



European Medicines Agency
Evaluation of Medicines for Human Use

July 2009
Doc.Ref.: EMEA/334517/2009

CHMP ASSESSMENT REPORT

FOR

Biferonex

International Nonproprietary Name: **Interferon beta-1a**

Procedure No. EMEA/H/C/000901

CHMP Assessment Report as adopted by the CHMP with all information of a commercially confidential nature deleted.

This should be read in conjunction with the “Question and Answer” on the withdrawal of the application.

TABLE OF CONTENTS

1.	BACKGROUND INFORMATION ON THE PROCEDURE.....	3
1.1	Submission of the dossier	3
1.2	Steps taken for the assessment of the product.....	3
2	SCIENTIFIC DISCUSSION.....	5
2.1	Introduction.....	5
2.2	Quality aspects.....	5
2.3	Non-clinical aspects.....	10
2.4	Clinical aspects	16
2.5	Pharmacovigilance.....	32
2.6	Overall conclusions, risk/benefit assessment and recommendation	32

1. BACKGROUND INFORMATION ON THE PROCEDURE

1.1 Submission of the dossier

The applicant BioPartners GmbH submitted on 24 July 2007 an application for Marketing Authorisation to the European Medicines Agency (EMA) for Biferonex, through the centralised procedure falling within the Article 3(1) and point 1.

The legal basis for this application refers to:

Centralised / Article 8(3) / Known active substance.

The applicant applied for the following indication treatment of relapsing-remitting multiple sclerosis.

Scientific Advice

The applicant did not seek scientific advice at the CHMP.

Licensing status:

Biferonex was not licensed in any country at the time of submission of the application.

The Rapporteur and Co-Rapporteur appointed by the CHMP and the evaluation teams were:

Rapporteur: **Jaana Kallio**

Co-Rapporteur: **Barbara van Zwieten-Boot**

1.2 Steps taken for the assessment of the product

- The application was received by the EMA 24 July 2007.
- The procedure started 15 August 2007.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 5 November 2007. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 5 November 2007.
- During the meeting on 10-13 December 2007, the CHMP agreed on the consolidated List of Questions to be sent to the applicant. The final consolidated List of Questions was sent to the applicant on 14 December 2007.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 23 July 2008.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 5 and 9 September 2008. The revised version of the Clinical part was circulated on 23 September 2008.
- During the CHMP meeting on 22-25 September 2008, the CHMP agreed on a list of outstanding issues to be addressed in writing and in an oral explanation by the applicant. The list of outstanding issues was sent to the applicant on 26 September 2008.
- The applicant submitted the responses to the list of outstanding issues on 21 October 2008.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to list of outstanding issues to all CHMP members on 4 and 5 November 2008.

- During the CHMP meeting on 17-20 November 2008, outstanding issues were addressed by the applicant during an oral explanation on 19 November 2008 before the CHMP.
- During the CHMP meeting on 17-20 November 2008, the CHMP agreed on a second list of outstanding issues to be addressed in writing and in an oral explanation by the applicant.
- The applicant submitted the responses to the second list of outstanding issues on 15 January 2009.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to second list of outstanding issues to all CHMP members on 4 February 2009.
- During the meeting on 16-19 February 2009, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a negative opinion for granting a Marketing Authorisation to Biferonex on 19 February 2009.

2 SCIENTIFIC DISCUSSION

2.1 Introduction

Multiple sclerosis (MS) is the most common, non-traumatic cause of neurological dysfunction in young adults. Approximately 80% of MS patients have relapsing-remitting MS (RRMS) at the onset of the disease, characterized by acute exacerbations, with interceding returns to the pre-crisis baseline, and secondarily by the appearance of a disability that may worsen after each crisis.

The mechanism of action of interferon (IFN) beta in the treatment of MS remains unclear. It is not known if the clinical benefits are due to the same mechanisms involved in its antiviral effect. In several multicenter studies, involving several hundred patients with MS, IFN beta-1a three times per week significantly delayed sustained disease progression, reduced the annual exacerbation rate, brain lesion volume and number of new lesions as measured by MRI.

The medicinal product formulation of Biferonex (interferon beta-1a) does not contain human serum albumin (HSA) present in approved formulations of commercial interferon-beta containing products.. Biferonex (interferon beta-1a) is supplied as a sterile liquid formulation in ready-to-use syringes. IFN beta-1a is indicated for the treatment of ambulatory patients with relapsing remitting multiple sclerosis who had at least 2 relapses with in the last two years. The recommended dosage of IFN beta-1a is 6 MIU (equivalent to the contents of one pre-filled syringe) to be administered subcutaneously three times per week.

No CHMP Scientific Advice has been provided. The Applicant refers the relevant CHMP Guidance.

The marketing authorisation application for the HSA-free formulation of IFN beta-1a has been submitted by BioPartners as a stand-alone application, pursuant to Article 6 of Council Regulation (EEC) No 2309/93, as amended, and Article 8(3)(i) of Directive 2001/83/EC, as amended.

This is a mixed application for a biological product, including limited number of non-clinical and clinical data on the product to be marketed, HSA-free formulation of IFN beta-1a, supported with literature. Biferonex is derived from the same genetically modified cell line/MCB that was used to produce clinical trial batches used for the approval of Avonex (Jacobs' trial). The majority of data were derived from publications. Due to the stand-alone type of application, this assessment report focuses mainly on the data on the product intended to be marketed and the bridging evidence to the previous formulation.

2.2 Quality aspects

Introduction

Beta-Interferon is a recombinant purified interferon beta-1a (IFN beta-1a) active substance solution produced by Rentschler Biotechnologie GmbH. The medicinal product is marketed by BioPartners. IFN beta-1a is produced in Chinese hamster ovary (CHO) cells and purified using chromatographic methods. The natural hIFN beta-1a consists of 166 amino acid residues with a molecular weight of approximately 22.5 kD. The secondary structure is dominated by five α helices (A-E), along with short stretches of 3_{10} helix in adjoining sequences and connecting loops. The molecule contains a disulphide bridge extending between Cys 31 of loop AB and Cys 141 of loop DE and a free Cys at position 17. The AB and DE loops are believed to play important roles in receptor interactions, and the disulphide linkage may act to coordinate their structure. IFN beta-1a is glycosylated at a single site, Asn 80 at the end of helix C. The amino acid sequence of IFN beta-1a produced by Rentschler Biotechnologie GmbH is identical to the endogenous counterpart.

Rentschler started its interferon development program in 1974 and subsequently in its subsidiary Bioferon biochemische Substanzen GmbH & Co. (Bioferon), founded in 1981 which developed recombinant interferon beta-1a (IFN beta-1a). Biogen became a partner to this project in 1984. A

suitable interferon-producing cell clone was isolated in 1986 and was designated BIC 8622. A clinical program in patients with relapsing remitting multiple sclerosis (RRMS) was initiated.

In November 1990, Bioferon supported the performance of a randomised, placebo-controlled, multi-center pivotal Phase III study with material from the BIC 8622 cell line. The material for this study was named BG9015. It was the first clinical study with recombinant interferon beta in multiple sclerosis. In this study, a dose of six million international units (MIU) was applied intramuscularly once per week.

In 1993, while the clinical study was still ongoing, the joint venture between Bioferon and Biogen ceased and production of BG9015 also ceased, however there was sufficient study material available and the clinical study was completed by Biogen [Jacobs et al. (1996)]. This study was the basis for the approval of Biogen's Avonex in the United States and subsequently in Europe.

Since that time, some process changes have been introduced to further refine production and purification of IFN beta-1a derived from the BIC 8622 cell line. One of the major changes was the change from the human serum albumin (HSA)-containing, lyophilised dosage form to the HSA-free liquid formulation.

Active substance

- **Manufacture**

The IFN beta-1a active substance is produced in CHO cells co-transfected with an expression plasmid containing the human full length gene for IFN beta-1a and a selection vector. The cell culture, harvesting and purification process have been validated.

Since the beginning of the process development in the mid 1980s, several changes have been introduced to both the cell culture and the purification processes. The current manufacturing process at pilot scale has been used for production of all batches for pivotal non-clinical and clinical trials. For earlier trials, also material from developmental manufacturing processes was used. In 2005 the pilot scale manufacturing process was scaled-up to achieve the current commercial production scale.

The Company has conducted comparability studies both between developmental manufacturing processes and between batches produced at pilot and commercial scale using the current manufacturing process. Comparability between the developmental lyophilised formulation and the current liquid formulation cannot be regarded as demonstrated because of the limitations of the analytical methods at the time of their manufacture and a lack of direct comparison of different material in the same test. For pilot and commercial scale batches of the current manufacturing process, the Company has compared results of in-process controls, characterisation data, batch release data and stability data. Generally, comparability between material from pilot and commercial scale production is regarded as proven.

Active substance has been characterised for structural characteristics (amino acid sequence and disulphide bridge pattern), glycosylation pattern (N-glycan fingerprint, antennary status, sialo status), physicochemical properties (molecular weight, charged isoform pattern, spectroscopic profile, extinction coefficient), and for specific activity. Analysis of disulphide bridge patterning revealed a fraction of potentially incorrectly paired cysteines. In response to major concern in the CHMP List of Questions (LoQ), the Company has quantitatively demonstrated that in pilot and commercial scale batches, more than 97% correct disulphide bridging can be seen. Therefore, further control of disulphide bridge patterning at batch release is not required. The characterisation of charged isoforms as well as the hydrophobic properties of the protein has been adequately conducted. The glycosylation pattern of IFN beta-1a was confirmed as fucosylated, biantennary and disialylated oligosaccharide with the composition NeuAc₂.Hex₅.HexNAc₄.Fuc. This is in accordance with the expected pattern based on previously published literature.

For impurity profiling, the Applicant has considered process- and product-related impurities. Process-related impurities arising from cell culturing and purification processes are properly described and analysed. In order to comply with the D120 major objection, the Applicant has characterised product-related impurities in required detail.

Suitability of the primary packaging has been demonstrated. The bulk DS solution is stored at 2-8°C which is not in line with the recommendation in the draft Ph. Eur monograph (recommended storage at -70°C). Storage at -70°C would however be preferable as rapid degradation by deamidation is detected at 2-8°C.

- **Specification**

The specifications for the active substance chosen cover either general EU or ICH-requirements for biotechnological products or pharmacopoeial requirements. In response to CHMP LoQ, the Applicant has introduced control for deamidated isoforms, control for impurities detected by RP-HPLC as well as control for antennary structures. The analytical methods used to analyse the new release parameters have been properly validated, except for one method for which the validation report should be provided for assessment prior to marketing authorisation. The modified specification is for most part regarded as acceptable. However, some minor modifications of the acceptance limits are requested in order to better correlate the specification with data obtained from batches used in clinical trials. In addition, the Applicant should commit to review the current acceptance limits once batch analyses data is available from 30 commercial scale active substance batches.

- **Stability**

Suitability of the primary packaging has been demonstrated. The bulk DS solution is stored at 2-8°C which is not in line with the recommendation in the draft Ph. Eur monograph (recommended storage at -70°C). Storage at -70°C would however be preferable as rapid degradation by deamidation is detected at 2-8°C. Even though the Applicant does not intend to change the storage temperature for the current manufacturing procedure, the Applicant commits to investigate storage of the DS at -70°C. As the DS is highly unstable with regard to deamidation, the currently proposed storage for 24 months at 2-8°C can only be accepted if it can be confirmed that the clinical studies were conducted with batches which are representative with respect to the degree of deamidation.

Medicinal Product

The medicinal product Biferonex is a liquid clear and colourless ready-for-use single dose solution with 6 MIU for parenteral application. The medicinal product contains the active substance interferon beta-1a produced by recombinant DNA technology, and the excipients sodium dihydrogen phosphate dehydrate, disodium phosphate, sodium citrate and DL-methionine. The concentration of the excipients is identical to the active substance bulk solution. Each single dose has a target volume (corresponding to the extractable volume) of 0.53 mL solution filled into 1 mL Hypak syringes, with a stainless steel needle. The mixing, formulation, filtration, sterile filtration and filling procedures are standard procedures commonly used for production of biotechnological pharmaceuticals. The commercial scale production process is in agreement with current guidelines.

- **Pharmaceutical Development**

There has been a major change in the formulation of medicinal product during development. The lyophilised presentation containing HSA was changed to the HSA- free liquid formulation. Presence of HSA may affect the stability of the formulation and is also likely to affect the immunogenicity, which should be considered when regarding the (pre)clinical studies performed with the HSA containing product.

The provided stability data does not justify a shelf life of 36 months at 2-8°C. Only batches produced at pilot scale have been analysed for the entire proposed shelf life, while data from commercial scale

batches are limited to one batch stored for 32 months and one batch for 18 months. Additionally, protein deamidation has not been studied.

- Adventitious Agents

Raw materials of non-animal origin, including the excipients are demonstrated to be safe regarding microbial contamination. The bioburden (bacteria and fungi) and the bacterial endotoxin content are tested as defined in Ph. Eur. The purification columns are sanitised and no microbial contamination has been detected. The materials of biological origin used during manufacturing are the cell banks, FCS and trypsin. FCS is manufactured from foetal bovine blood harvested in abattoirs from healthy dams fit for human consumption. Serum has been used during generation of the cell banks and is also used in routine cell culture as supplement of the growth medium. The FCS used for establishment of cell banks was either of Australian, USA or Canada origin. No incidents of TSE were reported in these countries at the time establishment of the cell banks. The gamma-irradiated trypsin of porcine origin is routinely controlled for parvo- and circoviruses by the vendor. The trypsin used is considered free of TSE risk

The cell banks have been tested for microbial contamination and mycoplasma and shown to be clean. Non-viral safety is adequately addressed during the manufacturing. The TSE risk of the cell banks is considered negligible. Viral testing of the cell banks have been performed on MCB, WCB 1, WCB 3 and post production cells PPC 1, originating from WCB 1. Adventitious viruses are tested both *in vitro* and *in vivo*, bovine viruses are tested *in vitro* and specific rodent viruses are indicated by mouse and hamster antibody production tests. Retroviruses and retrovirus like particles are detected by S⁺/L⁻ focus and XC plaque assays and by transmission electron microscopy. The cell banks have been demonstrated to be free from adventitious agents.

In addition to direct testing, the viral safety is assured by viral clearance studies. The viral inactivation and removal steps are considered effective, the log reductions in the individual steps are >4 logs while the overall reduction is >10 logs. The choice of spiking viruses for clearance studies is fully justified and acceptable. The nanofiltration is demonstrated effective in reducing all selected viruses, as well as the prion protein like agent PrP^{res}. In addition, inactivation at low pH has been adequately addressed.

In conclusion, the Applicant has properly followed the ICH Q5A (R1) and CPMP/BWP/268/95 guidelines on adventitious agent safety of biotechnology products. The adventitious agent risk associated with the product is therefore considered as small.

- Manufacture of the Product

Stability of the formulated bulk of eight weeks at 2-8°C and 4 weeks at 25±2°C (60±5% relative humidity) was demonstrated. Data of formulated bulk and product release was submitted and consistency demonstrated.

- Product Specification

The proposed specifications for the medicinal product include pharmacopoeial tests for interferon and additional tests for purity/impurities.

- Stability of the Product

The stability data provided were sufficient to justify a shelf life of 30 months at 2-8°C.

- GMO

Not applicable

Discussion on chemical, pharmaceutical and biological aspects

The assessment of the Company's responses submitted to the CHMP List of Questions and subsequent list of outstanding issues has revealed that there are still concerns remaining in the area of Quality pertaining to the medicinal product acceptance criteria and stability of the finished product.

Although the Avonex batches used in Jacobs trial originate from the same MCB as Biferonex and the batch analyses data submitted do not reveal considerable qualitative differences between the active substances of Avonex and Biferonex, full comparability on the quality level between the two products can not be assured. Data from Jacobs' trial batches can only be considered as supportive.

2.3 Non-clinical aspects

Introduction

All pharmacokinetic studies except the study assessing pharmacokinetic drug interactions were conducted in compliance with the GLP regulations. All toxicological studies except one of the genotoxicity studies, an *in vitro* embryotoxicity study and one of the antigenicity studies were conducted in compliance with the GLP regulations.

Pharmacology

- Primary pharmacodynamics

No pharmacodynamic studies had initially been performed with Biferonex. In response to major objections raised by the CHMP, the Applicant performed an additional PK/PD study in Cynomolgus monkey. Neopterin and 2'5'-OAS, the pharmacodynamic markers for interferon beta action, were increased in response to Biferonex. These data demonstrate that Biferonex is pharmacologically active in Cynomolgus monkey, the species used in toxicity studies. However, despite the fact that a clear PD response was discernible the dose-response relationship was weak, and correlation between PD and PK parameters was not evident, especially after repeated dosing every two days for two weeks. The Applicant interpreted these observations as normal inter-individual variation and as similar to what has been observed with another commercially available interferon beta 1a product.

All the other data assessing pharmacodynamics are derived from journal articles produced by various research labs using HSA-containing formulation of interferon beta-1a. Consequently, none of the studies were GLP-compliant. Due to the species-specificity of interferon beta-1a most of the pharmacodynamic investigations were performed in *in vitro* systems using human cell cultures assessing antiviral, antiproliferative and immunomodulating effects. As there is only indirect evidence on the mechanism of action of beta-interferons in MS, the submitted data was aimed to provide information on the anti-viral, anti-proliferative and immunomodulating activities known for interferons. Therefore, these were considered as secondary pharmacodynamics. None of the experiments were performed with the HSA-free interferon beta-1a formulation for which the marketing authorisation is applied.

Eight journal articles, two of which appeared to be meeting abstracts, were submitted to provide information on the antiviral activity of interferon beta. In these publications, interferon beta-1a exhibited inhibitory effect on herpes simplex virus, vesicular stomatitis virus, hepatitis B virus, coxsackievirus B3 and human immunodeficiency virus.

The antiproliferative activity of interferon beta-1a was investigated in several human cell lines: melanoma cells, haematopoietic progenitor cells and peripheral blood mononuclear cells, Kaposi's sarcoma cells and fibroblasts and keratinocytes. Further investigations concerned the antiangiogenic effect of interferon beta-1a in fertilized egg, as well as the radiosensitizing effect of interferon beta-1a in different cell lines: squamous cells, lung carcinoma cells and sarcoma cells.

The immunomodulating activity of IFN beta-1a was evaluated by investigating influence on synthesis, degradation or expression of several molecules: 2',5' oligoadenylate synthetase, neopterin, tryptophan, HLA class I and II antigens, β 2 microglobulin, interleukin 1 receptor antagonist protein, IFN gamma and other cytokines, interleukin 2 receptor, ICAM-1 and metalloproteinases. Also, the influence of IFN beta-1a on natural killer cell activity and antibody dependent cytotoxicity was investigated.

- Secondary pharmacodynamics and Safety pharmacology programme

No separate studies were conducted in animals concerning safety pharmacology. However, the effects of interferon beta-1a on different functions were monitored during the single-dose toxicity studies in mice, rats, guinea pigs and monkeys and repeat-dose toxicity studies in monkeys. The limited safety pharmacology observations in these repeated-dose toxicity studies revealed that Rentschler interferon beta-1a had only minor effects and was similar in this respect to other interferon beta-1a preparations.

According to the Applicant, due to species-specificity of interferons as a result of interactions with their specific receptors, classic safety pharmacology studies were considered of limited value. However, primates are considered the most relevant species and pharmacological activity of beta-interferons has been shown in monkeys. In the repeat-dose toxicity studies in cynomolgus monkeys, increase in body temperature following administration of IFN beta-1a was not observed. This is in contrast to what has been observed with another interferon beta-1a product. This divergence was considered to be a result of natural variation.

Clinical observations produced evidence for an interaction between heparin (-Ca) and natural IFN beta in the sense of an increased anticoagulatory effect with increased risk of bleeding. Therefore, an animal study was performed to elucidate if such an interaction might also occur under IFN beta-1a. The results implicate that recombinant interferon beta-1a may not have increased anticoagulatory effect in combination with heparin as the natural interferon beta.

Initially, the CHMP was concerned that no information related to e.g. specificity of receptor binding and affinity had been provided. This information is considered valuable in assessing the relevance of pharmacokinetic data produced with human serum albumin (HSA)-containing interferon beta-1a. Furthermore, the possibility that removal of HSA from the formulation may affect receptor binding properties and thereby may have a significant impact on the pharmacokinetics and toxicity could not be excluded without further data.

In response to the above CHMP concerns, additional studies have been initiated by the applicant addressing comparative receptor binding and potency. Due to technical difficulties, functional and receptor binding studies using human and monkey PBMCs did not produce conclusive results. Instead, the company provided reports in which bioactivity in terms of induction of IFN-dependent gene expression and real-time binding to immobilised interferon receptor subunits were evaluated. These data compared Biferonex and Biferonex supplemented with human serum albumin (HSA) to two commercially available IFN beta-1a products. These data suggest that the presence of HSA does not affect the pharmacological behaviour of Biferonex.

- **Pharmacodynamic drug interactions**

Clinical observations produced evidence for an interaction between heparin (-Ca) and natural IFN beta in the sense of an increased anticoagulatory effect with increased risk of bleeding. Therefore, an animal study was performed to elucidate if such an interaction might also occur under IFN beta-1a.

In a rat study, the combination of heparin with natural IFN beta, but not with IFN beta-1a at the dose of 0.5 MIU/kg led to a significant prolongation of the thrombin time in comparison to heparin alone.

These results suggest that recombinant interferon beta-1a may not have increased anticoagulatory effect in combination with heparin as the natural interferon beta.

Pharmacokinetics

Due to species-specificity of interferon beta-1a, studies on pharmacokinetics have been restricted to the Cynomolgus monkey. Serum levels were measured as integral parts of both the single and repeated dose toxicity studies in this species.

Absorption

Absorption after single dose was evaluated in three studies using intravenous, subcutaneous and intramuscular routes of administration at three dose levels 0.6, 3.0 and 15.0 MIU/kg. Absorption after repeated administration was evaluated in two studies using intramuscular and subcutaneous routes of administration. These studies were performed with a formulation of interferon beta-1a containing HSA. The original file for the Biferonex MAA contained only one study in which the HSA-free formulation intended for marketing of interferon beta-1a was used. In this study absorption after single dose subcutaneous administration at one dose level was evaluated. A new study with repeated subcutaneous administration of the HSA-free formulation was initiated in response to the CHMP List of Questions (LoQ). A validated cytopathic effect reduction assay (CPE) was used for the determination of interferon beta-1a levels in monkey serum. All studies except b/PC-518 and b/PC-521 were conducted in compliance with the GLP regulations.

After single-dose administration IFN beta-1a exhibited linear single dose pharmacokinetic characteristics. A dose-proportional increase in exposure was observed after subcutaneous and intramuscular administration when the dose was increased from 3.0 to 15.0 MIU/kg. After intravenous administration however, the exposure rose over-proportionally with increasing doses. AUC rose by a factor of 8.4 when the dose was increased five-fold from 0.6 to 3.0 MIU/kg, and by a factor of 6.3 when the dose increased from 3.0 to 15.0 MIU/kg. Consequently, decrease in total clearance by a factor of 2 was observed. $T_{1/2}$ was estimated to be 3.4h at the low dose and 6.4 and 13.9 h at the medium and high dose, respectively. All further parameters (mean residence time, volumes of distribution) showed corresponding systematic changes with increasing dosage levels. There was no observable difference between serum levels in male and female animals.

Single-dose pharmacokinetics of the HSA-free formulation of interferon beta-1a was evaluated after subcutaneous administration at the dose level of 3.0 MIU/kg. The pharmacokinetic results for the HSA-free formulation and the HSA-containing formulation were similar when comparing the results of two different studies in which IFN beta-1a was administered subcutaneously in a dose of 3.0 MIU/kg. On average, IFN beta-1a activity reached a maximum 6-7 hours after dosing and returned to the baseline level after 48 h with a mean half-life of 6-8 hours. However, the comparison of kinetics from two different studies is an unsound basis to draw conclusions on the comparability of the different formulations.

Pharmacokinetic data after repeated dosing with HSA-containing formulation of interferon beta-1a have been produced in the toxicity studies using subcutaneous and intramuscular administration routes at dose levels 0.6, 3.0 and 15.0 MIU/kg. After four weeks subcutaneous dosing every second day, a considerable increases in C_{max} and AUC values were observed. Interferon beta-1a seemed to accumulate after repeated dosing, the mean accumulation factors being 3.8 at the low dose and 4.1 at the high dose. No accumulation was observed after intramuscular dosing. T_{max} seemed to double in the high dose group after the eighth compared to the first dose. The half-lives remained unchanged in all groups. In many animals, the accumulation was attenuated by the development of neutralising antibodies. After the last dose, complete neutralisation of serum interferon beta-1a activity was observed in most of the animals. The appearance of the neutralising antibodies is likely to interfere with the interpretation of the PK data. Despite the apparent accumulation $T_{1/2}$ remained unchanged which may indicate that formation of immune complexes may have had an effect. Due to the high inherent variability of the biological response to beta-interferon and the bioassay used for determination of interferon beta-1a serum levels, the number of animals used in these studies is considered very small.

The unexpected considerable increase in C_{max} and AUC values after repeated subcutaneous dosing has not been explained. In response to the CHMP LOQ an additional PK/PD study was performed in cynomolgus monkeys with the HSA-free formulation after repeated dosing. In this study too a considerable increase in C_{max} and AUC was observed, but only at the low dose (3 MIU/kg; 3.5-fold and 7.3-fold, respectively), and not or much less at the high dose (12 MIU/kg; 0.9-fold and 1.7-fold for C_{max} and AUC, respectively). It is noted that interindividual differences in PK response are very large, making a clear interpretation of the data difficult. Clinical PK data with Biferonex (HSA-) after repeated subcutaneous dosing are limited to six healthy subjects. In that study, the accumulation factors were determined to be 1.1 and 1.9 for AUC and C_{max} , respectively. However, given the small number of subjects and large variation (CV% ranging from 46% to 81%) the value of these results is minimal. In conclusion, data on the non-clinical and clinical pharmacokinetics of Biferonex after repeated subcutaneous administration show a dose-response relation after single dose, but are highly variable after repeated dosing.

Distribution

No studies were performed to investigate distribution. However, tissue distribution studies could have been valuable. Due to species-specificity the only suitable species would have been monkey, and performing such studies in monkey would not be practical.

Metabolism and Excretion

No studies were performed to investigate metabolism or excretion. As laid down in the ICH guideline CPMP/ICH/320/95, the absence of metabolism and excretion studies with Biferonex is justified.

The effect of interferon beta-1a on hepatic drug metabolising enzymes was studied in the rat and guinea pig. In guinea pigs, slight effects in reducing metabolic capability were seen and therefore, a note regarding the possible effect of interferon beta on the cytochrome P-450 system has been added in the SPC.

Toxicology

Single-dose toxicity was evaluated in mouse, rat, guinea pig and monkey and repeat-dose toxicity in monkey. Genotoxic potential was assessed in *in vitro* studies, embryotoxicity was assessed in chicken eggs, and local tolerance was evaluated in guinea pig, rabbit and monkey. Formation of neutralising antibodies was evaluated in mice and monkey. Human serum albumin (HSA)-free formulation of interferon beta-1a, the formulation intended for marketing, has been used in a comparative local tolerance study in rabbit and in an immunogenicity assessment in monkeys. All other studies were performed with HSA-containing formulation of interferon beta-1a. All studies except b/PC-512, Chen (1997b, 1999) and Mertens (1993) were conducted in compliance with the GLP regulations. Studies in animals, especially those in non-primates are of limited value due to the species-specificity of the actions of interferon beta-1a.

- Single dose toxicity

Single-dose toxicity after intravenous administration of interferon beta-1a at dose level of 39 MIU/kg was evaluated in mice and rats. After 14 days, no signs of toxicity could be detected at the chosen dose level. In Cynomolgus monkey, intravenously administered interferon beta-1a appeared to have no drug-related toxic effects at dose levels up to 15 MIU/kg. The animals were observed for six weeks, and specific anti-HSA and anti-interferon beta-1a antibodies were detected in animals in the mid (3.0 MIU/kg) and the high dose (15.0 MIU/kg) groups from week 2 onwards. Toxicity after subcutaneous route was evaluated in guinea pigs using multiple dose levels ranging from 2 to 18 MIU/kg. After 72 hours of observation no treatment-related signs of toxicity were noted.

- Repeat dose toxicity (with toxicokinetics)

Repeat-dose toxicity was tested in Cynomolgus monkeys up to 15 MIU/kg for 4 weeks administered by the subcutaneous (once every second day) and intramuscular (once a week) routes. Following subcutaneous administration every two days for 29 days, the effects seen could be characterised as primary effects due to the pharmacological activity of interferon beta-1a and secondary effects consequent to ongoing immunological or inflammatory reactions. Primary effects observed included haematological changes – decrease in eosinophils, and, at the high dose, in neutrophils, platelets, haematocrit, haemoglobin and mean corpuscular volume. Antibodies to both HSA and IFN beta-1a were detected at all dose levels and those to IFN beta-1a were characterised as neutralising in 2/4, 3/4 and 4/4 animals at the low, mid and high dose levels, respectively. The biochemical changes observed in high dose animals (decrease in serum albumin, increase in serum globulins and increase in plasma fibrinogen) were considered secondary to ongoing inflammatory reactions resulting from the formation of immune complexes by the anti-HSA and/or anti-IFN antibodies. These complexes were the likely cause of the decrease in total complement haemolytic activity seen at the high dose and also the increase in iliac lymph node weight associated with lymphoid hyperplasia, together with the periarteritis/arteritis seen in the pancreas, kidneys and at the injection site. A single animal with high antibody titres had more extensive periarteritis affecting many organs. Similar effects were noted in animals treated once weekly by intramuscular injection, although the reactions to treatment were less marked than in the subcutaneous study. Anti-HSA and anti-IFN antibodies were found with the latter being neutralising, however these appeared later and at lower levels, as compared to the subcutaneous study.

NOEL could not be determined for either administration routes. Formation of neutralising antibodies may complicate toxicity assessment. As the neutralising antibodies could eliminate the drug, potential toxic effects could remain undetected. Moreover, due to this problem no long-term toxicity data could be produced in animals using the HSA-containing formulation at the dosages applied. Where relevant

data from animal studies cannot be obtained, the focus of risk assessment is laid in the clinical studies, and a need for careful monitoring of patients receiving Biferonex is evident.

- Genotoxicity

Genotoxic potential was evaluated in three *in vitro* studies. Potential for gene mutations and chromosomal aberrations was assessed using bacterial Ames test and *in vitro* cultured human peripheral blood lymphocytes. Interferon beta-1a was found not to induce gene mutations or have clastogenic effects.

- Carcinogenicity

Specific carcinogenicity studies were not performed with Rentschler Biotechnologie produced IFN beta-1a. No signals emerged from testing for genotoxic potential. Moreover, there is no indication from knowledge of the pharmacology of interferon beta-1a that would indicate support for or induction of proliferation of transformed cells. This is in line with the recommendations in the ICH guideline (CPMP/ICH/302/95), and therefore, the absence of carcinogenicity studies is considered justified.

- Reproduction Toxicity

No *in vivo* reproductive and developmental toxicity studies were conducted with Rentschler Biotechnologie produced interferon beta-1a. Induction of abortions has been observed with two beta-interferons currently on the market. These effects are considered as a likely class effect, and they are recognised in the proposed SPC where the initiation of treatment with Biferonex is contraindicated in pregnancy. However, an *in vitro* study evaluating embryotoxicity of interferon beta-1a was performed using the hatched egg test (HET). A dose-dependent increase in embryoletality was observed. The relevance of these data is not known. In general, the data available for reproductive and developmental toxicity of beta-interferons seems to be very limited.

- Toxicokinetic data

Accumulation of interferon beta-1a was observed after repeated subcutaneous dosing. Neutralising antibodies were detected from week 2 onwards which may have had an impact on the interpretation of PK results. The PK results in Cynomolgus monkey after repeated subcutaneous and intramuscular administration are presented in the following **table**:

Table. AUC data after repeated dosing in monkeys

Route	Dose (MIU/kg)	C _{max} (IU/mL)		AUC (IU·h/mL)	
		First	Repeat	First	Repeat
sc	0.6	83	314	1284	4818
	3.0	545	1239	8359	21914
	15.0	2969	8311	43153	177935
im	0.6	153	180	1323	1545
	3.0	1751	1316	14601	14443
	15.0	10455	9601	70824	72173

However, the appearance of the neutralising antibodies in the serum may affect the PK and toxicity results, and thus the data on pharmacokinetics and toxicity have to be interpreted with caution. The possible elimination of the drug by the neutralising antibodies may lead to a situation where toxic effects remain undetected.

- Local tolerance

Local tolerance to IFN beta-1a was investigated in guinea pigs, rabbits, and monkeys. Intraarterial, intravenous, paravenous and intramuscular administration routes were evaluated in rabbits. Subcutaneous tolerance of the HSA-containing formulation was evaluated in rabbits and in monkeys.

No sensitising properties were observed in guinea pigs after multiple epidermal applications of HSA-containing formulation of interferon-beta-1b. Intravenously administered HSA-containing formulation of interferon beta caused slight reddening of the congested vein after single dose in rabbit, whereas no findings of local irritation could be seen after intra-arterial, intramuscular or paravenous administration. No significant difference in local irritation between the HSA-containing and HSA-free formulations of interferon beta-1a after a single subcutaneous administration in rabbit could be seen. Serious injection site reactions were observed after repeated subcutaneous dosing in the toxicity study in Cynomolgus monkey. Periarteritis/arteritis was noted in high dose group animals. This was considered as a secondary reaction to immune complex formation. However, this was not tested in the absence of HSA in this species. Severe injection site reactions have not been observed in clinical studies, and therefore this observation may have clinical relevance only in patients having high titres of anti-IFN beta 1a antibodies.

- Other toxicity studies

Immunogenicity

The potential immunogenicity of interferon beta-1a has been studied in two specific studies, as well as being evaluated as an integral part of the toxicity studies. The antibody formation was evaluated in Cynomolgus monkey. Binding antibodies against both HSA and interferon beta-1a were detected in most of the animals at dose level of 3.0 and 15.0 MIU/kg after intramuscular and subcutaneous dosing, and after sc administration also at the dose level of 0.6 MIU/kg. The anti-interferon beta-1a antibodies were shown to have a neutralising capacity in the *in vitro* cytopathic effect reduction assay (CPE assay). The CPE assay used for determination of neutralising antibodies was adequately validated. Immunogenicity data with HSA-free formulation of interferon beta-1a is very limited. Formation of antibodies has been studied in six monkeys after a single subcutaneous dose. Removal of HSA from the formulation might possibly have an effect on the immunogenicity of the protein, e.g. new epitopes may be exposed leading to increased antigenicity. Deamidation and formation of aggregates may also have an effect on the immunogenic potential of the active substance. The majority of the non-clinical data has been produced with the HSA-containing formulation of IFN beta-1a and true bridging data could and can not be produced, as the interferon beta 1a product used in non-clinical studies is no longer available. Thus, although the immunogenic potential of IFN beta 1a with and without HSA may differ in cynomolgus monkeys, it is unlikely that such subtle differences could be detected in monkeys. Whether the immunogenic potential of Biferonex in humans differs significantly from other IFN beta containing medicinal products needs to be established based on clinical data.

Ecotoxicity/environmental risk assessment

As a protein, Biferonex is not expected to pose any risk for the environment, in accordance with the CHMP guideline on environmental assessment.

Discussion on the non-clinical aspects

The main concern has been the lack of adequate bridging studies with the albumin-containing formulation and the albumin-free formulation intended for marketing. Due to technical reasons and unavailability of the original interferon beta 1a product used in non-clinical studies, the applicant cannot produce the required bridging data.

However, the additional data provided suggest that pharmacological behaviour of Biferonex is not affected by the presence of HSA. Moreover, considerable quality-related differences other than deamidation, between HSA-containing commercial interferon beta 1a batches and Biferonex were not detected. However, full comparability could not be ensured. In the absence of true bridging data, and without a possibility to produce it, certain level of uncertainty remains. Regarding safety, this uncertainty would concern any potential difference in immunogenic potential. This, however, is not feasible to be investigated in animals and therefore a need for further animal studies is not foreseen. From a non-clinical point of view and within the limitations of non-clinical models used, the available non-clinical safety data can be considered to sufficiently support clinical use of Biferonex.

2.4 Clinical aspects

Introduction

The pivotal clinical trial was performed in accordance with GCP and ICH as claimed by the applicant.

The applicant has provided a statement to the effect that clinical trials conducted outside the community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

All studies were conducted with the approval of Ethics Committees or Institutional Review Boards. Informed consent was obtained for all subjects, and the studies were performed in accordance with the version of the Declaration of Helsinki that applied at the time the studies were conducted.

The assessment of the clinical documentations did not raise concerns about compliance with GCP. A GCP inspection in regard to the clinical program is not considered necessary.

Pharmacokinetics

Out of several clinical trials only one PK study (study BP IFN beta 004) conducted in healthy volunteers is considered relevant for this application.

Methods

Study BP IFN beta 004 was a randomized, open, single centre study, which was conducted in 24 healthy subjects (aged 21-42 years) to compare the pharmacokinetic profile of three different iv formulations (Biferonex (HSA-), Biferonex with added albumin (HSA+), and Rebif (in a HSA+ solution) as well as to establish the steady state pharmacokinetics of Biferonex (HSA-) after 4 subsequent sc doses of 18 MIU given at 48 hour intervals vs. Rebif. This study was divided in two parts:

Part A consisted of a 3-way crossover design during which 12 subjects received in 3 periods one single intravenous dose of 18 MIU IFN beta-1a as three different iv formulations; the three formulations IFN beta-1a were compared by means of an analysis of variance (ANOVA) with gender, subject, treatment, period and sequence as main effects in the model on the following Log-transformed PK parameters: C_0 , $AUC_{(0-inf)}$, MRT, $t_{1/2}$, CL, V_1 , V_{ss} and V_z .

Part B was a randomized parallel design during which 12 subjects received 4 x 18 MIU at 48-hour interval of Biferonex (HSA-) or Rebif. In this second part, six (6) subjects received each treatment. The comparison of PK profile after BioPartners HSA- formulation and Rebif by the s.c route was also performed by ANOVA, but was complicated by the very low concentrations measured, thus, providing only imprecise quantitative estimations.

The bioassays, ELISA and CPE, used for measurement of interferon beta-1a in serum samples are well validated and suitable for analysis of IFN beta-1a potency in different samples including human and primate serum samples. The CPE assay is, however, not specific for IFN beta-1a since it detects the antiviral activity, i.e. interferon potency, in the samples using virus-induced lysis of the target cells as outcome measure.

- Absorption

Whatever the formulation, the interferon beta-1a serum concentration profile after a single iv injection was described by a bi-exponential process constituted by a rapid decrease in serum concentrations in the 20 minutes post-injection followed by a slower one up to 2 hours after dosing. Mean half-lives of the corresponding phases were 0.040 and 0.55 hours. These short half-lives resulted in a rapid and marked decrease in interferon beta-1a: 20 minutes after injection, the interferon beta-1a concentrations represent less than 2% of those measured 2 minutes after injection.

After single iv injection, the two Biferonex formulations with added or without HSA compared rather well as shown by point estimates on pharmacokinetic parameters ranging from 86 to 121 %. However, the study was neither oriented nor powered to compare formulations using the bioequivalence approach.

- **Distribution and Elimination**

Single 18 MIU sc doses of Biferonex (HSA-) or Rebif did not elicit any consistent curve in the PK profile. In order to assess PK parameters and absolute bioavailability after s.c injection, each undetectable or low concentration was set at 3 IU/mL, i.e. the average LLOQ of the ELISA assay bound approach. The increase is very modest and quite variable between subjects and within subjects.

- **Dose proportionality and time dependencies**

Repeated s.c doses of 4x 18 MIU at 48-hour intervals did not result in significant accumulation of interferon beta-1a, whatever the formulation, but the data is not exclusive due to highly variable coefficient of variation% (CV%) ranging from 46% to 57% and 7.1 – 10 % for mean AUC of Biferonex and Rebif, respectively. Therefore, the possible accumulation of Biferonex following repeated administration cannot be excluded based on a limited PK data from only 6 patients.

- **Special populations**

No investigations have been performed in the paediatric population nor considered necessary by the Applicant at this point. The use of Biferonex in this population will have to be addressed at a later stage, including a PK analysis, in case of marketing authorisation.

- **Pharmacokinetic interaction studies**

No clinical interaction studies have been performed with Biferonex.

According to the literature, multiple administration of interferon beta-1a (Rebif) in healthy subjects did not affect the activity of CYP19, CYP2D6, and CYP3A4, with a marginal but not significant increase for CYP1A2 activity (Buchwalder et al 2000). However, it has been demonstrated that interferon beta treatment significantly reduces total hepatic CYP-450 in mice (Carelli et al 1996).

IFN beta-1a inhibitory impact on CYP450 isoenzymes has been confirmed clinically during chronic therapy with already marketed interferon beta-1a, probably triggered by a complex interaction with released cytokines. Consequently, care should be applied for patients with concomitant therapy, especially if these drugs are substrates, inhibitors and inducers of CYP450. Care is especially warranted with substances with a narrow therapeutic index like antiepileptics and many antidepressants

In conclusion, the evidence on pharmacokinetic comparability between the studied three formulations (i.e. Rebif, an EU-licensed IFN beta-1a formulation containing HSA and beta-INF with or without HSA) is considered questionable. The comparative PK data obtained from the Study BP IFN beta 004 for Biferonex without and with added HSA suggests that HSA does not affect the pharmacokinetic profile of INF-beta-1a, and that there are no significant differences in PK parameters between any of the studied three formulations following a single i.v. administration, however the trial was not statistically powered to compare different treatments using 90% Confidence Interval (CI) approach. With this limitation in mind, the question on the comparability of the PK of HSA-containing and HSA-free formulations in subcutaneous administration has been sufficiently addressed.

Pharmacodynamics

- **Mechanism of action**

The mechanism of action of IFN beta in the treatment of MS remains unclear.

Interferons are species-specific, intercellular signalling proteins. After the binding of an interferon to its receptor, an increased transcription and translation of at least 100 genes in the target cell occurs. Therefore, a number of biological effects - antiviral, antiproliferative, radiosensitising, immunomodulating and neuroimmunological - have been described for interferon *in vitro* and *in vivo*. Biological activity of IFN beta can be monitored by measuring the concentrations of surrogate serum markers 2',5'-oligoadenylate synthetase (2',5' OAS), neopterin, and Beta2-microglobulin and hMx protein.

It is not known if the clinical benefits are due to the same mechanisms involved in its antiviral effect or whether other mechanisms are involved. In several multicenter studies, involving several hundred patients with MS, IFN beta-1a three times per week significantly delayed sustained disease

progression, reduced the annual exacerbation rate, brain lesion volume and number of new lesions as measured by MRI.

- **Primary and Secondary pharmacology**

There were two PD or PK/PD studies (BP IFN beta-001 & -004) conducted with the applied formulation, BP IFN beta-1a HSA-, that was used in pivotal clinical trial.

BP IFN beta-001 Study

This study was designed to assess the pharmacodynamic profile of Biferonex (HSA-) after i.v. and s.c. administration as well as to create the link with available surrogate markers investigated with HSA+ and HSA- formulations and to gather further information on safety and tolerability of Biferonex over dose range. Six male and 6 female subjects were included. Four different doses of Biferonex from 2 of the 4 possible pairs of treatments: 1. 0.5 MIU i.v. and 1.5 MIU s.c., 2. 1 MIU i.v. and 3 MIU s.c., 3. 2 MIU i.v. and 6 MIU s.c., 4. 4 MIU i.v. and 12 MIU s.c. Serum concentrations of 2',5'-oligoadenylate synthetase (2',5' OAS), neopterin, and Beta2-microglobulin were measured.

For all 3 markers selected, treatment elicited a dose dependent increase with Emax (maximum serum response) after i.v. dosing for all three markers with similar profile for both routes. Concentrations were back to baseline within 5 to 6 days of treatment. For dose response analysis with log-log model, significance for dose was high for all markers: neopterin and beta2-microglobuline ($p < 0.0001$ for all) and 2',5'OAS ($p = 0.002$ and 0.008).

The BP IFN beta-004 Study

This study was conducted to establish the pharmacokinetic profile of Biferonex (HSA-) after i.v. administration at 18 MIU. The possible impact of albumin on pharmacokinetic profile was also studied by comparing 3 different i.v. formulations: Biferonex (HSA-), Biferonex (HSA+), and Rebif. Steady state pharmacokinetic profile of Biferonex (HSA-) after 4 subsequent s.c. doses of 18 MIU given at 48 hour intervals was tested against Rebif.

The study design was open, single centre study with 2 parts:

- Part A (12 subjects) was a randomized, latin square, 3-way crossover design with the 3 different formulations i.v. 18 MIU.
- Part B (12 subjects) was a randomized parallel design of multiple 4 x 18 MIU at 48h intervals s.c. doses of either Biferonex in HSA-free solution or Rebif.

Serial blood samples were collected over 7 days to measure serum concentrations of neopterin in Part A and B. In Part A (12 subjects), the neopterin serum profiles were identical with a marked increase for Emax noted after i.v. administration between 24 and 48 hours and a return to baseline in 7 days. There were no significant differences between treatments (Bioferon (HSA-), Bioferon (HSA+) and Rebif). In Part B, maximal effect was achieved at 24 hours and initially maintained but the response decreased after the third and fourth s.c. doses (48h intervals). Pharmacodynamic parameters did not statistically differ between Bioferon (HSA-) and Rebif.

In conclusion, the pharmacodynamic characteristics Biferonex (interferon beta-1a), (HSA-) are rather similar after i.v. and s.c administration with slightly more adverse events with the s.c. route. Pharmacodynamic properties of Biferonex (HSA-), Biferonex (HAS+) and Rebif also appear similar on the basis of these studies with no significant differences in tolerance.

Clinical efficacy

Biferonex, which is the product intended to be marketed differs from the already approved products Rebif and Avonex as it is manufactured without human serum albumin and has a pH-value of 6.8. These characteristics may lead to a better or worse systemic and local tolerability as well as to a change in immunogenicity due to a higher deamination grade and subcutaneous route of administration where, in contrast to the intramuscular route, the product is directly presented to the dendritic cells. Thus, the value of literature references in supporting the efficacy or safety of the test product is unknown. As emphasised in the quality assessment the product has to be assessed *ex-novo*

As this is a standalone application, a full comparability exercise to any of the marketed beta IFNs has not been performed by the applicant.

- Dose response studies

No clinical dose response study for Biferonex was conducted. Only previous dose response studies of Rebif and Avonex were cited. The CHMP was concerned considering the uncertain bioequivalence of the albumin-containing and albumin-free formulations.

- Main study

Study BP IFN 003: A randomised, parallel group, placebo-controlled, double-blind phase III study of Biferonex (interferon beta-1a) in the treatment of relapsing-remitting multiple sclerosis (RRMS) (ORIGIMS)

METHODS

Study Participants

Main inclusion criteria were the diagnosis of RRMS (clinically definite or laboratory supported relapsing-remitting multiple sclerosis according McDonald criteria 2001), an at least a 2-year history of MS before trial entry, a history of 2 or more exacerbations in the 2 years prior to trial entry, an EDSS score of 0 to 5.0 and a stable neurological state for at least 8 weeks at the time of the pre-trial evaluation.

Excluded were patients with an exacerbation within 60 days before inclusion, prior immunosuppressive therapy with the exception of corticosteroids for treatment of relapses, contraindications for immunotherapy and any comorbidities/co-medication that could interfere with study outcome.

Treatments

The study was a randomized, parallel group, placebo-controlled, double-blind phase III study of Biferonex in the treatment of relapsing-remitting multiple sclerosis (RRMS). Biferonex was administered in liquid solution in prefilled 0.53 mL syringe 6 million units (MIU), three times a week, 104 weeks (24 months), subcutaneously (sc).

Following the double blind trial period, treatment was extended for a further year, and all patients on placebo were switched to active treatment. This extension provided 3-year long-term data on safety and efficacy of Biferonex treatment. Data of the active extension phase (OATE) were presented to CHMP in response to the List of Questions.

Objectives

The study objective was designed to demonstrate efficacy and safety of Biferonex versus placebo (2:1 ratio) in patients with RRMS. The conduct of this study was in accordance with the relevant CHMP guideline.

Outcomes/endpoints

The primary efficacy variable was:

- The number of exacerbations over the study period of 24 months

Secondary efficacy variables were:

- Proportion of patients free from exacerbations
- Expanded disability status scale (EDSS) progression
- Integrated disability status scale (IDSS) progression
- Nine-hole peg test
- Proportion of patients with progression
- Time to exacerbation
- Total number of exacerbations
- Magnetic Resonance Imaging (MRI) disease activity

Randomisation

Randomization was adequate. There were no notable differences in the placebo group vs. Biferonex group, except in the mean age of female patients, where there was a significant difference between the two treatment groups in the PP population (38.4 ± 7.7 years in the Biferonex group compared with 35.3 ± 7.5 years in placebo group, [$p=0.0021$]). The blinding of the study continued until the final data were released. Study medication preparations were identical in appearance, packaging, and labelling. MRI analysis was performed centrally in a blinded manner. The treating physician and evaluating physician were different. Potential injection sites were covered with a dressing prior to the assessment of exacerbations and prior to the evaluation of disability by the evaluating physician. If required, paracetamol, ibuprofen or other non-steroidal anti-inflammatory agents could be given concomitantly with the study medication for relief of fever, myalgia or other flu-like symptoms.

Statistical methods

The analyses for efficacy variables were performed for both the ITT and PP data sets. The ITT set consisted of all randomised subjects who received at least one dose of study medication. The PP set consisted of all subjects from the ITT set who received at least four weeks of study medication and for whom no major protocol deviations were observed. The primary analyses were performed for the ITT set.

The primary efficacy variable was analyzed using Poisson log linear regression with the total number of exacerbations as the dependent parameter and with terms in the model for the number of exacerbations within the last 24 months, treatment and center, as well as an offset, which was the logarithm of the number of years (calculated as number of days divided by 364) the patient had been on study.

In order to assess the robustness of the model the same Poisson log linear regression analysis was done, but excluding all patients who discontinued the study prematurely. Since one interim analysis was planned, a modified two-sided significance level of $\alpha = 0.0294$ for both the interim and the final analyses were used, giving an overall 15% type I error rate.

A hierarchical test procedure was applied to the two secondary efficacy variables, MRI - disease activity and sustained progression. In case a p-value ≤ 0.0294 was calculated for the primary efficacy variable, a confirmatory analysis was done for the first secondary variable using the same significance level $\alpha = 0.0294$. If a p-value ≤ 0.0294 was calculated for the first secondary efficacy variable, a confirmatory analysis was done for the second secondary variable using the same significance level. All other tests were performed on a descriptive level with a two-sided significance level of $\alpha = 0.05$.

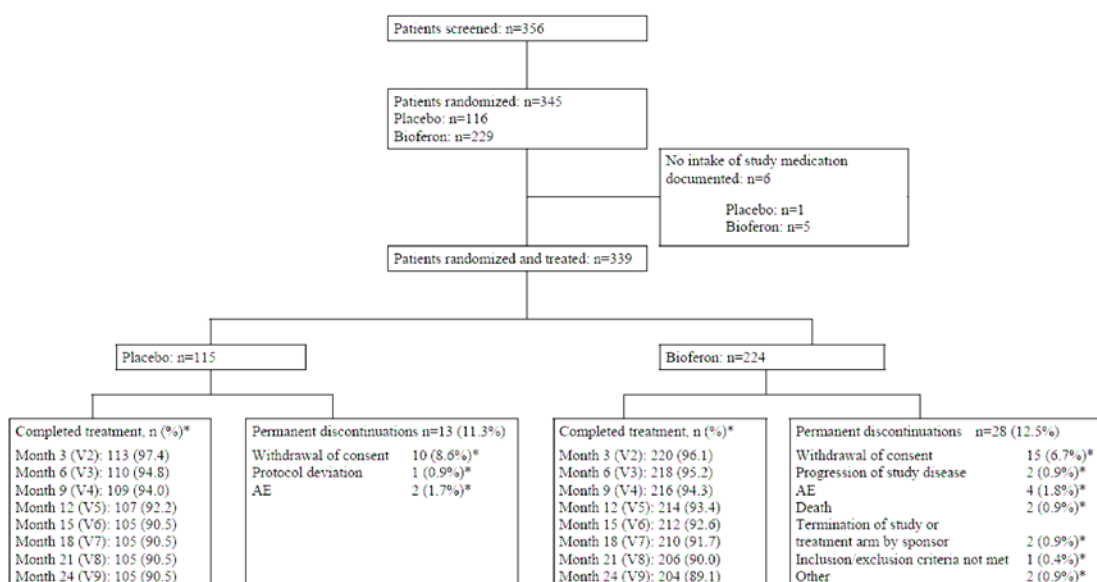
MRI-disease activity was analysed with Cochran-Mantel-Haenszel test with stratification adjustment for center. The comparison at V9 was used for confirmatory testing. In addition, a non-parametric extended Mantel-Haenszel statistic was calculated with covariate adjustment for the number of enhancing lesions at baseline and stratification adjustment for center. Time to sustained progression was analysed using Kaplan-Meier estimates and stratified log-rank test. Center was the stratification factor.

No formal statistical tests will be done for the safety parameters. For the incidence rates of the adverse events Cochran-Mantel-Haenszel tests stratified for centres will be presented but the results will only be interpreted descriptively.

Numerous further exploratory analyses were foreseen for hypotheses generating purposes.

RESULTS

Participant flow



*Calculated using number of patients randomized as denominator; AE=adverse event; N=number of patients used to calculate all percentages; n=number of patients

Source: Section 14.1, Tables 1.1 and 1.2.1 and Section 16.2, Listing 22

Apparently there is no differential drop-out. It was noted that in the active group there were two drop outs due to disease progression and none in the placebo arm.

Recruitment

The study was conducted in 4 countries, Czech Republic (8 patients), Poland (115 patients), Romania (27 patients) and Serbia (189 patients). In total 115 patients were randomized to placebo and 224 to Biferonex. The mean duration of MS from first symptoms was 8.07 years (range 2-29 years). It should be noted that in these countries, the treatment of RRMS may differ from the clinical practice used in Western Europe.

Conduct of the study

Baseline data

ORIGISMS N of patients/baseline features, results			
Patients disposition / Baseline features			
	Placebo	INF- β -1a 6-MIU	Comments
n-randomized	116	229	
n-ITT	115 (99.1%)	224 (97.8%)	
n-at month 24	105 (90.5%)	204 (89.1%)*	
Baseline features			
Age	36.6 (8.2)	35.5 (7.5)	(yrs, mean, SD)
Time since diagnosis	4.3 (3.6)	5.04 (4.6)	
Duration from 1 st symptoms	7.84 (5.77)	8.19 (5.77)	(yrs)
Exacerbations / past 2 years	2.0	2.0	Median
EDSS score at baseline	3.0	3.0	Median
Range	1 -5	1-6	
n of Gd+ lesions			
Mean (SD)	2.5 (6.3)	2.2 (4.6)	
Median	0.0	0.0	
Range	0 ; 52	0 ; 52	

There were no relevant differences between the study arms with respect to demography, medical history, concomitant diseases or co-medication.

Numbers analysed

A total of 339 randomized patients (98.3%) received at least one dose of study medication, and were therefore included in the ITT population. Of the 339 patients in the ITT (intent-to-treat) population, 332 did not have any major protocol deviation (112 in placebo and 220 in the Bioferon group) and received at least 4 weeks of study medication, and were therefore included in the PP (per protocol) population.

Outcomes and estimation

The primary efficacy variable was the number of exacerbations per patient over the study period of 24 months (table below).

Table

Exacerbations, n (%)	Placebo [n=115]	Bioferon [n=224]	p-value
24 months (whole study period)			0.3967 ^a
None	61 (53.0)	128 (57.1)	
1	28 (24.3)	54 (24.1)	
2	15 (13.0)	25 (11.2)	
3	7 (6.1)	11 (4.9)	
4	1 (0.9)	3 (1.3)	
5	2 (1.7)	3 (1.3)	
6	1 (0.9)	-	

^a Cochran-Mantel-Haenszel test with stratification adjustment for center.

The difference in this primary endpoint was not statistically significant between the Bioferon group and placebo group (p=0.397).

The differences in the secondary endpoints were:

A. Difference between the treatment groups in proportion of patients remaining free from exacerbations

	Placebo [n=115]	Bioferon [n=224]	p-value ^a
6 months			0.0242
No exacerbation	88 (76.5)	193 (86.2)	
12 months			0.2588
No exacerbation	77 (67.0)	163 (72.8)	
18 months			0.3931
No exacerbation	68 (59.1)	143 (63.8)	
24 months			0.4188
No exacerbation	61 (53.0)	128 (57.1)	

B. Difference between the treatment groups in expanded disability scale (EDSS) progression (time to sustained progression)

Time to sustained progression.	Placebo n=112	Bioferon n=220
With sustained progression	26 (23)	35 (16)
Censored	86 (77)	185 (84)

Log-rank test with stratification adjustment for center: p = 0.0560.

Cochran-Mantel-Haenszel test with stratification adjustment for center: p = 0.1134

C. Integrated disability status scale (IDSS) progression.

The mean IDSS (the area under the EDSS curve above baseline, adjusted for time on study) was higher in the placebo group (110.99) than in the Bioferon group (91.34) but the difference was not statistically significant.

D. Nine-hole peg test.

Mean times needed to perform the Nine-Hole Peg Test with the dominant hand were slightly lower in the Biferonex group than in the placebo group at every time point including baseline but there were no statistically significant differences between the two groups in scores.

E. Proportion of patients with progression.

There was no significant difference between the treatment groups.

F. Time to exacerbation.

Log-rank test with stratification adjustment for center: $p = 0.2070$.

G. Total number of exacerbations.

There was no significant difference between the treatment groups.

H. MRI related end-points

Volume of T2-lesions: In the Biferonex group, the mean volume of T2 lesions decreased with time throughout the study, whereas it increased in the placebo group. Changes from baseline were statistically significant between the treatment groups at every time point.

Volume of T1-Hypointense lesions: In the Biferonex group the mean volume of T1-hypointense lesions decreased, whereas it increased in the placebo group. The difference in the mean changes from baseline values between the two groups was statistically significant at Month 24.

Volume of gadolinium-enhanced lesions: The mean volume of gadolinium-enhanced lesions was significantly smaller in the Biferonex group than the placebo group at Months 6, 12 and 24. In both groups, the mean volume of gadolinium-enhanced lesions decreased with respect to baseline; however this decrease was statistically significantly greater in the Biferonex group than the placebo group.

The table below summarised these results.

ORIGIMS- EFFICACY		Primary and main secondary endpoints		
		Placebo n-ITT=115	INF-β-1a n-ITT=224	Comments
Exacerbations				
Annualized exacerbation rate				Primary endpoint
Mean (sd)		0.50 (0.79)	0.39 (0.58)	$p=0.397$
Median (range)		0.0 (0.0 ; 5.06)	0.0 (0.0 - 2.50)	CMH adjusted for centre
Distribution of exacerbations				
None		53.0%	57.1%	$p=0.40$
1 exacerbation		24.3%	24.15	CMH adjusted for centre
2 exacerbations		13.0%	11.2%	
3 exacerbations		6.1%	4.9%	
4 exacerbations		0.9%	1.3%	
5 exacerbations		1.7%	1.3%	
6 exacerbations		0.9%	-	
Exacerbation rate over time				
At 6 months		0.59	0.30	$p= 0.02$
At 12 months		0.51	0.37	$p= 0.20$
At 18 months		0.49	0.37	$p= 0.31$
At 24 months		0.50	0.39	$p= 0.34$
Exacerbation free over time				
At 6 months		77%	86%	$p= 0.0242$

At 12 months	67%	73%	p= 0.2588
At 18 months	59%	64%	p= 0.3931
At 24 months	53%	57%	p= 0.4188
EDSS derived variables			
Progression			
Survival rate at 12 months	0.87	0.92	
Survival rate at EOS	0.76	0.84	LRT p=0.056
n with sustained progression	26/112 (23%)	35/220 (16%)	p=0.1134 CMH adj. for centre
EDSS score at baseline	2.94	2.88	Mean
Δ EDSS score EOS (mean)	0.24	0.15	p=0.63 CMH adj. for centre
Δ EDSS score EOS (median)	0.0	0.0	
MRI-variables			
Volume of T2 lesions (cm ³)			
Baseline	7.536	9.609	
Change (mean)	1.152	-0.488	
Difference	-1.492 (CI _{95%} - 2.246 ; -0.7380)	p=0.0001	
Volume of hypodense T2 lesions (mm ³)			
Baseline	1802.9	2408.6	
Change (mean)	272.3	-242.9	
Difference	-336.6 (CI _{95%} - 621.9 ; -51.3)	p=0.02	
Volume of G+ lesions (cm ³)			
Baseline	220.6	169.8	
Change (mean)	-30.1	-99.3	
Difference	-100.9 (CI _{95%} 1.83.3 ; -18.5)	p=0.02	
Cum. number of active lesions			
Mean (SD)	13.1 (16.3)	6.9 (22.2)	p<0.001
Median (Range)	8.0 (0; 83)	2.0 (0; 302)	

Ancillary analyses

- Analysis performed across trials (pooled analyses and meta-analysis)

To respond to the CHMP concerns in order to investigate the robustness of the results previously presented, the applicant has performed a sensitivity analysis including missing data allocation by the half and the double of the pre-trial exacerbation rates.

The sensitivity analysis is based on nine methods of Missing data allocation (MDA) which are summarised in the legend of table 1. Particularly sensitivity analysis of the results was conducted by using half or double of the pre-trial exacerbation rate as imputation as requested.

The robustness of the results was evaluated with the help of several models taking into account potential adjustments effects. These also are summarised in the legend of table 1.

Each endpoint was analysed with each MDA on two main populations: Intent To Treat population (n=338) which was the sample associated with the main analysis and the Per Protocol Population (n=332) is considered as supportive.

Results

In table 1 main results are summarised. Analysis of the 4 endpoints related with exacerbations

Table 1 mean risk ratio, mean 95% upper limit and mean p-values for exacerbation rate (NX) and moderate to severe exacerbation rate (NX2, excluding exacerbations ≤ 1 on the EDSS)

	NX (n of exacerbations / 24 months)			NX2 (n of severe exacerbations / 24 months)		
	Mean Risk ratio	Mean 95% upper limit	p-value	Mean Risk ratio	Mean 95% upper limit	p-value
Main model^{1, 2}						
WS	0.78	0.99	0.036	0.74	0.93	0.013
SWS	0.79	1.00	0.047	0.74	0.95	0.019
½ pre-trial	0.81	1.03	0.088	0.77	0.99	0.038
2 pre-trial	0.75	0.93	0.009	0.68	0.87	0.002
Tradeoff k=0	0.79	1.00	0.049	0.75	0.95	0.018
Tradeoff k=0.25	0.79	0.99	0.042	0.75	0.94	0.015
Tradeoff k=0.50	0.79	0.99	0.042	0.74	0.94	0.015
Tradeoff k=0.75	0.78	0.98	0.035	0.74	0.93	0.012
k proportional to duration	0.78	0.98	0.035	0.74	0.93	0.012
Models¹						
Main	0.78	0.99	0.036	0.74	0.93	0.013
Completer	0.79	1.00	0.049	0.78	0.95	0.017
NX _{pre}	0.79	1.00	0.046	0.76	0.95	0.017
Optimal	0.77	0.97	0.027	0.75	0.90	0.007
Samples						
ITT	0.78	0.99	0.036	0.74	0.93	0.013
PP	0.76	0.96	0.019	0.69	0.89	0.005
MDA-all²						
WS	0.77	0.98	0.032	0.73	0.91	0.006
SWS	0.78	0.99	0.038	0.73	0.93	0.004
½ pre-trial	0.81	1.03	0.084	0.77	0.98	0.023
2 pre-trial	0.73	0.91	0.007	0.67	0.85	0.000
Tradeoff k=0	0.78	0.99	0.041	0.74	0.93	0.000
Tradeoff k=0.25	0.78	0.98	0.036	0.74	0.92	0.000
Tradeoff k=0.50	0.78	0.98	0.036	0.74	0.92	0.000
Tradeoff k=0.75	0.77	0.97	0.030	0.73	0.91	0.000
k proportional to duration	0.77	0.97	0.030	0.73	0.91	0.000
Mean trade Off	0.77	0.98	0.032	0.73	0.91	0.001

¹Missing data allocation

SWS: Systematic Worst Case

Each dropout is systematically assigned to therapy failure. The expected in-trial exacerbation rate is calculated by adding to the in-trial exacerbation rate the expected exacerbation rate (baseline exacerbation rate adjusted for duration of the post-interruption).

WS: Corrected of Alternative Worst Case

Pre-trial values may overestimate exacerbation the expected in-trial exacerbation rate as it was observed that the pre-trial rate is generally higher than the in-trial rate. For this reason, in the Alternative Worst Case Scenarios the systematic worst case pre-trial conditions for all the patients is allocated except for dropouts with either a follow-up of at least one year without signs of disease progression and for dropouts at least 6 months without signs of disease progression and a reason of interruption clearly independent of the disease. For early dropout, LOCF pre-trial conditions are highly probable.

WS/2: WS in using Half of pre-trial value & WS2: WS in using double of pre-trial value

Some concern remained on WS justification due to the allocation of pre-trial conditions for in-trial allocation. As observed in historical trials, RX₀ was found overwhelmingly larger in pre-trial compared to RX values during the trial. For this reason values with half (RX₀/2) and double (RX₀*2) of the pre-trial values was allocated.

Trade-off in-Pre with k=0 & k=0.25 & k=0.50 & k=0.75 & k=proportional to Trial duration.

For dropouts the expected in-trial exacerbation rate lies somewhere in between observed in-trial exacerbation rate (RX_i) and pre-trial exacerbation rate (RX₀) accordingly $RX = RX_i + k(RX_0 - RX_i)$

The robustness of the results for four equally spaced RX values in selecting $k=0, 0.25, 0.5, 0.75$ in equation 2. For patients with longer follow up, RX_i becomes more likely and at the opposite RX_0 is more expected when follow up is small. Hence the last analysis used a k value proportional to the duration of being in the trial. $RX = RX_i + (\max\{(730 - \text{Duration})/550, 0\} * (RX_0 - RX_i))$

²Poisson regression models

NX estimated exacerbation rate, NX_0 baseline exacerbation rate,

Main model: $NX = \text{treatment} + \text{center}$

Completer model: $NX = \text{treatment} + \text{center} + \text{completer} + [\text{completer} * \text{treatment}] + \epsilon$

Baseline model: $NX = \text{treatment} + \text{center} + NX_0 + [NX_0 * \text{treatment}] + \epsilon$ (NX_0 = baseline exacerbation rate)

Optimal model: $NX = 0.262 + 1.34 * \text{treatment} + 1.87 \log \text{MRI} + \log \text{VTI} + \epsilon$

The files provided included the original statistical analysis files to provide evidence that the new imputed Poisson regression has been performed similarly as the original Poisson regression analysis without imputation for the primary endpoint (relapse rate = number of exacerbations / study duration in years). This is indeed the case and the analysis could be repeated with the imputed data. The only difference is that the values in the second analysis have been imputed with two imputation methods Systematic Worst Case Scenario (SWS) and Alternative Worst Case Scenario (WS). In the SWS method of imputation of the primary endpoint relapse rate value 2 years before the initiation of the study has been used for all dropouts. In the WS method this imputation has been done only to some of the dropouts.

The presented imputation method is a closely similar method to LOCF method, SWS might have been an acceptable method (in contrary to WS method), since it contains fewer on-trial evaluations.

By using WS/SWS imputation the variation of the endpoint is naturally smaller although the difference between means is similar than in the original analysis:

Results of primary endpoint by different statistical methods of ORIGIMS (24 MONTHS): Mean (SD)

	Placebo	Biferonex	p-value
<i>Original Analysis(ITT)</i>	<i>0.495 (0.788)</i>	<i>0.395(0.577)</i>	<i>0.1754</i>
<i>WS(ITT)</i>	<i>0.52 (0.64)</i>	<i>0.41(0.577)</i>	<i>0.036</i>

By SWS method the p-value is 0.047

The primary endpoint values (number of exacerbations / study duration in years) can be calculated for each patient, independent of completing the study. The withdrawals can bias the results, if the reasons for withdrawals are connected to the treatment effect and there is difference in the number of withdrawals between the treatment groups. Now, the proportion of withdrawals is similar. However, the submitted data files do not contain the individual non-imputed data, where one could easily compare the original and the imputed primary endpoint values of each patient. Also the number of withdrawals (i.e. the imputed patients in the second analysis) is different (20 in Biferonex-group and 11 in placebo group) from the original number of withdrawals in the study report (28 in Biferonex-group and 13 in placebo group).

The confirmatory principle for the primary endpoint analysis is that the primary analysis method of primary endpoint is well described in the study protocol. Now, the imputation methods have been developed after opening the study code and knowing the results. This is in strong disagreement with the above mentioned principle. There is also only one pivotal study, and therefore, these results remain very unconfirmatory. It is also unclear why the imputation has not been performed for all withdrawals.

- Clinical studies in special populations

No studies have been conducted in special populations nor considered necessary at this point by the applicant. The use of Biferonex in this population will have to be addressed at a later stage in case of marketing authorisation.

- Supportive studies

The published JACOBS trial was submitted (as well as other publications). The results of this trial, using the lyophilized formulation, are considered supportive for the claimed indication for the to-be-marketed product.

- Discussion on clinical efficacy

This application is supported by one pivotal clinical trial. In this pivotal clinical trial, in the initial and predefined analysis, the primary efficacy end-point (the number of exacerbations per patient over the study period of 24 months) was not reached. The clinical secondary end points were not met either. Only MRI lesions related secondary end-points were different between the treatment groups at statistically significant level. The lack of a relationship between MRI and clinical outcome is debatable. The lack of a clinically measurable effect was, however, considered a major concern. The greatest benefits of IFN beta are expected in the early phases of MS. The failure of this study may be related to the selection of the patients since many of them had already relatively advanced MS. Alternative explanations for the results may be product related i.e. the efficacy of the product is different. Thus, robust evidence for clinically meaningful efficacy is lacking.

The Applicant has provided a comparative analysis between the pivotal trial and the Jacobs trial, which was the basis of the approval of Avonex. This trial was performed with the lyophilized formulation and therefore can be considered only as supportive.

In the response to the CHMP concerns, the Applicant has elaborated on apparently unexpected deficiencies including error in sample size calculations and unfortunate patient selection given that the annual exacerbation rate was lower as compared to older trials. It is noted that the major shortcomings apparently were only discovered after the results were known to be unfavourable. A new independent statistical analysis of the original data by a third party revealed large differences between the original analysis and adapted analysis. The new analysis remains a post-hoc analysis and data-driven decisions could have been made. Moreover the magnitude of the difference observed did not change. The effect size remains clinically non-significant.

As a response to the CHMP List of outstanding issues, the company provided additional data on the analytical methods. However, the second analysis is still considered only as a sensitivity analysis for the original primary analysis. The analyses give some evidence that the Biferonex treatment may decrease the annual exacerbation rate, but justification of the sensitivity analyses performed and additional sensitivity analyses were requested.

In response to the CHMP 2nd List of outstanding issues, the company provided additional data. The initial assumption that early dropouts with no relapse are all a treatment success was perceived as an unrealistic scenario. For these dropouts the exacerbation rate over the missing observation period was estimated based on the pre-trial exacerbation rate using different approaches. For the primary endpoint i.e. annual exacerbation rate results of the sensitivity analysis were consistent i.e. the risk ratio was stable about 0.78 (range 0.73-0.81). However, the upper limit of the 95% confidence interval was also stable ranging from 0.97 to 1.03. This was also reflected in the modest p-values. However these p-values were not adjusted for the interim analysis as should have been done. The underlying assumption that the pre-trial exacerbation rate is predictive for the future exacerbation rate could not be verified. Sensitivity analysis on *post hoc* responder definitions gave the same picture.

Clinical safety

Safety data for the applied formulation, Biferonex (HSA-) are available from five clinical trials:

- the pivotal Phase III study with RRMS patients (BP IFN beta 003),
- one Phase II study with patients suffering from chronic viral cardiomyopathy (Study b/P13-131), and
- three Phase I studies with healthy volunteers (Studies b/PH-132, BP IFN beta 001, and BP IFN beta 004).

The safety database includes 114 placebo-controlled and 224 active-controlled patients.

- Patient exposure

The most relevant safety data with respect to the intended formulation comes from the pivotal phase III trial (Study BP INF 003) of 2 years duration. It was a placebo-controlled trial with a total of 339 RRMS patients (n = 224 in Beta-INF group and n = 115 in placebo group). Patients received either Biferonex 6 MIU s.c three times a week or placebo. The mean time ($\pm$ standard deviation) time on Beta-INF (HSA-) and on placebo was 690.5 ($\pm$ 137.1) days and 681.6 ($\pm$ 158.5) days, respectively. This is in accordance with the recommendations given in the MS guideline (CHMP/EWP/561/98, rev. 1).

- Adverse events

In comparison to AEs reported for Rebif (commercially available beta-IFN 1a for s.c administration) the two years safety data from pivotal phase III placebo-controlled trial with a total of 339 RRMS patients (n = 224 in Beta-INF Rebif group and n = 115 in placebo group) did not identify any significant new AEs, significantly different intensities nor higher frequencies in any of the reported AEs.

However, new cardiovascular and urogenital abnormalities were observed in physical examination more often in Biferonex group than in placebo group (i.e. 10 patients (4.5%) receiving Biferonex vs. two (1.7%) receiving placebo and 10 patients (4.5%) receiving Biferonex vs. none receiving placebo, respectively).

The most common AEs included chills, pyrexia, and headache and injection site reactions.

The overall percentage of patients experiencing Treatment-Emergent Adverse Events was higher in Biferonex group than in placebo group (i.e. 221 patients (98.7%) vs. 108 patients (93.9%), respectively). Taking into account that the dose used in the pivotal clinical trial equals to the minimum recommended dose of Rebif (6 MIU s.c three times a week) and that none of the subjects had received a maximum recommended dose (12 MIU three times a week), the higher incidence of TEAEs that are typically associated with INF treatment, in active arm than in placebo arm indicates that Biferonex has some biological activity.

The data are summarised in the table below:

Table 38: Summary of adverse events reported in $\geq 5\%$ of patients in either treatment group (ITT Population – whole study period)

System Organ Class Preferred Term	Number (%) of Patients Experiencing Event {Number of Events}						p-value ^a
	Placebo [N=115]		Bioferon [N=224]		Overall [N=339]		
	n (%)	{n}	n (%)	{n}	n (%)	{n}	
Any TEAE	108 (93.9)	{993}	221 (98.7)	{3146}	329 (97.1)	{4139}	0.0204
General Disorders and Administration Site Conditions	84 (73.0)	{309}	199 (88.8)	{1161}	283 (83.5)	{1470}	<0.0001
Fatigue	69 (60.0)	{203}	161 (71.9)	{421}	230 (67.8)	{624}	
Pyrexia	23 (20.0)	{29}	142 (63.4)	{290}	165 (48.7)	{319}	
Chills	29 (25.2)	{51}	102 (45.5)	{351}	131 (38.6)	{402}	
Asthenia	8 (7.0)	{11}	32 (14.3)	{45}	40 (11.8)	{56}	
Influenza-like illness	4 (3.5)	{4}	15 (6.7)	{22}	19 (5.6)	{26}	
Musculoskeletal and Connective Tissue Disorders	71 (61.7)	{213}	162 (72.3)	{519}	233 (68.7)	{732}	0.0291
Myalgia	63 (54.8)	{178}	148 (66.1)	{412}	211 (62.2)	{590}	
Musculoskeletal stiffness	9 (7.8)	{13}	22 (9.8)	{30}	31 (9.1)	{43}	
Back pain	7 (6.1)	{7}	20 (8.9)	{27}	27 (8.0)	{34}	
Psychiatric Disorders	63 (54.8)	{113}	129 (57.6)	{308}	192 (56.6)	{421}	0.6657
Depressed mood	26 (22.6)	{47}	64 (28.6)	{127}	90 (26.5)	{174}	
Depression	28 (24.3)	{41}	50 (22.3)	{131}	78 (23.0)	{172}	
Anxiety	6 (5.2)	{10}	14 (6.3)	{22}	20 (5.9)	{32}	
Nervous System Disorders	42 (36.5)	{104}	117 (52.2)	{384}	159 (46.9)	{488}	0.0019
Muscle spasticity	21 (18.3)	{59}	58 (25.9)	{214}	79 (23.3)	{273}	
Headache	11 (9.6)	{16}	61 (27.2)	{107}	72 (21.2)	{123}	
Infections and Infestations	48 (41.7)	{90}	93 (41.5)	{189}	141 (41.6)	{279}	0.9778
Upper respiratory tract infection	14 (12.2)	{17}	21 (9.4)	{29}	35 (10.3)	{46}	
Influenza	11 (9.6)	{15}	23 (10.3)	{38}	34 (10.0)	{53}	
Nasopharyngitis	16 (13.9)	{20}	9 (4.0)	{13}	25 (7.4)	{33}	
Urinary tract infection	5 (4.3)	{6}	15 (6.7)	{17}	20 (5.9)	{23}	
Pharyngitis	2 (1.7)	{2}	12 (5.4)	{20}	14 (4.1)	{22}	
Investigations	28 (24.3)	{52}	75 (33.5)	{160}	103 (30.4)	{212}	0.0563
ALT increased	10 (8.7)	{13}	28 (12.5)	{33}	38 (11.2)	{46}	
Platelet count decreased	4 (3.5)	{5}	22 (9.8)	{25}	26 (7.7)	{30}	
AST increased	2 (1.7)	{2}	16 (7.1)	{17}	18 (5.3)	{19}	
Haemoglobin decreased	4 (3.5)	{6}	12 (5.4)	{20}	16 (4.7)	{26}	
Blood and Lymphatic System Disorders	10 (8.7)	{12}	69 (30.8)	{125}	79 (23.3)	{137}	<0.0001
Leukopenia	5 (4.3)	{6}	49 (21.9)	{81}	54 (15.9)	{87}	
Lymphopenia	4 (3.5)	{4}	18 (8.0)	{22}	22 (6.5)	{26}	
Gastrointestinal Disorders	16 (13.9)	{21}	29 (12.9)	{43}	45 (13.3)	{64}	0.6720
Skin and Subcutaneous Tissue Disorders	7 (6.1)	{8}	37 (16.5)	{61}	44 (13.0)	{69}	0.0092
Reproductive System and Breast Disorders	9 (7.8)	{13}	33 (14.7)	{46}	42 (12.4)	{59}	0.0753
Vascular Disorders	7 (6.1)	{7}	19 (8.5)	{24}	26 (7.7)	{31}	0.4632
Eye Disorders	7 (6.1)	{7}	12 (5.4)	{15}	19 (5.6)	{22}	0.8311

- Serious adverse event/deaths/other significant events
- A summary of serious adverse events is summarised in the table below.

BioPartners GmbH
BP IFN beta 003 (RB b/S06-204)

**Table 43: Summary of serious treatment emergent adverse events
(ITT Population)**

System Organ Class Preferred Term	Number (%) of Patients Experiencing Event (Number of Events)						p-value ^a
	Placebo [N=115]		Bioferon [N=224]		Overall [N=339]		
Any TEAE	4 (3.5)	{5}	16 (7.1)	{21}	20 (5.9)	{26}	0.1938
Gastrointestinal Disorders	-		1 (0.4)	{1}	1 (0.3)	{1}	0.4795
Peritonitis	-		1 (0.4)	{1}	1 (0.3)	{1}	
Infections and Infestations	2 (1.7)	{3}	3 (1.3)	{3}	5 (1.5)	{6}	0.7157
Appendicitis	1 (0.9)	{2}	-		1 (0.3)	{2}	
Tonsillitis	-		1 (0.4)	{1}	1 (0.3)	{1}	
Urinary tract infection	1 (0.9)	{1}	1 (0.4)	{1}	2 (0.6)	{2}	
Urosepsis	-		1 (0.4)	{1}	1 (0.3)	{1}	
Injury, Poisoning and Procedural Complications	-		1 (0.4)	{1}	1 (0.3)	{1}	0.5127
Limb injury	-		1 (0.4)	{1}	1 (0.3)	{1}	
Neoplasms, Benign, Malignant and Unspecified (incl. Cysts and Polyps)	-		2 (0.9)	{2}	2 (0.6)	{2}	0.3206
Uterine leiomyoma	-		1 (0.4)	{1}	1 (0.3)	{1}	
Dermatofibrosarcoma	-		1 (0.4)	{1}	1 (0.3)	{1}	
Nervous System Disorders	-		1 (0.4)	{2}	1 (0.3)	{2}	0.4795
Epilepsy	-		1 (0.4)	{2}	1 (0.3)	{2}	
Pregnancy, Peripartum and Perinatal Conditions	1 (0.9)	{1}	-		1 (0.3)	{1}	0.1573
Pregnancy	1 (0.9)	{1}	-		1 (0.3)	{1}	
Psychiatric Disorders	1 (0.9)	{1}	5 (2.2)	{5}	6 (1.8)	{6}	0.3732
Completed Suicide	-		2 (0.9)	{2}	2 (0.6)	{2}	
Depression	-		2 (0.9)	{2}	2 (0.6)	{2}	
Suicide attempt	1 (0.9)	{1}	1 (0.4)	{1}	2 (0.6)	{2}	
Reproductive System and Breast Disorders	-		3 (1.3)	{4}	3 (0.9)	{4}	0.2045
Menometrorrhagia	-		1 (0.4)	{1}	1 (0.3)	{1}	
Metrorrhagia	-		1 (0.4)	{1}	1 (0.3)	{1}	
Ovarian cyst	-		2 (0.9)	{2}	2 (0.6)	{2}	
Skin and Subcutaneous Tissue Disorders	-		2 (0.9)	{3}	2 (0.6)	{3}	0.3206
Decubitus ulcer	-		1 (0.4)	{1}	1 (0.3)	{1}	
Urticaria	-		1 (0.4)	{1}	1 (0.3)	{1}	
Urticaria generalised	-		1 (0.4)	{1}	1 (0.3)	{1}	

^a Cochran-Mantel-Haenszel test with stratification adjustment for center.
n = number of patients.
{n} = number of TEAEs.
Percentages are based on the total number of patients in the ITT population.

Source: Section 14.3, Table 6.3.1.1

A total of 20 patients (5.9%) experienced a treatment emergent SAE; 16 (7.1%) occurring in the Biferonex group and 4 (3.5%) in the placebo group. There were two completed suicides both of which occurred in Biferonex group. The causality between the death and INF treatment was possible in both cases.

The other SAEs in Beta-INF recipients included depression (2 cases), suicide attempt (1 case), epilepsy (1 case) and generalized urticaria (1 case). These SAEs were considered to have a possible relationship to study medication except for the other case of depression, in which the causality was deemed probable by the treating investigator.

Other SAEs that were reported only in Biferonex group and the causality of which were considered unlikely included: uterine leiomyoma (1 case) and dermatofibrosarcoma (1 case). In the placebo group one suicide attempt was considered to have a possible relationship to study medication. No new SAEs were recognized in the pivotal placebo-controlled phase III study conducted with Biferonex, but as a new biological product its long-term safety needs to be studied further as post-approval commitment as discussed in the risk management plan.

- Laboratory findings

Anemia, leukopenia, thrombocytopenia, lymphopenia and asymptomatic increases in transaminases were experienced by patients receiving Biferonex as expected.

- Safety in special populations

No clinical trials have been conducted in special populations with the applied INF beta formulation and thus, the knowledge on safety of Biferonex is limited to the anecdotal cases and literature review. Additional safety data is not considered necessary, but the general warnings related to INF beta use in aged population, in patients with renal or hepatic insufficiency must be implemented into the product information as done with the licensed INF beta products.

- Immunological events

Approximately 17.9% of patients receiving Biferonex developed neutralizing antibodies (NAB) during 18 month follow up. The results are in line with the information reported for Rebif; 24% of the patients in the 6 MIU group vs. 0 (placebo) and 15% (12 MIU) tested positive (i.e. "positivity" maintained for at least two consecutive visits). Of note is that one patient in the placebo group also had an IFN NAB serum titer > 20 NU/mL.

The CHMP was concerned that the development of NAB+ on a substantial proportion of patients might explain inconclusive results of the ORIGIMS study, especially as the difference in relapse rate was statistically significant at 6 months but disappears thereafter. The HSA-free product might have changed the immunogenicity of INF-1-beta-a. As the data presented by the applicant show that no differences in clinical outcome between NAB+/- subjects could be observed, this explanation is unlikely. (see table below)

Table 7: Total number of exacerbations over 24 months by maximal NAB category reached (ORIGIMS)						
	Total number of exacerbations - N (%)					
Max NAB category reached	0	1	2	3	4	5
NAB-	102 (58.3)	41 (23.4)	19 (10.9)	8 (4.6)	3 (1.7)	2 (1.1)
NAB+ low 20-200 NU/ml	7 (38.9)	5 (27.8)	5 (27.8)	1 (5.6)	-	-
NAB+ medium 201-1000 NU/ml	7 (70.0)	2 (20.0)	-	1 (10.0)	-	-
NAB+ high > 1000 NU/ml	12 (57.1)	6 (28.6)	1 (4.8)	1 (4.8)	-	1 (4.8)

Comparison between categories using Poisson Regression did not reveal any statistical significance ($p = 0.463$).

- Safety related to drug-drug interactions and other interactions

No interactions between Biferonex and concomitantly used medications have been reported as AEs.

- Discontinuation due to adverse events

Discontinuation rate due to AEs has been relatively low in both Biferonex group and placebo group (1.8% and 1.7%, respectively).

- Post marketing experience

Not applicable

- Discussion on clinical safety

The safety profile of the interferons beta has already been well characterized, both from clinical studies and post-marketing data. In comparison to AEs reported for Rebif, the two years safety data from the ORIGIMS study with Biferonex did not identify any significant new AEs, significantly different intensities nor higher frequencies in any of the reported AEs.

The most common AEs included chills, pyrexia, headache and injection site reactions.

However, the current data are insufficient for a comparison of the safety profile with the marketed beta IFNs.

The most important potential risks with this therapy are haematological abnormalities, changes in liver function, psychiatric reports of depression and potential immunogenicity. The identified and potential risks involved are similar to the class of interferons beta.

2.5 Pharmacovigilance

Detailed description of the Pharmacovigilance system

The CHMP considers that the Pharmacovigilance system as described by the applicant fulfils the legal requirements and provides adequate evidence that the applicant has the services of a qualified person responsible for pharmacovigilance and has the necessary means for the notification of any adverse reaction suspected of occurring either in the Community or in a third country.

Risk Management Plan

The Applicant has submitted a risk management plan (RMP) with the application for marketing authorisation of Biferonex. The RMP was prepared according to the new EMEA guidelines.

Should Biferonex be authorised, the pharmacovigilance activities such as studies on long-term safety and immunogenicity will be further discussed in view of a possible marketing authorisation.

The CHMP, having considered the data submitted in the application was of the opinion that it was not appropriate to consider risk minimisation activities at this time.

2.6 Overall conclusions, risk/benefit assessment and recommendation

Quality

The assessment of the applicant's responses submitted to the CHMP List of Questions and subsequent list of outstanding issues has revealed that there are still concerns remaining in the area of Quality pertaining to the medicinal product acceptance criteria and stability of the finished product regarding deamidation.

Although the Avonex batches used in Jacobs trial originate from the same MCB as Biferonex and the batch analyses data submitted do not reveal considerable qualitative differences between the active substances of Avonex and Biferonex, full comparability on the quality level between the two products can not be assured.. Data from Jacobs' trial batches can only be considered as supportive.

Non-clinical pharmacology and toxicology

The main concern has been the lack of adequate bridging studies with the albumin-containing formulation and the albumin-free formulation intended for marketing. Due to technical reasons and

unavailability of the original interferon beta 1a product used in non-clinical studies, the applicant cannot produce the required bridging data. However, the additional data provided suggest that pharmacological behaviour of Biferonex is not affected by the presence of HSA. Moreover, considerable quality-related differences other than deamidation, between the HSA-containing commercial interferon beta 1a batches and Biferonex were not detected. However, full comparability could not be ensured. In the absence of true bridging data, and without a possibility to produce it, certain level of uncertainty remains. Regarding safety, this uncertainty would concern any potential difference in immunogenic potential. This, however, is not feasible to be investigated in animals and therefore a need for further animal studies is not foreseen. From a non-clinical point of view and within the limitations of non-clinical models used, the available non-clinical safety data can be considered to sufficiently support clinical use of Biferonex.

Efficacy

In the pivotal trial, the primary efficacy end-point (the number of exacerbations per patient over the study period of 24 months) was not reached and neither were any of the clinical secondary end-points. Only MRI related end-points were different at statistically significant level between the Biferonex group and placebo group. However, the clinically efficacy remains questionable.

In the response to the CHMP concerns, the Applicant has elaborated on apparently unexpected deficiencies including error in sample size calculations and unfortunate patient selection based on the fact that the annual exacerbation rate was lower as compared to older trials. It is noted that the major shortcomings apparently were only discovered after it was discovered that the results were known to be unfavourable. A new independent statistical analysis of the original data by a third party revealed large differences between the original analysis and adapted analysis. The new analysis remains a *post-hoc* analysis and data-driven decisions could have been made. Moreover the magnitude of the difference observed did not change. The effect size remains clinically non-significant.

Safety

The most common AEs included chills, pyrexia, and headache and injection site reactions.

The overall percentage of patients experiencing Treatment-Emergent Adverse Events was higher in Biferonex group than in placebo group (i.e. 221 patients (98.7%) vs. 108 patients (93.9%), respectively), indicating that Biferonex has some biological activity.

A total of 20 patients (5.9%) experienced a treatment emergent SAE; 16 (7.1%) occurring in the Biferonex group and 4 (3.5%) in the placebo group. There were two completed suicides both of which occurred in Biferonex group. The causality between the death and INF treatment was possible in both cases.

Approximately 17.9% of patients receiving Biferonex developed neutralizing antibodies during 18 month follow up. The results are in line with the information given for Rebif; 24% of the patients in the 6 MIU group vs. 0 (placebo) and 15% (12 MIU) tested positive (i.e. "positivity" maintained for at least two consecutive visits).

- User consultation

Not applicable

Risk-benefit assessment

In the single pivotal trial, the primary efficacy end-point (difference in the number of exacerbations per patient over 24 months) was not reached and neither were any of the clinical secondary end-points. Only MRI related end-points were different at statistically significant level between the Biferonex group and placebo group. Therefore, the clinically efficacy remains questionable. Whether this is due to the study design or intrinsic product characteristics (lesser efficacy due to the larger deamidation grade and subcutaneous route of administration) cannot be established on the basis of the available

data. Compared to interferon beta products on the market, Biferonex is of a different quality in terms of deamidation. Given this quality difference, the clinical efficacy and safety has to be demonstrated convincingly for the Biferonex product itself.

The overall benefit-risk balance of Biferonex is negative.

Grounds for refusal

The therapeutic efficacy of Biferonex has not been demonstrated.

- Compared to Interferon beta products already approved, Biferonex is different in terms of protein deamidation. Given this quality difference, the clinical efficacy has to be demonstrated convincingly for the Biferonex medicinal product itself. The single pivotal clinical trial failed to show adequate evidence of efficacy. Whether this is due to the study design, robustness of the results or intrinsic product characteristics cannot be established on the basis of the available data.

Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered by consensus that the risk-benefit balance of Biferonex in the treatment of relapsing-remitting multiple sclerosis characterized by two or more exacerbations in the previous two years was unfavourable and therefore did not recommend the granting of the marketing authorisation.