overall findings in this study are considered representative of the pk properties of darbepoetin alfa.

An additional intravenous (iv) arm was intended to support the potential iv administration of darbepoetin alfa in future studies. As expected, darbepoetin alfa peak serum concentrations were substantially higher following iv versus sc dosing. The PK properties of darbepoetin alfa remained constant over time, and no drug accumulation was observed.

Study 980290 A

This was a randomised open-label dose-finding, active-controlled multicentre study of sc darbepoetin alfa for the treatment of anaemia in subjects with solid tumours receiving multicycle chemotherapy. The primary objective was to assess the safety of darbepoetin alfa administered once a week. PK assessment was a secondary objective. Seven dose levels of darbepoetin alfa were evaluated (0.5, 1.0, 1.5, 2.25, 4.5, 6 and 8 µg/kg) administered weekly for a period of 12 weeks. Subjects randomised to receive rHuEPO received 150 U/kg 3 times/week with a dose doubling at week 8 if the initial response to therapy was inadequate. No such dose increases were permitted for the darbepoetin alfa dose cohorts.

The efficacy assessments included change in the hgb concentration from baseline, the proportion of subjects who achieved a hgb response (increase of ≥ 2g/dl from baseline without a RBC transfusion in the preceding 28 days) and the time to hgb response. Since subjects in the darbepoetin alfa groups were not permitted to increase dose during the study treatment period whereas subjects in the rHuEPO control arm were allowed to increase dose in the case of lack of response, direct comparison of efficacy between the darbepoetin alfa dose cohorts and the rHuEPO control is confounded.

The intent-to-treat (ITT) analysis set is the primary data set. PK data were generated in a subgroup of patients at trough and 48 hours post-dosing.

Results

Pharmacokinetics

Trough and 48-hour post-dose data from 211 patients were analysed. Both mean trough serum concentrations and 48-hour post-dose serum concentrations at week 12 were higher (about 1.6 fold) than at week 4 for all dose groups. Overall, results were consistent with dose-linearity, generally time-linear pk and minimal accumulation of darbepoetin alfa.

Two hundred and eighty eight patients were randomised, 269 received at least one dose of study drug and 175 subjects completed study treatment. A total of 216 patients received darbepoetin alfa, whereas 53 subjects received rHuEPO. Baseline demographics and disease characteristics such as primary tumor type or disease stage were similar for rHuEPO- and the all-darbepoetin alfa group.

The mean duration of exposure was approximately 10 weeks in both treatment arms and the mean weight-adjusted average weekly dose for all darbepoetin alfa groups was very close to the planned doses.

Safety

Deaths were comparable between the treatment groups (8% darbepoetin alfa vs.11% rHuEPO) and were mainly due to disease progression. Two subjects in the darbepoetin alfa group experienced bilateral pulmonary emboli and discontinued study drug. Serious adverse events (SAE) were reported in a higher proportion of subjects in the rHuEPO group (43% vs.29%). A higher proportion of subjects in the 8.0 µg/kg/week cohort reported SAEs compared with the other darbepoetin alfa dose cohorts. Two SAEs were considered treatment related (darbepoetin alfa) by the investigators: deep vein thrombosis and gastrointestinal bleeding.

Overall, the incidence and type of adverse events were comparable among treatment groups. Treatment related injection site pain was noted (mostly mild to moderate in intensity) and this finding was reflected in the SPC.

No dose-response relationship was observed for the incidence of adverse events. No clear dose limiting toxicities were apparent for the administered doses. No serum samples were reactive in the anti- darbepoetin alfa antibody screening.

Efficacy
An apparent dose-response effect at doses of 0.5 to 4.5 µg/kg was observed for the Kaplan-Meier proportions of subjects achieving a hgb response and for the Kaplan-Meier proportion of subjects who achieved a hgb correction, with the maximum effect observed in the 4.5 µg/kg/week dose cohort and no apparent additional benefit at doses of 6.0 and 8.0 µg/kg/week. Weekly doses of 2.25 µg/kg resulted in at least 50% of subjects achieving a hgb response. There was no apparent dose-response relationship for the proportion of patients receiving a transfusion from week 5 to the end of treatment (EOTP).

Due to the confounding influence of rHuEPO dose increases, the study was not designed to allow for formal comparisons of efficacy between rHuEPO and darbepoetin alfa. However, the dose regimen of 4.5 µg/kg once weekly appeared to be more active [e.g. mean (SE) change in hgb concentration from baseline of 1.9g/dL (0.32)] compared with the rHuEPO 150 IU/kg 3 times a week/group (1.1g/dL (0.25), whereas the 1.5 and 2.25 µg/kg doses (1.1g/dL (0.28) and 1.3g/dL (0.23) respectively) appeared to have similar activity compared to rHuEPO.

Clinical efficacy: pivotal trial

The pivotal trial 980297 was a double-blind, placebo-controlled, randomised study of darbepoetin alfa for the treatment of anaemia in lung cancer subjects receiving multicycle platinum-containing chemotherapy. Three hundred and twenty patients with either non-small cell lung cancer (NSCLC) or small-cell lung cancer (SCLC) were included into the trial in Europe, Australia and Canada. Patients with hgb concentrations of ≤ 11.0 g/dl could participate if their anaemia was predominantly due to cancer or chemotherapy. Patients who had received more than 4 RBC transfusions within the last 4 weeks or any transfusion within 2 weeks before randomisation were excluded. Darbepoetin alfa was administered at 2.25 µg/kg s.c. weekly for a maximum of 12 weeks. Dose doubling (4.5 µg/kg weekly) was performed in patients whose hgb concentration had increased ≤ 1.0 g/dl from baseline at the beginning of week 6.

The primary *objective* was to show superiority of darbepoetin alfa over placebo by assessing the proportion of subjects who receive RBC transfusions from week 5 to EOTP. Secondary objectives compared the effectiveness of darbepoetin alfa with placebo based on: the number and proportion of subjects achieving a hgb response; the correction of anemia; the timing and quantity of transfusions; HRQOL scores and safety.

Safety endpoints included the incidence and severity of adverse events, the incidence of death as well as the maximum increase of hgb, the proportion of subjects with hgb concentrations > 15.0 g/dl (men) or > 14.0 g/dl (women), information on tumour response and the presence of anti- darbepoetin alfa antibodies.

Results

Three hundred and twenty subjects were randomised, 314 received study medication (darbepoetin alfa = 156, placebo = 158) and were included in the ITT analysis set and 219 subjects completed the study. The primary analysis data set, used for the primary endpoint, consists of 297 patients (darbepoetin alfa = 149, placebo = 148). The demography and baseline characteristics were well balanced between the groups.

The results are presented in the table below:

Table 3: Efficacy of Darbepeotin alpha in reducing RBC transfusions (Kaplan-Meier Proportions presented)

Endpoint	Estimate of Percentage	Placebo	darbepoetin alfa 2.25 (µg/kg/wk)	Difference in Percentages darbepoetin alfa - Placebo	P-Value
RBC Transfusion during weeks 5 to EOTP*	(95% CI)	51 (43, 60)	21 (15, 28)	-24 (-35, -13)	<0.001
RBC Transfusion during weeks 1 to EOTP	(95% CI)	60 (52, 68)	26 (20, 33)	-25 (-35, -14)	<0.001

* Primary endpoint

Darbepoetin alfa reduced the Kaplan-Meier proportion of subjects receiving RBC transfusions from week 5 to the EOTP from 51% in the placebo group to 21% in the darbepoetin alfa group. This weighted (by tumour type and region strata) difference of 24% is highly significant ($p < 0.001$) and was consistently demonstrated regardless of the methods used to account for subject withdrawals or the statistical analysis methods. The "proportion of subjects with either a hgb ≤8.0 g/dl or a RBC transfusion from week 5 to the EOTP", was also assessed. The estimated proportions are slightly higher using this alternative definition: 62% placebo vs. 29% darbepoetin alfa. This represents a weighted difference of 30% (95% CI: 19, 41; $p < 0.001$) in the darbepoetin alfa group and is consistent with the results from the primary analysis. Thus, the effect of darbepoetin alfa in reducing the proportion of subjects receiving a RBC transfusion is robust to differences in transfusion policies among centres.

Independent effects of tumour type, region, and baseline hgb were observed. However, despite these effects, darbepoetin alfa significantly reduced the proportion of subjects requiring a RBC transfusion. In addition, darbepoetin alfa prolonged the time to the first transfusion; the 25th percentile was reached at 8.0 and 12.7 weeks in the placebo and darbepoetin alfa groups, respectively. Darbepoetin alfa also reduced the mean (SE) number of standard units of RBCs transfused: darbepoetin alfa 0.67 (0.14) units, placebo 1.92 (0.27) units. The mean (SE) number of days when a transfusion was required was also reduced: darbepoetin alfa 0.3 (0.1) days, placebo 0.9 (0.1) days. No difference in transfusion rates was found in patients receiving carboplatin only when compared to patients receiving cisplatin as well.

The proportion of patients achieving a hgb response was significantly different in favour of the darbepoetin alfa group [difference between groups: 29 % (95 % CI: 18, 40) ($p < 0.001$)]. The proportion of patients achieving a hgb correction was significantly different in favour of the darbepoetin alfa group [difference between groups: 43 % (95 % CI: 32, 53) ($p < 0.001$)]. Hence, the results for the secondary endpoints are consistently in favour of darbepoetin alfa and support the results for the primary endpoint.

Finally, the effects on tumour progression/death were followed in a long-term follow-up (median 11 months). The analyses stratified by tumor type (NSCLC or SCLC). There was no evidence that patients in the darbepoetin alfa group had an increased risk relative to subjects in the placebo group with respect to time to disease progression, time to death, and time to disease progression or death.

Safety

The number of deaths was comparable (22 darbepoetin alfa, 19 placebo), with most of them due to disease progression and none considered treatment related. Similar numbers of patients withdrew due to an AE (10 darbepoetin alfa, 13 placebo), mostly due to disease progression. Overall the incidence

and type of AEs were similar, with nausea, vomiting, asthenia and fatigue being the most commonly reported ones. Similar proportions of patients reported serious adverse events (60 darbepoetin alfa, 58 placebo), only one of these was regarded related to darbepoetin alfa: worsening of leg ulcers, peripheral edema, maculopapular skin efflorescence (necrotic ulcerative vasculitis, unconfirmed diagnosis).

Treatment-related AEs were reported for more patients treated with darbepoetin alfa (13) compared to placebo (8). The most frequent AEs were injection site pain (6 darbepoetin alfa, 3 placebo, all but 1 mild intensity) and nausea (3 darbepoetin alfa, 1 placebo). Treatment-related severe AEs were reported for 4 patients receiving darbepoetin alfa (thrombocytopenia, dyspnoea, maculo-papular rash, deep vein thrombosis) and for 2 patients in the placebo group (fatigue, deep vein thrombosis).

No evidence for anti- darbepoetin alfa antibodies was found following assays at baseline and at least once during treatment.

Clinical Efficacy: Supportive Studies

Study 990114
This was a randomised, double-blind, parallel placebo-controlled, dose-finding study of darbepoetin alfa for the treatment of anemia in 66 subjects with lymphoproliferative malignancies receiving chemotherapy. Patients were randomised in a 1:2:2:1 ratio to receive darbepoetin alfa at doses of 1.0, 2.25, or 4.5 μg/kg once weekly or a matched volume of placebo for 12 weeks.

The primary *objective* was to assess the relationship between darbepoetin alfa dose and hgb response. The primary *endpoint* was the time to sustained hgb response (defined as an increase in hgb of ≥ 2.0 g/dl from baseline, sustained for at least 28 days or until EOTP in the absence of RBC transfusion during the period of sustained response and the preceding 28 days).

Results

Higher response rates were observed in the darbepoetin alfa group relative to the placebo group for hemoglobin-based endpoints. While this study was not designed to make formal comparisons between groups, statistically significant differences between darbepoetin alfa and placebo were observed for hgb response ($p = 0.003$) and correction ($p = 0.029$). In addition, the incidence of transfusions from week 5 to EOTP was lower in the darbepoetin alfa dose groups than in the placebo group.

No deaths occurred on study and no patient withdrew due to an adverse event. Similar proportions of patients reported adverse events (darbepoetin alfa 95 %, placebo 91 %).

Due to the size of this study the clinical dataset was considered too small to approve the use of darbepoetin alfa in these patients (e.g. in patients with Hodgkin's Disease and non-Hodgkin's Lymphoma). This is reflected in the approved indication i.e. "solid tumours (non-haematological malignancies)" and the "warnings and special precautions section" of the SPC.

Study 980290 B
Study 980290 B, was a randomised, open label, rHuEPO controlled, dose-finding study of sc darbepoetin alfa once every two weeks for the treatment of anemia in subjects with solid tumors receiving multicycle chemotherapy. One hundred and seventy six patients were randomised to one of the four darbepoetin alfa groups or to 40000 U/wk rHuEPO, 160 received at least one dose of study drug, and 104 subjects completed 12 weeks of study treatment. Baseline demographics with respect to gender, age and race were similar for rHuEPO and the darbepoetin alfa groups.

Darbepoetin alfa was active at this schedule of administration and that there was no apparent loss of efficiency moving from once weekly to once every 2 week administration (Section 5.1 of the SPC mentions the fact that darbepoetin alfa had similar effectiveness when administered as a single

injection either weekly or once every 2 weeks, in general, without any increase in total dose requirements).

The percentage of deaths on study was comparable between the treatment groups (8% NESP vs.16% rHuEPO) and were mainly due to disease progression. Fifteen percent of subjects in the NESP group and 6% in the rHuEPO group were withdrawn from the study for an AE.

Study 980291
This was a randomised, double-blind, placebo-controlled, dose-finding study of sc darbepoetin alfa once every three weeks for the treatment of anemia in subjects with solid tumors receiving multicycle chemotherapy. The primary objective was to assess the safety of darbepoetin alfa.

The number of deaths was comparable (NESP, 5 %; placebo: 8 %) and none was considered treatment related. No patients withdrew due to treatment-related adverse events. Similar proportions of patients reported AEs (NESP 96 %, placebo 94 %) and SAEs (NESP 29 %, placebo 35%). Only one SAE was regarded related to NESP (pulmonary embolism).

Overall the incidence and type of adverse events were similar, with nausea, fatigue, vomiting, constipation and diarrhoea being the most commonly reported ones. The efficacy results from this study indicated that darbepoetin alfa is active at dosing schedules of every 3 weeks in this population.

Discussion

Efficacy
Given the many possible combinations of tumour type and chemotherapy regimens in tumour-associated anaemia, it cannot reasonably be expected to investigate the efficacy of darbepoetin alfa in a confirmatory trial for each and every combination. A certain degree of generalisation will always be necessary. The MAH chose lung cancer representing a population of those receiving platinum based chemotherapy and showed superiority over placebo for these patients in a tightly controlled confirmatory study. This information was then generalised by using supportive trials for tumour-associated anaemia in other forms of tumours and different chemotherapeutic agents (e.g. studies 980290 and 990114). The data generated through the darbepoetin alfa programme provide evidence that the efficacy and safety of darbepoetin alfa are independent of the type of malignancy in patients receiving platinum and non-platinum containing chemotherapy. Since the efficacy of darbepoetin alfa is mediated by the same mechanism of action and the same receptor as rHuEPO, literature regarding the treatment of tumour-associated anaemia with rHuEPO can be considered as supportive data. In this respect, several recent large studies totalling almost 10,000 patients indicate that the correction of anaemia by rHuEPO seems to be independent of tumour type and chemotherapeutic agents. For these reasons the generalisation from lung cancer patients receiving platinum-based chemotherapy to the treatment of anaemia in solid tumours receiving any kind of chemotherapy is acceptable. The notable exception is Hodgkin's Disease and non-Hodgkin's Lymphoma, where the size of the clinical dataset is too small to approve the use darbepoetin alfa in these patients. This is reflected in the approved indication i.e. "solid tumours (non-haematological malignancies)" and the "warnings and special precautions section" of the SPC.

The supportive trials are neither confirmative in character nor are they powered to prove differences between darbepoetin alfa and the corresponding control group (placebo in 990114 and 980291 and rHuEPO in 980290B) due to their design as phase I/II trials. Nonetheless, the development plan to investigate the clinical effectiveness in the intended population with trials of high internal quality is acknowledged. In addition, the design of controlled, blinded dose-response trials in this complex therapeutic situation with a variety of tumour types and cytotoxic regimens allows to differentiate between AEs related to the concomitant chemotherapy and the study treatment more accurately than would have been possible in uncontrolled phase I/II trials.

The pharmacokinetic results are consistent with dose linear pharmacokinetics over a wide dose range (0.5 to 8 µg/kg weekly and 3 to 9 µg/kg every two weeks). Pharmacokinetic parameters did not change on multiple dosing over 12 weeks (dosing every week or every two weeks). There was an

expected moderate (< 2 fold) increase in serum concentration as steady state was approached, but no unexpected accumulation upon repeated administration. These findings are reflected in section 5.2 of the SPC. The recommended *dose regimen* is 2.25 µg/kg administered weekly, as used in the pivotal trial. The increase in dose is covered in section 4.2 of the SPC, where it is indicated that the dose should be doubled if the increase in hgb is less than 1 g/dl after 4 weeks. Section 5.1 of the SPC mentions the fact that darbepoetin alfa had similar effectiveness when administered as a single injection either weekly or once every 2 weeks, in general, without any increase in total dose requirements.

Regarding the pivotal trial, the primary endpoint is considered relevant and the results are sufficiently consistent between the different study centres, thus ruling out any severe intra- and inter-investigator effects. As regards the trial design, the CPMP requested a non-inferiority trial to obtain information on the relative efficacy and safety of darbepoetin alfa vs. rHuEPO. The data presented have demonstrated efficacy of darbepoetin alfa against placebo or rHuEPO controls in various clinical settings. More importantly, due to the variations in physiological responses of patients to EPO, any clinical study which reflects "real life" conditions must incorporate titration rules regarding the applied dose based on the patient's response and hgb level. In the case of a non-inferiority trial, where both trial arms are pharmacological active, such a titration against the study endpoint would "normalise" the results of both the active and the control arm and tend to hide any potential differences between the arms, thus limiting the information that could be derived from such a trial. Taking this into consideration and the fact that the pivotal study has demonstrated the efficacy of darbepoetin alfa compared to placebo on both transfusion and hgb endpoints, the divergence from the Scientific Advice is acceptable.

Safety Overview
As defined in the original MAA, the modification of the amino acid sequence in darbepoetin alfa compared to human erythropoietin raises a theoretical concern regarding potential immunogenicity. Although there has been no evidence of any treatment related antibodies to darbepoetin alfa, the seriousness of developing neutralising antibodies with severe sequelae has been highlighted by the recent increase in the incidence of Pure Red Cell Aplasia, mostly in patients receiving s.c. Epoetin alfa. Whilst the BIAcore assay developed by the MAH has been recognised as current 'state of the art', there are limitations to sensitivity. The MAH has committed to further develop and improve the sensitivity or specificity of the currently available battery of immunoassays (RIP, BIACore 3000 and cell based neutralising bioassay), and will continue to seek advice on an ongoing basis from both immunology experts in European regulatory agencies and other external experts to determine the most relevant assays to monitor potential antibody formation. The MAH intends to perform post-marketing studies in the nephrology and oncology indications, in which antibody formation will be monitored.

The clinical trials supporting this indication did not include sufficient patients with long-term survival expectancy receiving adjuvant and neo-adjuvant chemotherapy in early stage tumours. In view of the recent increase in the use of highly myelotoxic therapeutic regimens in such patients and given the lack of experience in the use of recombinant human erythropoietin in this setting, a warning has been added to section 4.4 of the SPC to advise caution in the use of erythropoetic agents in this population.

The incidence of AEs and SAEs reported were generally similar between darbepoetin alfa, placebo and rHuEPO and there was no increase in incidence with the higher dosage groups of darbepoetin alfa.

The incidences of arthralgia, peripheral oedema and injection site pain were higher in darbepoetin alfa patients than in placebo patients. These AEs have been added to section 4.8 of the SPC.

7. Treatment of anaemia in adult cancer patients with non-haematological malignancies receiving chemotherapy

Introduction

The anaemia caused by renal impairment is mainly due to limited production of endogenous EPO, whereas anaemia in cancer is multifactorial. The incidence of anaemia severe enough to result in blood transfusions may be as high as 60 % in certain tumour types. The causes may include intrinsic

factors, such as proliferative defects in stem cells, multiple intrinsic causes such as chronic disorders, marrow infiltration, or nutritional deficiency. There may also exist extrinsic causes like mechanical erythrocyte destruction such as microangiopathic haemolytic anaemia; chemically induced anaemia (e.g. by cancer chemotherapy); infection-induced anaemia; antibody-mediated processes; hypersplenism and occult or overt blood loss.

The use of erythropoietic agents is an alternative treatment option for patients with anaemia undergoing treatment with chemotherapy. Similar effects have been reported across all tumour types in randomised, placebo-controlled trials where administration of erythropoietic agents has increased haemoglobin levels, reducing RBC transfusion rates, and increasing subjects' quality of life. The substantial data reported have led to erythropoietic agents being indicated for use in treating chemotherapy-induced anaemia.

In the original oncology application, a phase II dose-finding study (study 990114) with a limited number of subjects (n=66) with lymphoproliferative disease was included. The number of subjects was limited, and although no efficacy or safety concerns were identified for darbepoetin-alfa in these subjects, the size of the dataset was not considered large enough to support an initial indication in this patient group.

To provide further data on the efficacy and safety of darbepoetin alfa in this patient population, an additional, pivotal, randomised, placebo-controlled, phase III study in patients with lymphoproliferative malignancies receiving chemotherapy has now been submitted Study NESP-20000161 in Lymphoproliferative Malignancies. The data generated through the darbepoetin alfa programme provide evidence that the efficacy and safety of darbepoetin alfa are independent of the type of malignancy in patients receiving platinum and non-platinum containing chemotherapy. Since the efficacy of darbepoetin alfa is mediated by the same mechanism of action and the same receptor as rHuEPO, literature regarding the treatment of tumour-associated anaemia with rHuEPO has been considered as supportive data.

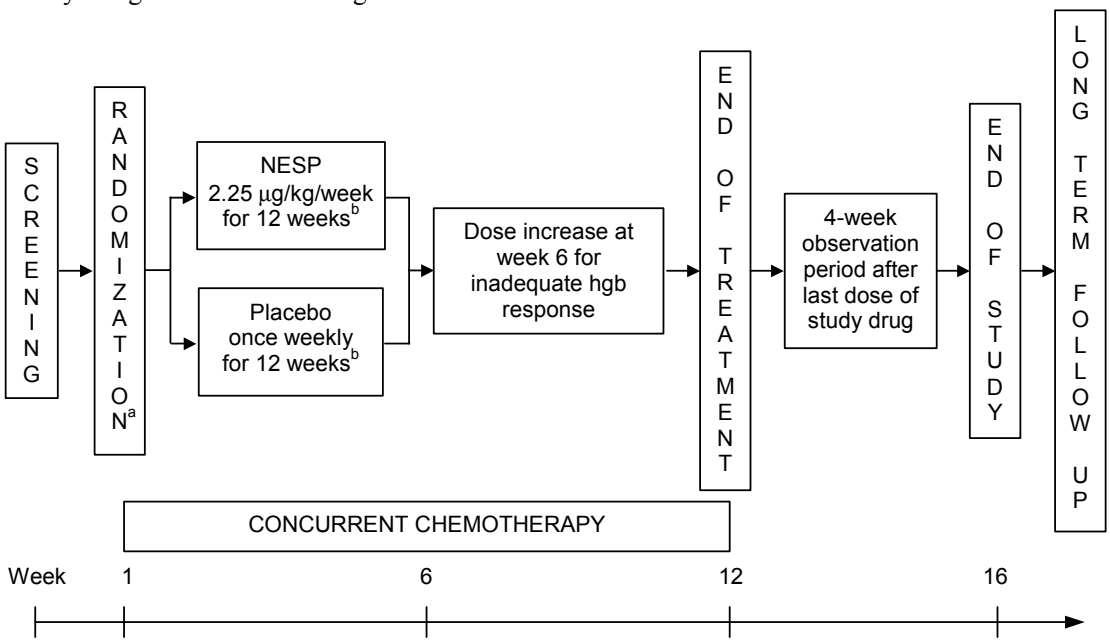
2 Clinical aspects

Study NESP-20000161 in Lymphoproliferative Malignancies

Study Design

This is a double-blind, randomised, placebo-controlled parallel-group study designed to confirm the safety and demonstrate the efficacy of 2.25 µg/kg once weekly in anaemic subjects (haemoglobin ≤ 11.0 g/dl) with lymphoproliferative malignancies receiving chemotherapy

The study design is shown in the figure below:



Study Population

Three hundred and forty nine anaemic subjects (haemoglobin ≤ 11.0 g/dl) with lymphoproliferative malignancies receiving chemotherapy were randomised to receive darbepoetin alfa at a dose of 2.25 µg/kg once weekly or matching placebo. Overall, 173 (50%) subjects had myeloma and 171 (50%) had lymphoma (non-Hodgkin's lymphoma, chronic lymphocytic leukaemia, Hodgkin's disease, or Waldenström's macroglobulinaemia). Most subjects (71%) had stage III/IV disease, although more subjects in the darbepoetin-alfa group (50%) had progressive disease at baseline, compared to placebo (43%). Eighty two percent had received prior chemotherapy, 15% had received prior radiotherapy, and 27% were defined as heavily pretreated with chemotherapy

The *primary efficacy endpoint* was the proportion of subjects who, by the end of treatment phase, achieved a haemoglobin response (defined as an increase in haemoglobin of ≥ 2.0 g/dL) over baseline in the absence of RBC transfusions in the 28 days before laboratory sampling.

The main *secondary endpoint* was the proportion of subjects who received a RBC transfusion from week 5 to the end of the treatment phase, estimated using the Kaplan-Meier method. The first 4 weeks of treatment were not included in the main RBC transfusion analysis as historical data suggest that statistically significant reductions in RBC transfusion have only been reported after the first month of treatment. Furthermore, there was a Health-related Quality of Life (HRQOL) secondary endpoint estimated as the mean change from baseline to the end of treatment phase for the Functional Assessment of Cancer Therapy (FACT) Fatigue Scale score.

Possible antibody formation to darbepoetin alfa was evaluated. A battery of validated and approved assays were used: a radioimmunoprecipitation (RIP) screening assay to detect seroreactivity to darbepoetin alfa and a BIAcore assay and cell-based bioassay to detect neutralising or inhibiting effects on the activity of darbepoetin alfa.

The main safety endpoint in this study was to assess if the proportion of adverse events was consistent with that expected for subjects with lymphoproliferative malignancies who are receiving chemotherapy.

Results

Efficacy

Darbepoetin alfa increased the Kaplan Meier proportion (95% CI) of subjects achieving a haemoglobin response from 18% (95% CI 12, 24) in the placebo group to 60% (95% CI 52, 68) in the darbepoetin alfa group, a significant difference of 42% (95% CI 32, 52) ($P < 0.001$). When the data were analysed by malignancy type, a significant improvement in the rate of haemoglobin response with darbepoetin alfa relative to placebo was observed both in subjects with lymphoma (64% vs 13%, $P < 0.001$) and subjects with myeloma (56% vs 23%, $P < 0.001$). Significantly fewer subjects randomised to darbepoetin-alfa had a RBC transfusion from week 5 to the end of treatment phase (31%) compared with the placebo group (48%), an adjusted difference of -17% (95% CI: -28, -7; $p < 0.001$). This finding was consistently demonstrated regardless of the approach used to account for subject withdrawals or the use of an alternative definition of the endpoint (RBC transfusion or haemoglobin = 8.0 g/dL). Treatment with darbepoetin alfa also resulted in a statistically significant and clinically meaningful increase in the proportion of subjects achieving haemoglobin correction and the change in haemoglobin from baseline compared with placebo.

For the HRQOL endpoint, after adjusting for the effect of baseline score, improvements in fatigue, as measured by the Functional assessment of Cancer Therapy-fatigue (FACT-fatigue) scale were also observed ($P=0.032$).

Safety

The overall safety profile of darbepoetin-alfa in this study was similar to placebo and was consistent with that expected for subjects with lymphoproliferative malignancies who are receiving chemotherapy. Ten subjects (6%) in the darbepoetin-alfa group and 4 subjects (2%) in the placebo group died during the study or within 30 days after the last dose of study drug. No deaths were considered treatment related, and the most common cause of death was disease progression. Six subjects (3%) receiving darbepoetin-alfa and 7 subjects (4%) receiving placebo withdrew from the study because of a nonfatal AE. The incidence and severity of adverse events (AEs) was similar to that observed with placebo and was not related to haemoglobin concentrations, except for injection site pain (mild to moderate), which was more frequently reported on darbepoeitin-alfa, Serious adverse events (SAEs) were reported for 51 subjects (29%) receiving darbepoetin-alfa and 63 subjects (37%) receiving placebo. The incidence of SAEs considered related to blinded study drug by the investigator was 2% in both the darbepoetin-alfa and placebo groups. No relationship was observed between the rise in haemoglobin or the maximum haemoglobin concentration achieved and any particular AE or pattern of AEs. Changes in laboratory variables and vital signs were similar between the darbepoetin alfa and placebo groups. The proportion of subjects hospitalised was similar between treatment groups; however, the mean number of days hospitalised per subject was lower for the darbepoetin alfa group (5.3 days; SE: 0.8) than for the placebo group (7.2 days; SE: 1.0).

No antibody generation was observed.

Discussion

Darbepoetin alfa 2.25 μg/kg/week administered SC once weekly to subjects with lymphoproliferative malignancies receiving chemotherapy significantly increased the proportion of subjects achieving a haemoglobin response and decreased the proportion of subjects requiring a RBC transfusion compared with placebo. Furthermore, the Quality of Life data (HRQOL FACT-Fatigue score) suggest that darbepoetin alfa might reduce fatigue in these patients.

The data generated through the darbepoetin alfa development programme provide evidence that the efficacy or safety of darbepoetin alfa is independent of the type of malignancies in patients receiving chemotherapy containing platinum and non-platinum agents. This seems to be true also for patients with lymphoproliferative disease.

A dose of 2.25 µg/kg darbepoetin-alfa once weekly was effective in the majority of subjects with lymphoma or myeloma increasing the haemoglobin response rate significantly and reducing the percentage of subjects receiving RBC transfusions compared with placebo.
Regarding safety, the incidence of adverse events in subjects receiving darbepoetin alfa was similar to that of subjects receiving placebo and seems to be dominated by the underlying disease. The incidence of deaths on study was also similar in the darbepoetin alfa and placebo treatment groups. The incidence of severe or serious adverse events was also similar between treatment groups. Available long-term follow-up data do not indicate any adverse impact of darbepoetin alfa or other erythropoietic agents on either overall survival or progression-free survival. Results of the observational long-term follow-up study will be provided annually after approval, until three years after all patients completed the blinding phase of the study to check if there is a difference between patients receiving active drug or placebo.

For growth factors like erythropoietic agents, there is a theoretical risk of stimulating or inducing malignant cells in a clinically unfavourable way. However, no such effect has become evident until now and, in this respect, on the basis of the studies submitted, lymphoproliferative malignancies do not appear to be different from other forms of cancer. However emerging data regarding EPOR positive tumours will be submitted in order to assess the risk of cell line proliferation or tumour growth. With regard to antibody formation against darbepoetin alfa, negative results were obtained throughout the nephrology and haematology/oncology studies. This includes over 550 patients who have received darbepoetin alfa for over 2 years and were followed up as part of the post-licensing commitments for the nephrology indication. There is currently no evidence of antibody-mediated PRCA with darbepoetin alfa via any route of administration in any patient population.

Benefit/risk or conclusion

Overall, study NESP 20000161 together with study NESP 990114 provide confirmatory evidence that darbepoetin alfa is effective for the treatment of chemotherapy-induced anaemia in subjects with lymphoproliferative malignancies. Darbepoetin alfa appeared to be well tolerated and was not associated with any new safety concerns in this treatment setting.

The benefit – risk relation for expanding the indication for darbepoetin alfa to the treatment of anaemia in adult cancer patients with non myeloid malignancies receiving chemotherapy is favourable.