1 SCIENTIFIC DISCUSSION

1.1 Introduction

Natalizumab is a recombinant, humanized form of a murine monoclonal antibody that binds to the α4 subunit of α4β1 (also known as very late antigen 4 [VLA-4] or CD49d-CD29) and α4β7 integrin. The α4-integrins are expressed on all leukocytes, with the exception neutrophils, as well as haematopoietic progenitor cells, and mediate several homing and adhesive functions. This is accomplished through the binding of the α4-integrins to their cognate receptors vascular cell adhesion molecule-1 (VCAM-1) and mucosal addressing cell adhesion molecule-1 (MadCAM-1) expressed on endothelium. MadCAM-1 binding is specific to the α4β7-integrin expressed primarily on T lymphocytes and monocytes in the intestine. Additional α4-integren ligands include osteopontin and fibronectin (CS-1) expressed within the extracellular matrix.

Natalizumab effectively blocks the interaction of α4-integrins with their cognate receptors. Disruption of these molecular interactions prevents migration of mononuclear leukocytes across the endothelium and into inflamed tissue. Natalizumab may also suppress ongoing inflammatory reactions in diseased tissues by inhibiting α4-expressing leukocytes from binding with its ligands within the extracellular matrix.

Natalizumab is a full-length antibody of the IgG4 subclass. It consists of two heavy and two light chains connected by four inter-chain disulfide bonds. Like the IgG4 class of antibodies, natalizumab also demonstrates reduced binding to Fcγ receptors and lack of ability to fix complement in vitro.

Natalizumab is being co-developed by Biogen Idec Inc. and Elan Pharmaceuticals, Inc. for the treatment of relapsing multiple sclerosis (MS). In MS, lesions are believed to occur when activated T lymphocytes cross the blood-brain barrier (BBB). Leukocyte migration across the BBB involves the interaction between α4β1 and VCAM-1. Once in the tissues, α4β1 further interacts with CS-1 and osteopontin within the extracellular matrix. The result is further amplification of the inflammatory cascade and ultimately demyelination caused by leukocyte injury to the myelin sheath. This sequence of events makes MS a logical choice for treatment with natalizumab.

The *originally claimed indication* was" for treatment of relapsing forms of multiple sclerosis to reduce the frequency of clinical exacerbations". Natalizumab may be used as monotherapy in patients with relapsing multiple sclerosis. Natalizumab may also be used in combination with intramuscular interferon beta-1a once weekly for patients who continue to experience clinical exacerbations while receiving intramuscular interferon beta-1a.

The recommended dose of natalizumab is 300 mg IV every 4 weeks
After review of the dossier, the indication has been restricted on safety grounds as follows:

"Tysabri is indicated as single disease modifying therapy in highly active relapsing remitting multiple sclerosis for the following patient groups:

- Patients with high disease activity despite treatment with a beta-interferon (see 5.1);

or

- Patients with rapidly evolving severe relapsing remitting multiple sclerosis (see 5.1).

It is clarified (section 5.1 of SPC) that due to safety concerns treatment is restricted to the following patient groups:

- Patients who have failed to respond to a full and adequate course of a beta-interferon. Patients should have had at least 1 relapse in the previous year while on therapy, and have at least 9 T2-hyperintense lesions in cranial MRI or at least 1 Gadolinium-enhancing lesion.

or

- Patients with rapidly evolving severe relapsing remitting multiple sclerosis, defined by 2 or more disabling relapses in one year, and with 1 or more Gadolinium enhancing lesions on brain MRI or a significant increase in T2 lesion load as compared to a previous recent MRI.

1.2 Quality aspects

Introduction

Natalizumab is a purified, recombinant, humanized monoclonal antibody against the integrin α4-subunit (IgG_4/κ). Natalizumab is produced in non-immunoglobulin secreting (NS/0) murine myeloma cells. The molecular weight of glycosylated natalizumab is 149 kilodaltons. It consists of two heavy and two light chains connected by four inter-chain disulfide bonds. The α4-integrins are expressed on all leukocytes, with the exception of neutrophils. By binding to the α4-subunit of α4β1 (also known as very late antigen 4 [VLA-4] or CD49d-CD29) and α4β7 integrins, natalizumab blocks the interaction of α4β1 or α4β7 on leukocytes to their counter receptors (VCAM and MadCAM) and ligand (fibronectin), this acting as a selective adhesion molecule (SAM) inhibitor.

The drug product is a sterile, clear to slightly opalescent liquid concentrate for intravenous infusion, presented in type I borosilicate glass vials with bromobutyl stoppers and aluminium seals for which integrity has been demonstrated. Natalizumab concentrate is substantially free of particulate matter. Each 15 ml vial contains 300 mg natalizumab, sodium phosphate, monobasic, sodium phosphate, dibasic, sodium chloride, polysorbate 80 and Water for Injection (USP/Ph.Eur). For administration, the natalizumab concentrate is diluted in 100 ml saline. The diluted solution is to be infused intravenously over 1 hour at a rate of approximately 2 ml/minute.

Active Substance

Natalizumab is a full-length antibody of the IgG4 subclass. It consists of two heavy and two light chains connected by four inter-chain disulfide bonds. Antibodies of the IgG4 subclass are characterized by a shorter hinge region in comparison to antibodies of the IgG1 subclass, leading to a reduced flexibility of the hinge region. Like the IgG4 class of antibodies, natalizumab also demonstrates reduced binding to Fcγ receptors and lack of ability to fix complement *in vitro*.
The molecular mass of the intact deglycosylated natalizumab molecule, as measured by mass spectrometry, is 146 kDa. Each heavy chain has one potential N-linked glycosylation site. The structural characteristics of natalizumab are common among IgG antibodies, and a majority of antibody products share similar attributes.

Manufacture

Manufacturers
Natalizumab is manufactured in the "Large Scale Manufacturing (LSM) plant" owned and operated by Biogen Idec Inc. (at RTP, NC, USA) and routinely controlled at Biogen Idec's Quality Control laboratories (at RTP, NC, and at Cambridge, MA, USA, and San Diego, CA, USA).

Genetic development
The immunisation procedure and the cell line development are extensively described. The RAMOS cell-line used for the immunisation procedure is a well-characterized non-EBV releasing cell-line. The fusion partner SP2/0 –Ag14 is a widely used and well-characterised cell line for the generation of hybridomas for monoclonal antibodies. The humanisation by CDR grafting and the generation of the double gene expression vector is described comprehensively. The process of transfection and generation of the production cell line ATH-1 follows a reasonable strategy. The ability of the resulting

antibody (AN100226m) and the humanised antibody (AN100226) to bind to α4 integrin and to inhibit the α4 integrin dependent cell adhesion was demonstrated.

Cell Banking
The development of the cell line, the creation of the cell bank and its testing follow a scientifically sound scheme and are sufficiently described. The cell banking system is adequate for reliable manufacturing of the monoclonal antibody. Criteria for the establishment of new Working Cell Banks were provided. Testing of the cell bank was adequate. Generally the requirements of the relevant guidelines (Production and quality control of monoclonal antibodies, ICH Q5D, ICH Q5B and Ph. Eur. monograph 784 products of recombinant DNA technology) are met.

Starting materials
Serum is not used in the composition of the cell culture medium, including Working Cell Bank (WCB) cultivation and cell culture process media. Animal-derived raw materials are not used in the commercial manufacturing process of natalizumab and none of the components used are derived from animal sources. Bovine serum albumin (BSA) and human transferrin are used in the preparation of the Master Cell Bank (MCB) and WCBs. In addition, the freezing medium used for both MCB and WCBs contains foetal bovine serum and DMSO. Materials used in the manufacture process are adequately controlled.

Cell culture and purification
Natalizumab is expressed in a recombinant NS/0 (murine myeloma) cell line. NS/0 cells derived from a single vial of the Working Cell Bank are grown in increasing volumes of shaker flasks and bioreactors, to obtain an inoculum for the production bioreactor (volume of 15,000 litre). The contents of the production bioreactor are harvested and the conditioned medium is obtained using membrane filtration.

Natalizumab is purified using a sequence of affinity chromatography (recombinant Protein A Sepharose resin), anion-exchange chromatography and hydrophobic interaction chromatography. The final eluate is then concentrated and buffer is exchanged with the final formulation buffer using an Ultrafiltration/Diafiltration system.

In addition to the viral clearance achieved in the chromatography steps, further clearance is achieved by low pH treatment and 15 nm nanofiltration. The manufacturing process is designed to ensure a high degree of purity of the active substance, as well as freedom from adventitious agents.

This sequence of affinity chromatography, anion-exchange chromatography, and hydrophobic interaction chromatography, together with treatment at low pH and nanofiltration, is commonly used for other antibody products.

The manufacturing process and in-process controls have been sufficiently described. Establishment of controls for critical steps and intermediates are described in detail. Validation studies were presented, demonstrating clearance of process related impurities and consistency of the drug substance manufacturing process. Different processes have been used during the development of the manufacturing process. Data comparing commercial process with processes for clinical trial material were presented and demonstrate that biochemical and physicochemical characteristics are comparable, except for differences detectable in certain product attributes. It could be demonstrated sufficiently that these differences did not affect biological activity, pharmacokinetic and pharmacodynamic properties, or immunogenicity.

Characterisation
Natalizumab has been characterised extensively using a battery of modern analytical state-of-the-art techniques. Primary structure could be determined using Edman degradation and different methods of peptide mapping. Secondary and tertiary structure were analysed by circular dichroism and fluorescence spectroscopy. Additionally, amino-acid composition, molecular mass and disulfide bonds have been investigated. Glycan structure has been analyzed and different glycoforms have been quantified to determine the extent of post-translational modification. Other product-related impurities

have been identified and quantified, using reduced GelChip Capillary Electrophoresis (GelChip CE), size exclusion chromatography (SEC), ion exchange chromatography (IEC), and reverse phase high performance liquid chromatography (RP-HPLC). The amount of half-antibody was successfully quantified with non-reduced GelChip Capillary Electrophoresis.

Several biological characterization assays were developed to evaluate both the binding of the regions of natalizumab that contact VLA4 (functional end) as well as the immunoglobulin Fc region. The format of the assays that characterize the functional end of the molecule were specifically configured to reflect one of the proposed modes of action of natalizumab *in vivo*, i.e. binding to the VLA4 and blocking the binding of ligands VCAM and MadCAM to VLA4 and to the α4β7 integrin. Assays to characterise the Fc region of natalizumab focused on demonstrating the ability of natalizumab to bind Fcγ receptors I/II. In addition, natalizumab was also characterized in qualitative assays for its ability to bind to Fcγ receptor III and for its ability to mediate effector functions through the Fc region, namely, ADCC and CDC. These methods allowed a comprehensive characterisation of the antibody in terms of binding and functional properties of the molecule. The applicant followed the scientific advice given by the CPMP in 2002 to add an assay to characterise the binding of natalizumab to Fc receptors of cells involved in the clinical setting and to characterise the binding to MadCAM.

Process-related impurities have been analyzed by clearance validation and quantification in the active substance. As concentrations of DNA, recombinant Protein A and impurities from the cell culture process are below the limit of quantification, it is acceptable not to perform routine testing. As determination of host cell protein (NS/0) in more than 30 additional commercial scale batches demonstrated a consistent low level of host cell protein, it was justified, not to include this parameter in further batch testing of active substance.

Specifications

The analytical procedures and their validation in general are appropriate for the assessment of the active substance. The set of specification and limits were adequately justified on the basis of data obtained from 18 batches batch analysis and are typical for a monoclonal antibody. Specifications include: tests for identity, quantity, biological activity/potency, test for the consistency of the heterogeneity of the active substance, purity and impurities as well as bioburden and endotoxin. The biological potency of natalizumab is measured *in vitro* by its ability to bind α4-integrins and block its interaction with its co-receptor. An in-house reference standard (RS007-001) has been established for the use in this assay. Any future reference preparations will be qualified and compared to the in-use reference standard according to the same protocol used for the current standard and this protocol has been adequately described. The assay is considered acceptable and adequately validated.
Consistency in glycosylation is a common issue for monoclonal antibodies. Measure of the carbohydrate moiety is seen as a sensitive marker for the consistency of the production process and as an important part of the molecule. As a consequence, batches of active substance will be released on the basis of an interim specification for galactosylation and sialylation established based on characterisation data from 30 batches and subject to successful validation of the method. After testing of 20 commercial batches of active substance using the validated method, the commercial specification will be established.

Stability

The container closure system for storage of the active substance is sufficiently described and qualified. Samples for stability studies are appropriately held in polypropylene containers that are designed to mimic the actual storage vessels for drug substance and stability studies were performed in accordance with ICH requirements.
Real-time stability data were presented for four commercial process batches of active substance stored for 24 months at 5±3°C. Data from storage at 25°C were also presented. All batches were stable and specifications remained within the limits defined for end-of-shelf-life at the recommended storage condition at 5°C. No meaningful batch-to-batch differences in the rate of change of the lower pI isoforms, aggregates and purity were observed. Nevertheless, although all batches remained within

specification at 5°C, the lower pI isoforms, aggregates and purity were observed to change during accelerated storage at 25°C.
The data presented justifies the requested 24-month shelf life at 2-8°C for drug substance.

Medicinal Product

Pharmaceutical Development

The product is an aseptically processed liquid formulation filled in 15 ml borosilicate glass vials. Each vial contains a single 300 mg natalizumab dose, and is therefore intended for single-use. The quantitative composition is identical with the composition of the active substance.
During product development, five formulations of natalizumab have been used in clinical trials. The formulation used in most of the Phase II and all of the Phase III trials is identical to the formulation proposed for future commercialisation.

Manufacture

No additional excipients are needed for drug product manufacture as compared to the formulated drug substance. As no further formulation steps are necessary, manufacturing of drug product mainly consists of pooling, sterile filtration and aseptic filling of drug substance, followed by stoppering and sealing, labelling and packaging. Critical test and controls have been described and validation of process consistency has been demonstrated adequately with four full-scale batches, using the commercial process. Additionally container/closure integrity and shipping between sites in the USA has been validated sufficiently.
During evaluation, the lack of various limits for in-process controls and drug substance testing has been identified. Based on additional experience, which has been accumulated for most in-process parameters, a number of new action limits have been set. These action levels will be re-evaluated as needed and as additional manufacturing experience is obtained.

Product specification

In most cases, the tests and specifications for drug product are identical to those applied to drug substance. Tests being specific for the drug product are as follows: inspection of product in the intermediate packaging, test for extractable volume, lower pI isoforms and test for particulates. Sterility is controlled at the level of the drug product since no sterility claim is made for the drug substance (only bioburden is controlled at the level of the drug substance). Release test data for all lots used in non-clinical and clinical studies, including six batches manufactured at commercial scale, were presented. The same reference material established for the drug substance is used for control for the drug product, which is acceptable as the compositions of the drug substance and drug product is identical. The specifications have been appropriately justified and are acceptable. All release tests, including sterility, will be performed on importation into the EU at Elan Pharma (Athlone, Ireland).

Stability

The container closure system, consisting of type I borosilicate glass vials, bromobutyl stoppers and aluminium seals, has been appropriately described and its integrity has been demonstrated.

Real-time stability data were presented for 24 -month at 2-8°C for 5 commercial full-scale batches of natalizumab drug product. Stability was also studied under accelerated conditions at 25°C. Results were very similar to the results obtained for drug substance stability testing and no significant changes that could affect the efficacy and safety of the product were detected. Photo stability and in-use stability has been addressed sufficiently.
The data presented justifies the requested 24-month expiration date for drug product. The stability of these batches continues to be monitored as described by the study protocol.

Adventitious agents

TSE compliance
The drug substance is produced in a serum-free culture medium. No animal-derived material is added during fermentation of natalizumab. The MCB and WCBs, which have been established, are free from TSE-risk substances and TSE Certificates of Suitability have been provided for Bovine serum albumin and foetal bovine serum. Compliance with the Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products (EMEA/410/01 Rev. 2) has been demonstrated.

Virus safety
The fermentation process for production of natalizumab occurs in a serum-free medium. No animal-derived material is added during fermentation. This minimises a possible contamination with adventitious viruses. The cells used for production of natalizumab have been extensively screened for viruses. These tests did not show the presence of any viral contaminant in the MCB, with the exception of intracellular A-type and C-type retroviral particles which are well known to be present in murine myeloma cells (NS/O). This is acceptable since there is sufficient capacity within the manufacturing process of natalizumab for reduction of this type of viral particle and this is not a cause for concern.
The purification process of natalizumab includes several steps for inactivation/removal of enveloped viruses. The effectiveness of these steps has been sufficiently demonstrated. In addition, the following steps contribute to virus safety: purification on a Protein A column and hydrophobic interaction chromatography.
The removal capacity of small non-enveloped viruses (MVM) is mainly based on anion exchange chromatography and filtration. Removal by these chromatography steps is virus specific and has only some effectiveness for small non-enveloped viruses. Nevertheless, this is acceptable since virus screening for viruses including MMV is routinely performed at the end of the fermentation runs.
During the manufacture of natalizumab drug substance, column chromatography resins are used during purification. Viral clearance studies have been performed with unused and re-cycled chromatographic resins to determine the effect of continued resin re-use and to define an acceptable column lifetime.
In summary, the virus safety of the product has been sufficiently demonstrated.

Discussion on chemical, pharmaceutical and biological aspects

Consistency in glycosylation is a common issue for monoclonal antibodies. In the particular case of natalizumab, there is only one glycosylation site, which has been thoroughly characterised and which is situated outside the binding site of the monoclonal antibody. The dossier has demonstrated consistent production and a significant impact of variability in glycosylation on bioactivity is unlikely. The applicant proposed not to include analysis of the distribution of carbohydrate variants in the release tests because no impact was observed on immunogenicity or pharmacokinetics in the human bioequivalence studies conducted using natalizumab with different carbohydrate profiles.
However, the presented bioequivalence data were not entirely convincing as the studies were designed for a different purpose. Measure of the carbohydrate moiety is seen as a sensitive marker for the consistency of the production process and as an important part of the molecule, which should be analysed from batch to batch. As a consequence, batches of active substance will be released on the basis of an interim specification for galactosylation and sialylation established based on characterisation data from 30 batches and subject to successful validation of the method. After testing of 20 commercial batches of active substance using the validated method, the commercial specification will be established.

During evaluation, experimental testing of the drug product has been performed by an Official Medicines Control Laboratory (Paul-Ehrlich-Institut). Most of the methods to control the drug product have been performed, including general tests, tests for identity, protein concentration, biological activity, purity and impurities. Biological activity has been determined with the manufacturer's method (VCAM lysate Assay). The other tests are performed based on in-house methods of the Paul-Ehrlich-Institut. Three commercial scale batches have been analysed, using standard materials of the manufacturer. The overall conclusion was, that all tested samples complied with the specifications. On the basis of the results that were obtained, consistency of lots was considered to be acceptable.

Overall, information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried out indicate satisfactory consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in the clinic.

At the time of the CHMP opinion, there were a number of minor unresolved quality issues having no impact on the Risk-benefit balance of the product. The applicant gave a Letter of Undertaking and committed to resolve these after the opinion, within an agreed timeframe.

1.3 Non-clinical aspects

Introduction

The non-clinical evaluation programme addresses in various degrees issues around the indications MS and inflammatory bowel disease (IBD), specifically, Crohn's disease (CD). Clinical dosing in these indications has been intravenous (IV) every 4 weeks at doses up to 6 mg/kg or at a fixed dose of 300 mg. The fixed dose of 300 mg is the intended dose for marketing.

Pharmacology

- Primary pharmacodynamics

Primary pharmacodynamics were examined in 9 studies, showing the effects of natalizumab in a guinea pig model of EAE (animal model of multiple sclerosis), the cross reactivity of natalizumab in a variety of animal species, the specificity of natalizumab against α4 Integrin and comparable binding of natalizumab and its murine parental antibody (AN100226m) to human lymphocytes of healthy individuals and patients with MS.

The studies in the guinea pig EAE model on the efficacy of natalizumab were performed at 3 mg/kg dose (MAD) administered s.c. on day 7 and 14 days after EAE induction. Natalizumab induced beneficial effects on active EAE, as a model of MS.

The main natalizumab effect (at serum levels above approximately 1-5 µg/ml) in all examined species is the induction of a reversible increase of WBC derived primarily by elevation in lymphocytes counts due to the block of α4 integrin. This increase is in the range of 1.1 to 2.8 fold of controls, consistent with human data. The normalization in WBC count is reached when serum natalizumab levels fall below of 1-5 µg/ml value.
Species specificity of AN100226m was determined by fluorescence-activated cell sorting (FACS) analysis. The following species are reacting with the antibody: Rhesus and cynomolgus monkey, pig, ferret, guinea pig, and dog. Lymphocytes from rat, gerbil, hamster, rabbit, or marmoset monkey are not reactive.

The affinity of AN100226m and AN100226 (the humanized form of the antibody) for guinea pig lymphocytes was very similar to that for human lymphocytes (Kd = 0.3 nM).

The specificity of the binding of natalizumab including the lack of cross reactivity with non-α4 integrin chains was determined by the binding to α4 and non-α4 integrins of transfected cell lines.

The binding characteristics of AN100226m in terms of its ability to inhibit α4-integrin mediated receptor binding with MadCAM-1, osteopontin, and fibronectin was demonstrated.

The α 4 integrin expression on lymphocytes isolated from healthy volunteers was compared to that of lymphocytes isolated from patients with multiple sclerosis (MS) by using an indirect method

employing FACS analysis to evaluate the relative expression of α 4 integrin on lymphocytes from the healthy volunteers and patients with MS. The expression was comparable between the two groups.

The studies in the guinea pig EAE model on the efficacy of natalizumab were performed. At 3 mg/kg dose (MAD) administered s.c. on day 7 and 14 days after EAE induction natalizumab induced beneficial effects on active EAE, as a model of MS.

- Secondary pharmacodynamics

Secondary pharmacodynamics were evaluated in several studies, where the potential effects on the lymphoproliferation of whole blood lymphocytes and the ability to produce cytokines in response to phytohemagglutinin (PHA) or anti-CD3 antibody stimulation, the profile of cytokines produced upon stimulation, natural killer (NK) cell activity, and T cell cytolytic function were assessed. Under the *in vitro* exposure conditions utilized in this study, natalizumab did not appear to significantly alter immune regulatory and effector cell functions in normal human lymphocytes or monocytes in the above described parameters.

Minor effects on the immune cell function were observed in a second study to evaluate the subacute toxicity of natalizumab after intravenous infusion in cynomologus monkeys (PHA stimulated proliferation of PBMC´s and spleen cells).

- Safety pharmacology programme

The potential effects of IV infusion of natalizumab on cardiac and respiratory parameters were studied in a standard safety pharmacology study on cardiovascular parameters in beagle dogs (3 doses: 0.3; 3mg and 30mg/kg). Decreases in systemic and left ventricular pressures with associated decreases in ventricular contractile indices were seen in one dog treated with 3 mg/kg and two dogs treated with 30 mg/kg natalizumab. The effects were transient and returned to baseline by the end of the infusion or shortly thereafter. Further evaluations were performed during chronic applications of natalizumab to cynomolgus monkeys, where also cardiovascular parameters were determined.

- Pharmacodynamic drug interactions

Pharmacodynamic drug interactions of natalizumab were determined in combination with interferon 1β (Avonex) in rhesus monkeys after intravenous infusion. No statistically significant differences were found between animals treated with natalizumab alone or in combination with Avonex.

Pharmacokinetics

- Methods of analysis

Presence of natalizumab and antibodies to natalizumab was measured using ELISA assays. The limit of quantisation (LOQ) of the fluorometric assay was 5.0 µg/mL and the colorimetric assay was 0.5 µg/mL. It is not clear whether the assay as it is performed is appropriate to analyze anti-natalizumab antibodies. The applicant commits to further investigate other methods to facilitate more sensitive anti-drug antibody detection but with reduced sensitivity to interference by the presence of free drug.

For one non-clinical study, sera samples that showed the presence of anti-natalizumab antibodies were also tested in a second ELISA assay (anti-idiotypic) to further characterize the antibody response. The limit of quantisation (LOQ) of this assay was 1.0 µg/mL.

- Main pharmacokinetic parameters

Natalizumab shows a pharmacokinetic (PK) typical profile of monoclonal antibodies, with dose-dependent but not dose-proportional increases in Cmax and AUC values and increases in elimination

half-lives with increasing dose that were accompanied by decreasing clearance rates. This profile is probably the result of saturation of the major antibody clearance pathway (Fc-mediated phagocytosis). No gender-related differences in disposition were observed across species, nor did pregnancy appear to significantly alter disposition in guinea pigs or cynomolgus monkeys.

PK analysis of a series of four studies were intended to enable the design and verification of a repeat-dose intraperitoneal (IP) dosing regimen in mice to be further used for human tumour xenograft cancerogenicity studies in athymic (nude) mice and severe combined immunodeficiency (SCID) mice. Mice data are considered of limited relevance since natalizumab is not binding to its α 4 integrins. The mean $t_{½}$ for the iv route (10mg) was 77 hr, and 97 hr for the ip route in mice.

The serum pharmacokinetics of AN100226 were characterized in male and female guinea pigs after single intracardiac dose or a multi-dosing study mimicking the dosing conditions used for the evaluation of reproductive toxicity in this species.

Several studies were performed to evaluate the PK profile of natalizumab in the cynomolgus monkey. The cynomolgus monkey represents a relevant non-human species to develop PK data that could be used for modelling of human PK parameters. A strong induction of antibodies to natalizumab after 11-14 day in most of the cynomolgus monkeys (detection limit 5µg/ml) was detected. Cynomolgus monkeys are not recognized as an adequate model to predict human immunogenicity due to species differences in the response of cynomolgus monkeys to a humanised protein from that of a human response to a humanised protein. The immunogenicity of natalizumab in cynomolgus monkeys is not dissimilar with what has been observed with some other humanized monoclonal antibodies in this species
PK profiles were determined after single and repeated administration and to compare different formulations or manufacturing sites/scales of natalizumab.

The main pharmacokinetic parameters after a single intravenous dose of natalizumab produced at the two different manufacturing sites in cynomolgus monkeys were comparable. The mean $t_{1/2}$ of the different studies was in the range of approx 60- 90h. Almost all monkeys in the PK as well as in the toxicology studies developed high anti-natalizumab titres (up to 1800 µg/ml) within 2-3 weeks after the administration.

The relative short $t_{1/2}$ and the high immunogenicity of the humanised mAb natalizumab are appropriately addressed within the SPC.

- Distribution

Distribution studies were included within the repeated dose toxicity studies.

- Metabolism and Excretion

No studies on the metabolism and the excretion were performed according to the proteinous nature of natalizumab.

Toxicology

- Single dose toxicity

The two studies where a single dose was applied suffered from severe technical problems.

- Repeat dose toxicity

In several repeated dose toxicity studies in cynomolgus monkeys the dosing of 3, 10, or 30 mg/kg of natalizumab was performed on alternate days through day 28 by IV injection.

A four-week combination toxicity study of natalizumab with Avonex followed by an eight-week recovery in rhesus monkey;

A 6-month weekly intravenous infusion toxicity study with natalizumab in cynomologus monkey with a 6-week recovery period;

A six-month toxicity study with natalizumab as weekly intravenous infusion in juvenile cynomologus monkeys with a 17-week recovery period

Slides of brain and spinal cord sections from three non-human primate studies (Biogen Study P00002-01-01; Elan Study 309-011-00; Elan Study 723-013-98) were forwarded to Charles River Laboratories Pathology Associates for review. There was no evidence of the simian variant of progressive multifocal leukoencephalopathy or any other demyelinating disease in any of the test animals from any of the studies. Additional analyses of completed primate toxicity studies with regard to pathology, and potential immunosuppression was performed. In two studies (723-013-98 and 309-011-00), one animal each had minimal to mild microscopic lesions in the brain characterized by perivascular mononuclear infiltrates and/or gliosis. In reviewing, these lesions were judged to be to be unrelated to the test article although an infectious etiology was not being ruled out.

Chronic toxicity studies of repeated doses of natalizumab over a 26-week period, followed by a 17 week dose-free period in cynomolgus monkeys and a 4 week study of repeated doses of natalizumab with and without Avonex were performed. Common and reproducible effects of natalizumab in cynomolgus monkeys were:

- dose related, significantly increased counts of WBC even at the end of recovery periods
- elevated reticulocyte counts
- severe increases in spleen weights
- very strong and fast induction of anti-natalizumab antibodies in almost all treated cynomolgus monkeys (especially at the doses intended for the treatment of humans)
- infusion related reactions including complement activation and shock-like reactions in some cynomolgus monkeys due to high anti-natalizumab antibodies levels

- Genotoxicity

Natalizumab was tested in two genotoxicity studies utilising two standard assays for the testing of the mutagenic potential of the mAb. There were no indications for a mutagenic potential as expected for a protein-like monoclonal antibody nor for potential contaminants or product excipients in the drug product.

- Carcinogenicity

Standard short or long term carcinogenicity studies were not performed due to the proteinous nature of the test article.

Two *in vitro* and three *in vivo* studies were performed to study the effects the natalizumab on human α 4 expressing tumour cell lines in vitro and in vivo (as transplants into SCID and nude mice).
No effects on in vitro tumour cell proliferation, either inhibition or enhancement, were seen following treatment with natalizumab over a 5 day period.

- Reproduction Toxicity

Several studies are submitted in order to evaluate the role of alpha 4 integrins on reproductive and developmental processes since many functions of α 4 during embryo development are described: in a α 4 knockout mouse, failures of placental and cardiac development even results in early gestation embryo lethality. Two cross-reactive species, the cynomolgus monkey and guinea pigs were used to

evaluate the reproductive and developmental toxicity of natalizumab. Standard but pharmacologically irrelevant, rodent species were not evaluated.

Fertility and early embryonic development

Studies addressing fertility and early embryonic development were performed in guinea pigs.

The suitability of the guinea pig as a reproductive model was shown including a negative control (mifepristone), which disrupted the pregnancies from approx. 75% (natalizumab group) to 5% (mifepristone group). Natalizumab sera levels in the females were 813.8+ 679.7 µg/ml. Transfer of natalizumab to the foetus was demonstrated by measuring 4.9µg/ml natalizumab in the foetuses sera.

Testis and epididymis were evaluated histological. Sperm analysis (motility and morphology) as well as the littering numbers was evaluated. No effects on male fertility, sperm function or reproductive organ histopathology were observed.
In one study, the males had to be replaced since several of the male guinea pigs, which had not been treated but had been co-housed with natalizumab-treated females as part of a female fertility study, died. The timing and symptoms of the deaths in the male fertility study were consistent with an immediate-type hypersensitivity (IgE-mediated) reaction to natalizumab. Natalizumab was not found in the sera of any of the 20 males tested. But all 10 males that had cohabited with females receiving 10 or 30 mg/kg of natalizumab were positive for anti-natalizumab antibodies, five at concentrations > 3 pg/mL. Anti-natalizumab antibodies were not found in the sera of the five males that had been cohabitated with females receiving placebo in Study 309-005-02 and at a low level in only one of five males, which had been cohabitated with females receiving 3 mg/kg natalizumab. The mechanism of the sensitation of the male guineas pig remains unclear.

In the female fertility study the influence of natalizumab on gestation length, gross pathology lesions, uterine and ovary weights in the females and the pregnancy rates were determined. Natalizumab treatment at 30 mg/kg resulted in a significant reduction (approx 50%) in pregnancy rates, an effect that was not seen at 0; 3 or 10 mg/kg. The significant reduction of the pregnancy rates is addressed in the SPC.

Embryo-foetal development

The objective of the 2 studies studying embryo-foetal development was to evaluate the potential effects of natalizumab treatment on the development of foetal guinea pigs in females treated prior (2 or 28 days) to implantation through the end of organogenesis.

Natalizumab treatment had no effect on gross pathology lesions or uterine weights in the females. No effects were seen on pregnancy rate, number of corpora lutea, number of implantations, number of early or late resorption, or number of dead and live foetuses. Pregnancy rates were 73, 67, 83 and 73% in the vehicle, 3, 10, and 30 mg/kg groups, respectively. Skeletal malformations and variations were seen in pups from all groups. No histological changes in the heart, thymus, liver, spleen, and intestinal tract were seen that were considered treatment related.

Alternate day dosing at doses up to 30 mg/kg was tolerated in pregnant female guinea pigs during the approximately 2 months of dosing. No significant abortion rates, fetotoxicity, or teratogenicity were observed following treatment with natalizumab prior to and through the period of organogenesis.

The transfer of natalizumab to the guinea pig foetuses as well as the induction on anti-natalizumab antibodies was demonstrated.

A study in pregnant *cynomolgus* monkeys treated with natalizumab during organogenesis (GD20-GD70) demonstrated natalizumab-related changes in the foetus that included mild anaemia, reduced platelet counts, increased spleen weights and reduced liver and thymus weights. These changes were associated with increased splenic extramedullary haematopoiesis, thymic atrophy and decreased

hepatic haematopoiesis. No abortifacient effects, fetotoxicity, or teratogenicity was observed in the study following treatment with natalizumab through the period of organogenesis.

Prenatal and postnatal development, including maternal function

In *cynomolgus* monkeys treated with natalizumab until parturition, low levels of natalizumab were detected in the breast milk of some animals, indicating the possibility for transfer of natalizumab into breast milk in humans.

One study (309-033-11) evaluated the potential for developmental effects in infants born to females treated with natalizumab and addressed the influence of natalizumab on the immune function. The study was designed to assess any effects in offspring following exposure during organogenesis and during full gestation in cynomolgus monkeys. Significant numbers of pregnancies were lost to abortions and stillbirths during the study.

- Other toxicity studies

Immunotoxicity

Four tissue cross-reactivity studies were performed with the parent antibody (AN100266m) and natalizumab:

- murine parent of natalizumab (AN100266m) to adult human tissues;
- cross-reactivity of natalizumab to adult human tissues including heart, kidney, liver, lung, and skeletal muscle tissue from MS patients in order to verify that inappropriate expression of α 4 was not occurring in major organ systems
- cross-reactivity to adult cynomolgus monkey and guinea pig heart tissues
- cross-reactivity of natalizumab to fetal tissues from humans and monkeys (rhesus and cynomolgus)

Antigenicity was detected in almost all animals within 2-4 weeks depending on the administered dose of natalizumab that is interfering with the anti-natalizumab antibodies detection assays. High titres of apparently neutralising anti-natalizumab antibodies up to 1600µg/ml are induced in cynomolgus monkeys.

In some toxicity studies shock-like reactions with complement activation and the formation of immune complexes due to high levels of anti-natalizumab antibodies were described in some cynomolgus monkeys.

Ecotoxicity/environmental risk assessment

The environmental risk assessment of natalizumab followed primarily the draft of guidelines related to this issue. From the results obtained, it is concluded that natalizumab for I.V.injection is of no immediate risk to the environment and no proposals for labelling provisions are necessary to reduce any potential environmental risks.

Discussion on the non-clinical aspect

Data on the effects of α4-integrin blockade on immune function in normal animals (primarily in cynomolgus monkey) and isolated human peripheral blood mononuclear cells has been obtained during the development program for natalizumab. During these studies specific immune function parameters were evaluated. The findings suggest that the immune function might be affected by natalizumab treatment.

Therefore, and additionally in the light of progressive multifocal leukoencephalopathy (PML) human cases, the applicant proposed studies to address this issue, as a follow-up measure.

1.4 Clinical aspects

GCP

The applicant has stated that all clinical trials were performed in accordance with GCP.

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.

Pharmacokinetics

The pharmacokinetics of intravenously administered natalizumab was systematically investigated in 3 single infusion studies with healthy volunteers, in 9 target-population studies with MS patients (4 single and 5 repeat infusions). In total, data from 486 patients could be gained. Additionally, two pharmacokinetic studies with Crohn's disease patients were conducted. These data provided evidence that the pharmacokinetics of natalizumab is non-linear, resulting in a fixed dose recommendation (300 mg).

After a single dose, natalizumab was absorbed in a linear manner reaching maximum serum concentrations in a dose-dependent manner 1 to 2 hours after the end of infusion. AUC increased in a dose-related manner and greater than dose proportional through 6 mg/kg. All pharmacokinetic studies submitted consistently show a volume of distribution of natalizumab in the range of 60-80 ml/kg, independent of the given dose, being consistent with a distribution in the vascular phase. The elimination parameters clearance and t/2 showed dose-dependent relationships predominantly at lower doses but appeared to be constant and independent of the actual administered dose at higher dose levels (3 and 6 mg/kg or 300 mg fixed dose).

Three studies evaluated the pharmacokinetics of natalizumab in the applied fixed 300mg target dose. The PK parameters determined for natalizumab were consistent with that observed following weight based dosing at 3 mg/kg and with the rationale for administering natalizumab via a fixed dose.

A population PK analysis was performed in MS-patients from 4 repeated dose studies. The analysis explored the effects of selected covariates including body weight, age, gender, hepatic and renal function, and presence of anti-natalizumab antibodies upon pharmacokinetics. Only body weight and the presence of anti-natalizumab antibodies were found to influence natalizumab disposition. The presence of persistent anti-natalizumab antibodies increased natalizumab clearance approximately 3-fold, consistent with reduced serum natalizumab concentrations observed in persistently antibody-positive patients.

For repeated dosing it remains unclear when steady state is reached. Based on a population pharmacokinetics analysis on samples from MS patients, including 581 patients who received a fixed 300 mg dose as monotherapy, the mean ± SD steady-state clearance was 13.1 ± 5.0 ml/h, with a mean ± SD half-life of 16 ± 4 days. This would indicate that steady state is reached within 12 weeks. However, natalizumab concentrations from the long-term studies indicate that steady state might be reached after about 36 weeks. The Applicant committed to further study the pharmacokinetics of natalizumab in MS patients in a post-marketing setting, in order to clarify the observed discrepancies.

- Special populations

No specific studies have been conducted in elderly patients or in patients with liver or renal impairment.

Pharmacodynamics

Natalizumab is a selective adhesion-molecule inhibitor and binds to the α4-subunit of human integrins, which is highly expressed on the surface of all leukocytes, with the exception of neutrophils. Specifically, natalizumab binds to the α4β1 integrin, blocking the interaction with its cognate receptor,

vascular cell adhesion molecule-1 (VCAM-1), and ligands osteopontin, and an alternatively spliced domain of fibronectin, connecting segment-1 (CS-1). Natalizumab blocks the interaction of α4β7 integrin with the mucosal addressing cell adhesion molecule-1 (MadCAM-1). Disruption of these molecular interactions prevents transmigration of mononuclear leukocytes across the endothelium into inflamed parenchymal tissue. A further mechanism of action of natalizumab may be to suppress ongoing inflammatory reactions in diseased tissues by inhibiting the interaction of α4-expressing leukocytes with their ligands in the extracellular matrix and on parenchymal cells. As such, natalizumab may act to suppress inflammatory activity present at the disease site, and inhibit further recruitment of immune cells into inflamed tissues.

With regard to its mechanism of action, i.e. to block the transmigration of leukocytes into inflamed tissue, the α4-integrin saturation on peripheral blood mononuclear cells (PBMC) and the total lymphocyte count were used to evaluate the pharmacodynamics of natalizumab. After a single dose, α4-integrin saturation increased immediately up to more than 90% and decreased faster in a dose-related manner 4 weeks later. Monthly dosing of natalizumab resulted in sustained α4-integrin saturation levels between 70 and 80%. Mean trough serum concentrations associated with a saturation level of 70% ranged from 15.3 to 25.9µg/ml. Elevations in absolute lymphocyte counts were within the normal range and relatively stable across studies. Saturation of α-integrin never fell below 70%, a circumstance sufficient to confer activity. However, phase III trial C-1802 shows a tendency for the repeated 300 mg dose of natalizumab to induce accumulation. This effect, less evident in the other studies submitted, indicates that the 300 mg dose may be too high in certain subjects.

Pharmacodynamic and dose-response characteristics of natalizumab have not been evaluated with regard to their relationship to efficacy endpoints.
The immunogenicity of natalizumab was investigated by screening blood samples for anti-natalizumab antibodies. All positive samples were analysed for their blocking/neutralizing effect and all positive samples were positive in both assays. In MS patients who received a single dose of the commercial material the overall observed incidence of anti-natalizumab antibodies (HAHA = human anti-human antibodies) after one single dose of natalizumab was 21% with a wide inter-trial-range and slightly higher than in healthy volunteers (12-16%) The response was visible within 5 weeks. After repeat infusions the HAHA incidence was reduced to 12%, and in the phase III studies to 10%. In 2-year controlled clinical trials in MS patients, persistent anti-natalizumab antibodies (one positive test reproducible on retesting at least 6 weeks later) developed in approximately 6% of patients. Persistent antibodies were associated with a substantial decrease in the effectiveness of natalizumab and an increased incidence of hypersensitivity reactions. Consequently, a warning is included in section 4.8 of the SPC, giving guidance to the prescriber when to measure anti-natalizumab antibodies and how to proceed in case of persistent positivity.

Clinical efficacy

The clinical development programme for the claimed indications comprises of three supportive phase II studies and two pivotal phase III trials. The study programme for natalizumab in MS treatment as a disease-modifying drug focused on two main branches, i.e. (1) the evaluation of natalizumab as monotherapy and (2) the evaluation as add-on therapy to intramuscular interferon-ß1a (Avonex). As main studies, data were presented from phase III trials (C-1801, C-1802), employing a fixed 300mg dose. C-1801 evaluated natalizumab as a monotherapy, C-1802 evaluated natalizumab as an add-on therapy to Avonex in patients still relapsing while on Avonex therapy (so-called “breakthrough disease”). Both phase III studies were two-armed studies with placebo (C-1802: Avonex monotherapy) as comparator.

- Supportive phase II studies

Study MS 231
Study MS 231 evaluated natalizumab monotherapy, comparing two dosages related to body weight (3mg/kg and 6mg/kg intravenously every 4 weeks, respectively) to placebo during 6 months with a follow-up of further 6 months after discontinuation of treatment. 213 patients (152 females, 61 males) were analysed (214 randomized, one erroneously) with 71 patients receiving placebo, 68 patients

receiving 3mg/kg natalizumab and 74 patients 6mg/kg natalizumab. Primary objective was to examine the effect of six monthly infusions of either 3mg/kg or 6mg/kg natalizumab on brain lesion activity as assessed by MRI, compared to placebo, in subjects with relapsing remitting MS or progressive MS. Secondary objectives were the assessment of safety, tolerability, immunogenicity and additional clinical and MRI efficacy parameters, as well as PK and PD evaluations in a subset of patients.

The study met its primary endpoint in the number of new Gd-enhancing lesions from baseline to month 6 for both dosage groups in favour of natalizumab ($p<0.001$). After cessation of treatment, i.e. in the 6-month follow-up period (months 7-12), no further treatment effect was observed. Also, the number of new Gd-enhancing lesions did not exceed that of the placebo group, i.e. no rebound effect was observed concerning the number of lesions.
Additionally, as a secondary endpoint in this study, the volume of new enhancing lesions was statistically significantly suppressed by natalizumab, again with a faster onset in the 3mg/kg group already significant at month 1 and slightly higher in terms of relative reduction (87% compared to placebo vs. 76% for 6mg/kg compared to placebo). While remaining stable in the placebo group, the number of persisting Gd-enhancing lesions was reduced in both natalizumab groups at month 6. Interestingly, after cessation of natalizumab, in both verum arms the number of persisting Gd-enhancing lesions remained reduced. Concerning T1 hypointense lesions, both number and volume of newly evolving lesions was suppressed in both dosage groups at month 6. Accordingly, both number and volume of T2 hyperintense lesions were reduced by both dosages. After discontinuation, the mean number of lesions approximated the placebo group, while the mean volume increase of these lesions exceeded that of the placebo group for both dosage groups (change of volumes between month 6 and 12: placebo group 1177.8±2290.21, natalizumab 3mg/kg group 1746.7±2979.87, natalizumab 6mg/kg group 2010.2±3773.79).

Subgroup analysis according to MS subtype (RRMS or SPMS, respectively)

The primary analysis according to MS subtype was defined post-hoc. For RRMS, statistically significant reductions in new Gd-enhancing lesions were observed for both dosages (mean numbers 12.1 for placebo, 0.6 for 3mg/kg and 0.6 for 6mg/kg). For SPMS, a statistical significance was only seen for the lower dosage group (mean number of lesions 5.4 for placebo, 1.0 for 3mg/kg [p=0.005] and 2.0 for 6mg/kg [p=0.083]).

Study MS 201
This was a randomised, double blind, parallel-group placebo-controlled study to evaluate the effect of natalizumab on MRI brain lesion activity. Ninety-nine patients with clinically definite RRMS (74%) or SPMS (26%) were randomized to receive two infusions (4 weeks apart) of either placebo or 3mg/kg natalizumab. Seventy-two patients were included in the ITT analysis (n=37 in the natalizumab arm, n=35 in the placebo arm). Primary efficacy endpoint was the number of new active lesions in MRI during the 12-week observation period following the first infusion. Active lesion was defined as either new enhancing lesion in T1-weighted scans or new or newly enlarging but not enhancing lesions on T2-weighted scans. Secondary efficacy endpoints were the number of new active lesions during the second 12 week observation period (i.e. weeks 12 – 24), new Gd-enhancing lesions, the evaluation of pre-existing enhancing lesions, persistent enhancing lesions, new and enlarging T2 lesions, new T1 hypointense lesions, number of exacerbations, EDSS and Guy's Neurological Disability Scale.

The study met its primary endpoint with statistical significance in favour of natalizumab (ITT population: 1.9±3.6 lesions with natalizumab vs. 3.5±4.9 lesions with placebo, p=0.042). For secondary MRI endpoints, the evaluation of the number of new active lesions during the weeks 12 - 24 showed that efficacy was no more observed and slightly more new lesions evolved in the natalizumab group (4.4 in the natalizumab arm vs. 3.6 in the placebo arm; estimated difference adjusted for baseline number of lesions 0.0, p=0.977). Accordingly, the number of new Gd-enhancing lesions was lower in the natalizumab group (mean number 1.7 for natalizumab, 3.2 for placebo) during weeks 0-12, being statistically significant after adjustment for baseline lesion count (p=0.017), while slightly higher in the natalizumab group with no obvious prolonged efficacy after discontinuation (weeks 12-24: mean 4.0 for natalizumab, 3.3 for placebo).

Study MS 202
This study was a 14-week, double-blind, placebo-controlled, dose-finding, parallel-group multi-centre study that enrolled a total of 180 patients to receive either placebo (n=63), 1mg/kg natalizumab (n=57) or 3mg/kg natalizumab (n=60) as a single infusion in acute exacerbation of MS.

Both RRMS and SPMS patients were included. Primary endpoint was the mean change from baseline to week 1 EDSS, secondary endpoints were EDSS change from baseline to week 2, SNRS (Scripps Neurological Rating Scale) mean change score from baseline to weeks 1 and 2, PGA (patient global assessment) at weeks 1 and 2, improvement in Gd-enhancing lesions at weeks 1 and 3, and the proportion of patients with steroid use during the study. The study was negative both in the primary (patients recovered equally) and the secondary endpoints assessed at weeks 1, 2 and 3. A higher number of patients in the natalizumab 3mg/kg group had fewer new Gd-enhancing lesions at both weeks 1 and 3 than patients in the placebo group, although this effect was not statistically significant. There was a statistically significant difference for the change from baseline to weeks 1 and 3 in the mean volumetric increase of Gd-enhancing lesions, being smaller in the combined natalizumab groups than in the placebo group.

- Pivotal studies

Methods

Both phase III studies were designed with two sets of objectives and endpoints, one set at one year (for the 1-year evaluation) and the other set at two years (for the 2-year evaluation), respectively.
For both studies, key inclusion and exclusion criteria were as follows:

Inclusion criteria

	C-1801	C-1802
Age range (years)	18-50	18-55
Diagnosis	RRMS (McDonald criteria)	
Relapse history	≥1 relapses in prior 1 year	≥1 relapses in prior 1 year despite Avonex therapy
Baseline EDSS	0.0 – 5.0	
Baseline MRI	Brain MRI demonstrating lesion(s) consistent with MS	
Baseline medication		Avonex therapy for at least 1 year

MAIN EXCLUSION CRITERIA

	C-1801	C-1802
MS types	PPMS/SPMS	PPMS/SPMS
Treatments not allowed at any time in the past	Total lymphoid irradiation, cladribine, T-cell or T-cell receptor vaccination, natalizumab or any other monoclonal antibody	
Prior immunomodulatory or immunosuppressant therapy time-frame drugs	≤ 6 months (patients excluded if history of ≥6 months on interferon)	≤ 6 months (with the exception that the patients required Avonex therapy for at least 1 year)
IV or oral glucocorticosteroids	≤ 50 days	
Factors in patient history	- MS exacerbation within 50 days - clinically significant infection within 30 days of enrolment	

	- history or abnormal laboratory results of significant cardiac, endocrinologic, haematological, hepatic, immunologic, metabolic, urologic, pulmonary, gastrointestinal, dermatologic, psychiatric, renal and/or other major disease - history of severe allergic or anaphylactic reactions - Inability to perform timed 25-foot walk, 9-HPT (9-Hole Peg Test) with both upper extremities and PASAT-3 (3-second Paced Auditory Serial Addition Test)

Study C-1801 randomized 942 subjects, 939 being dosed (3 subjects withdrew without dosing, all from the placebo group). In total, 627 subjects were randomized to receive 300 mg natalizumab, 315 to receive placebo. In study C-1802, 1196 patients were enrolled; after exclusion of 25 subjects from a closed site, 1171 subjects were included in the analysis: 589 received natalizumab as add-on therapy to Avonex, 582 placebo as add-on to Avonex.

Study	Design/control	Number of patients (female/male)	Posology	MS type(s) to be included	Efficacy endpoints
C-1801	Phase III Monotherapy Placebo-controlled 2 years of treatment	942 (660/282)	IV infusions every 4 wks Fixed dose Placebo (n=315) 300 mg (n=627)	RRMS as defined by McDonald criteria (2001)	1-YEAR Primary: ARR[5)] Secondary: - number of new or newly enlarging T2 lesions - number of Gd-enh. lesions - proportion of relapse-free subjects 2-YEARS Primary: EDSS progression Secondary: - Clinical relapse rate - Volume of T2 lesions - Number of T1 lesions - MSFC progression
C-1802	Phase III Combination therapy Placebo-controlled 2 years of treatment	1171 (862/309)	IV infusions every 4 wks as add-on to Avonex 30µg IM once weekly Fixed dose Placebo (n=582) 300 mg (n=589)	RRMS as defined by McDonald criteria (2001)	See C-1801

[1)] Gd, Gadolinium; [2)] MRI, Magnetic Resonance Imaging, [3)] EDSS, Expanded Disability Status Scale, [4)] MSFC, Multiple Sclerosis Functional Composite, [5)] ARR, Annualised Relapse Rate

In the phase III studies C-1801 and C-1802, a relapse was prospectively defined in the protocols as new or recurrent neurological symptoms, not associated with fever or infection, lasting for at least 24 hours, and accompanied by new objective neurological findings upon examination by an examining neurologist. The patient had to have objective signs on the examining neurologist's examination confirming the event as defined by the McDonald criteria (2001): New or recurrent neurological symptoms that evolved gradually over months were considered as disease progression, and were not counted as an acute relapse. New or recurrent neurological symptoms that occurred less than 30 days following the onset of a relapse were considered part of the same relapse and were not included into the calculation of the relapse rate.

Two sets of endpoints were defined, one set for the 1-year analysis and a set for the 2-year analysis:

	1-year analysis	2-year analysis
Primary endpoint	**Annualized relapse rate (ARR)** calculated as total number of relapses divided by total days of exposure, multiplied by 365	**Sustained (i.e. 12 weeks duration) EDSS increase from baseline**, defined as: 1. for baseline EDSS≥1.0: increase of ≥ 1.0 points 2. for baseline EDSS=0.0: increase of ≥ 1.5 points
Secondary endpoints (in order of decreasing relevance)	- mean number of new (or newly enlarging) T2 hyperintense lesions*) - mean number of Gd-enh. lesions - proportion of relapse-free patients	- relapse rate - mean volume of T2 hyperintense lesions - mean number of T1 hypointense lesions - MSFC progression
Key Tertiary endpoints[2)]	- VAS assessing patient´s global impression - MSQLI (MS Quality of Life Inventory, English only) or SF-36 (where available) - number of relapses requiring IV steroid use - number of hospitalizations	- effect on brain atrophy (brain parenchymal fraction in the MRI) - number/volume of Gd-enhancing lesions - proportion of relapse-free patients - volume of T1 hypointense lesions - extent of confirmed EDSS change - time to sustained progression - cognitive changes (PASAT-3) - visual function (Sloan letter chart) - number of hospitalizations

[1)] Compared between treatment groups using ordinal logistic regression (proportional odds model) in the ITT population. The analysis model included a term for treatment group, and for the number of T2 lesions at baseline (T2 lesions <9 versus ≥9). For subjects with missing data for the number of new or newly-enlarging T2 hyperintense lesions at 1 year, the mean value for the study population was used in the summaries and analyses as the imputed value. For the purpose of the statistical analysis, the number of lesions was classified as 0, 1, 2, 3, or ≥4. Imputed values were rounded before being categorized.

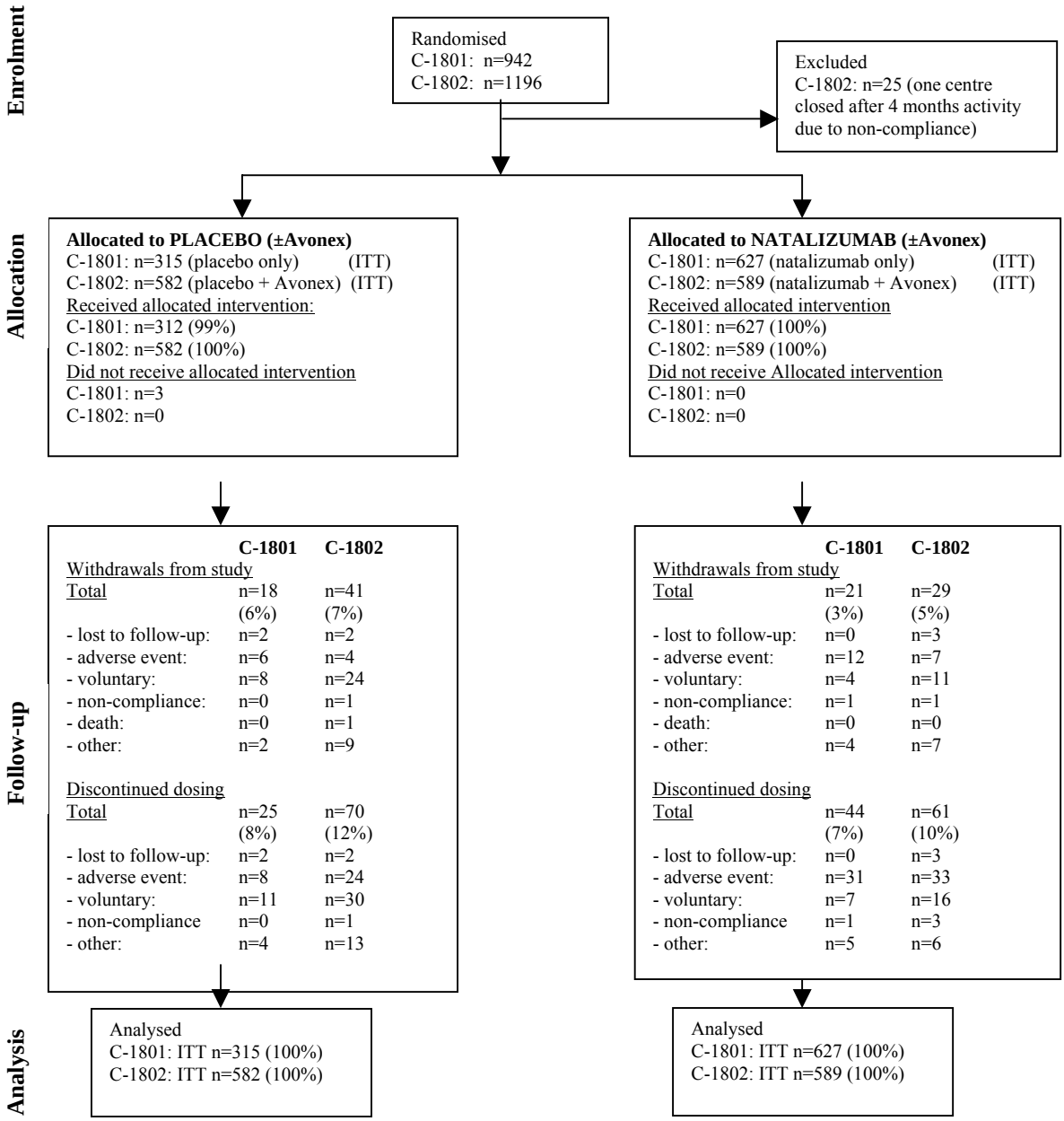
[2)] Note that for the tertiary endpoints of the studies for the 2-year analysis, some measures were not explicitly labelled as "2-year" endpoints in the study protocol. For these, a contribution to the 2-year analysis was assumed (e.g. effect on brain atrophy) from the lack of data presented after one year.

In studies C-1801 and C-1802 identical statistical methods were applied. Each study includes two primary endpoints; relapse rate analyzed at 1 year and disability progression to be analyzed at 2 years.

For the 2-year analysis, the primary objective focused on disability: The Expanded Disability Status Scale (EDSS) was measured at baseline, Week 12, and at 12-week intervals thereafter up to and including Week 120. The Multiple Sclerosis Functional Composite (MSFC) was evaluated at the same visits as EDSS. The change from baseline MSFC at 2 years was a secondary endpoint.

RESULTS

PARTICIPANT FLOW



Demographic and baseline disease characteristics were similar between the treatment groups in each study, except differences in gender in study C-1801 (slightly higher proportion of females in the verum group), longer disease duration in the placebo groups of both studies (one year) and a higher baseline EDSS (0.5 points) in the placebo group of trial C-1802.

Clinical response

One-year data

After one year, the annualized relapse rate was significantly reduced in both studies, and MRI activity markedly suppressed. In 96% of patients that received natalizumab in both studies, Gd-enhancing

lesions were completely absent after one year. Therefore, natalizumab, either as monotherapy or as add-on therapy to Avonex, showed profound efficacy. The results were consistent across most relevant subgroups (with the exception of T2 lesion load at baseline). Newer publications on the impact of relapses on MS progression suggest a relevant impact of relapses on the progression of disability in the initial phase of the disease (i.e. in EDSS ranges below 4.0). Although the suppression of MRI activity is clinically meaningful, given newer scientific data that show that extensive axonal damage takes place in active demyelinating lesions, the potential clinical and prognostic impact of this finding has to be further emphasised.

Table: Key efficacy data from phase III studies in RRMS after <u>one year</u>

	C-1801 (Monotherapy)		**C-1802 (Combination with Avonex)**	
	Placebo	Natalizumab	Placebo/Avonex	Nataliz./Avonex
PRIMARY ENDPOINT				
Annualized relapse rate (ARR)				
Absolute values[1)]	**0.805**	**0.261**	**0.816**	**0.383**
(95% CI)	(0.669-0.969)	(0.211-0.323)	(0.721-0.923)	(0.325-0.450)
% reduction		**68% (p<0.001)**		**53% (p<0.001)**
SECONDARY ENDPOINTS				
new (or newly enlarging) T2 hyperintense lesions[2)]				
- mean number	**6.1**	**1.2**	**2.1**	**0.5**
- % reduction		**80% (p<0.001)**		**76% (p<0.001)**
- % of patients developing no new lesions	22%	60%	40%	67%
- % of patients developing only a single lesion	13%	18%	29%	26%
- % of patients developing or enlarging ≥ 3 lesions	57%	16%	21%	3%
Gd-enhancing lesions				
- mean number	**1.2**	**0.1**	**0.8**	**0.1**
- % reduction		**92% (p<0.001)**[3)]		**88% (p<0.001)**[3)]
- completely absent (%)	68%	96%	76%	96%
proportion of relapse-free patients	53%	76% (p<0.001)[4)]	46%	67% (p<0.001)[4)]

[1)] from Poisson regression adjusted for the number of relapses in the one year prior to study entry, baseline EDSS (≤3.5 vs. >3.5), presence of Gd lesions (present vs. absent), and age (<40 vs. ≥40).
[2)] Missing values were imputed using the mean number of T2 hyperintense lesions in the study population. Both baseline and 1-year MRI data were available from 893 of the 942 randomized patients for study C-1801 and from 990 of 1171 patients for study C-1802, respectively, due to the timing of the 1-year analysis and due to withdrawal of some patients.
[3)] p-value for comparison based on ordinal logistic regression adjusted for baseline number of T2 lesions (<9 vs. ≥9 lesions).
[4)] logistic regression adjusting for the number of relapses in the previous year.

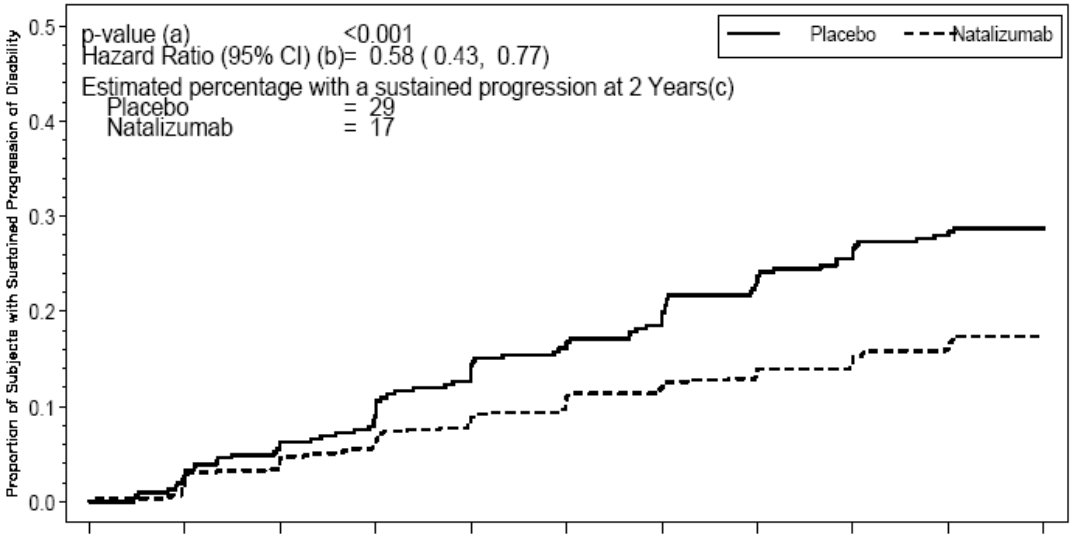
<u>Two-year data</u>

<u>Monotherapy (C-1801):</u>

Treatment with 300 mg natalizumab resulted in a 42% decrease in the risk of disability progression, as measured by sustained changes on EDSS, when compared to placebo over a 2-year period (p<0.001). These results were confirmed by an alternative scoring system, the Multiple Sclerosis Functional Composite (MSFC). The highly significant reduction in the annual relapse rate (ARR) that was the central argument for efficacy of the initial submission was confirmed after 2 years: Treatment with 300 mg natalizumab resulted in a 68% decrease in the annualized relapse rate versus placebo over both 1 and 2 years (p<0.001 for both time points). The effect was consistent across all subgroups regardless of age, gender, race, weight, baseline disease activity, and MS disease history.

The time to sustained progression of disability in the ITT population as measured in study C-1801 is depicted below.

Time to Sustained Progression of Disability as Measured by Increase in EDSS - ITT Population



Number of Patients at Risk

Placebo	315	296	283	264	248	240	229	216	208	200
Natalizumab	627	601	582	567	546	525	517	503	490	478

NOTE: Sustained progression of disability is defined as at least 1.0 point increase on the EDSS from a baseline EDSS >=1.0 sustained for 12 weeks or at least a 1.5 point increase on the EDSS from a baseline EDSS of 0 sustained for 12 weeks.

(a) Log Rank p-value.
(b) Hazard ratio (natalizumab/placebo) estimated from a Cox proportional hazards model adjusting for baseline EDSS and age (<40 versus >=40)
(c) Kaplan Meier estimate of the percentage of subjects expected to have sustained progression within 2 Years

The main features and results of the study are also tabulated in section 5.1 of the SPC (also including one year outcome data).

Resembling efficacy data at one year, the only subgroup in which the treatment effect on disability progression was not apparent was the subgroup with fewer than 9 baseline T2 hyperintense lesions. In the lower T2 hyperintense lesion burden subgroup of 44 subjects, the hazard ratio for sustained disability progression was 1.31 (CI 0.26-6.66), indicating a non-significant difference between placebo and active treatments groups. Possibly, this finding might again hint that in patients with lower disease activity, the impact of natalizumab treatment is markedly lower.

As regards other clinically relevant measures of disability, i.e. time to more severe disability (i.e. EDSS≥4.0) or even a need for walking aid (EDSS≥6.0), progression was statistically significantly reduced as compared to placebo. This is highly relevant not only for the patient, but also from a socioeconomic perspective, since these patients frequently become unable to work. The effect on disability was confirmed by the MSFC evaluation, both on the overall score and the scores for each component in a statistically significant manner. Also with visual function and quality of life measures (both on mental and physical component of the SF-36) significant improvements were detected as compared to placebo.

Through year 2, there was an 83% reduction in the number of new or newly-enlarging T2-hyperintense lesions in natalizumab-treated subjects compared to placebo ($p<0.001$). In the natalizumab group, 57% of subjects developed no lesions and 17% had a single new or enlarging T2-hyperintense lesion. In the placebo group, 15% of subjects developed no lesions and 10% had a single new or enlarging T2-hyperintense lesion. 96% of subjects in the natalizumab group were free of Gd-enhancing lesions after 1 year of treatment, and 97% after 2 years. The development of T1 hypointense lesions was reduced by 76%. It is estimated that half of acute T1 hypointense lesions

(which often correspond with inflammatory Gd-enhancing lesions) will evolve into chronic T1 "black holes" which correlate with disability progression (areas with axonal loss). Brain parenchymal fraction (BPF) was assessed as a marker of destructive pathologic processes ongoing in relapsing MS subjects, and changes indice a loss of brain tissue. During the first year of treatment, the effect of natalizumab on brain atrophy seemed to have been masked by a reduction in inflammation and edema, which caused an acute shrinkage in brain tissue ("pseudo-atrophy"). During the second year of treatment, the mean percentage decrease in BPF was significantly less in the natalizumab group as compared with the placebo group (0.24% vs. 0.43%; p=0.004). This might indicate a beneficial effect on brain atrophy that could in the overall two-year data be masked by "pseudo-atrophy" occurring in the first year of treatment (0.80% vs, 0.82%; p=0.822).

In the 6% of natalizumab-treated patients who developed persistent antibodies to natalizumab, efficacy was greatly diminished.

Combination therapy with Avonex (C-1802):

Treatment with 300 mg natalizumab when added to Avonex resulted in a 24% decrease in the risk of disability progression, as measured by changes on EDSS, when compared to Avonex monotherapy over a 2-year period (p=0.024). Treatment with 300 mg natalizumab when added to Avonex resulted in a 53% decrease in the annualized relapse rate over 1 year and in a 55% decrease in the annualized relapse rate over 2 years *versus* treatment with placebo plus Avonex in subjects inadequately responding to Avonex (p<0.001 for both time points). The effect was consistent across all subgroups regardless of age, gender, race, weight, baseline disease activity, and MS disease history. Again, as for study C-1801, the only subgroup where no apparent effect on disability progression was observed was the subgroup with fewer than 9 T2 hyperintense lesions at baseline.

The effect on disability was confirmed by the MSFC evaluation in the overall score and the scores for the components 25-foot walk (T25FW) and 9-hole peg test (9HPT) in a statistically significant manner (p<0.001), however not for the cognitive change as measured by PASAT 3 (p=0.159).

Through year 2, there was an 83% reduction in the number of new or newly enlarging T2-hyperintense lesions in the Avonex plus natalizumab group compared to the Avonex plus placebo group (p<0.001). In the Avonex plus natalizumab group, 67% of subjects developed no lesions and 13% had a new or enlarging single T2-hyperintense lesion. In the Avonex plus placebo group, 30% of subjects developed no lesions and 9% had a new or enlarging single T2-hyperintense lesion. 96% of subjects in the natalizumab plus Avonex group were free of Gd-enhancing lesions after both 1 and 2 years of treatment. The development of T1 hypointense lesions was reduced by 44%.

In the 6% of patients who developed antibodies to natalizumab, efficacy was diminished.

Add-on therapy to Avonex treatment

Comparison of the baseline number of Gd-enhancing lesions between monotherapy and combination therapy study showed a reduced number in the combination therapy study, which could point to some disease-modifying effect of Avonex in the time before entry. However, the efficacy outcomes both in the one-year and the two-year analyses show somewhat comparable efficacy of natalizumab in the verum groups of both studies. This had already with the one-year data led to the consideration that this could point to a dominating effect of natalizumab that might not necessarily justify the continuation of Avonex once natalizumab has been initiated. The design of the respective study allows only for the evaluation of the additive effect of natalizumab as add-on to Avonex compared to Avonex monotherapy, but does not allow the evaluation of the relative contribution of Avonex to this efficacy due to the lack of a natalizumab monotherapy arm.
It was further observed that patients in the control arm of study C-1802 might not have been adequately treated () . Since the relapse rate outcome after one year in the placebo groups of both trials was rather similar, this patient population was enriched by many primary interferon non-responders.

Finally, two cases of PML, one fatal, were observed in patients treated with the combination of natalizumab and Avonex for more than 2 years, rendering the combination therapy with Avonex obsolete**.**

Posology

Based on the 2-year data that were submitted by the Applicant, data suggest that efficacy is not substantially different among weight groups, neither in terms of progression of disability, nor in terms of relapse rate or MRI parameters. However, an impact of the relative overdose in subjects with low bodyweight on long-term safety cannot be excluded, and the Applicant committed to evaluate adverse events in relation to body weight on a regular basis as part of the PSURs.

- Subgroup analyses

Patients with low disease activity as measured by MRI

The finding that natalizumab treatment might show a smaller clinical benefit in patients with a lower lesion load (<9 lesions) was confirmed with the two-year data from both pivotal trials. While this finding could stem from methodological issues like a low relapse rate in the placebo group and the relatively small number of patients in this subgroup, patients with lower lesion load often exhibit a better prognosis that might not justify the use of an MS drug in any case during this stage of the disease, also given the potential occurrence of opportunistic infections including PML during long-term treatment with natalizumab.

Patients with progressive MS (SPMS)

Data supporting the claim for a broader indication (also including patients with progressive forms of MS that still relapse) are rather sparse and stem only from a limited number of patients from phase II studies MS 231 and MS 201. For study MS 231, statistical significance in the primary endpoint (number of new Gd-enhancing lesions) was only reached for the lower dose of natalizumab. No efficacy data exist in relapsing SPMS with the fixed dose regimen.

- Rebound after discontinuation

Data from the phase II study MS 231, where patients received 6 months of active treatment and a follow-up of additional 6 months after treatment discontinuation, suggested that there might be the danger of a subclinical rebound of MRI lesions, exceeding those in the placebo group. After discontinuation of natalizumab, the volume increase of T2 demyelinating lesions exceeded that of the placebo group for both dosage groups by more than double. Further, the volume increase from month 6 to month 12 of T1 hypointense lesions was approximately 3 times higher in the higher dose group than for placebo or the lower dose group, respectively. It still remains unclear if such findings might be hints for a possible MRI rebound after cessation, or if these are only chance findings due to MRI lesion natural variability and the short observation period. A slightly higher MRI lesion load (new active lesions) upon discontinuation was also observed in supportive study MS 201. Such rebound was not seen in the clinical measures, but such MRI rebound might not (yet) have become clinically obvious, since many MS lesions are clinically quiescent for a long period of time.

After cessation of dosage in all MS patients after the occurrence of the PML cases, considerable MRI data were obtained. The Applicant was asked to present relevant data from patients having completed their post-dosing MRI at least 2 months after cessation of dosing, stratified for adequate time intervals, to evaluate possible signs of MRI rebound. The available data, limited by some shortcomings by nature like no placebo comparator, differences in slice thickness, and varying time-points both in terms of last MRI and last dose of natalizumab, indicate that no major rebound may occur. However, the Applicant commits to evaluate further data in a post-marketing setting, e.g. as part of open label extension study C-1808.

- Discussion on clinical efficacy

Based on the results as presented, the conclusion can be drawn that natalizumab has a profound effect both on clinical activity (as measured by relapse rate and MRI endpoints) and progression of disability (as measured by EDSS and MSFC). The efficacy that was already apparent with the one-year endpoints was maintained at two years.

Clinical safety

- Patient exposure

Over 4,500 person-years of experience with natalizumab exist between the MS and Crohn Disease programmes. Of the 1,617 MS subjects treated with natalizumab in the placebo-controlled experience, the majority (1,271 or 79%) have received the fixed dose of 300 mg; 1,123 (69%) have been followed for at least 1 year, and 1,062 (66%) have been followed for over 2 years, satisfying, and indeed exceeding ICH safety database requirements.

All patients of C-1801 and C-1802 studies who received at least 1 dose of study drug and had at least 1 post baseline assessment were reported in the safety analysis. The safety data were based on the results from 8 target indication studies in relapsing Multiple Sclerosis (including both RRMS and SPMS), 9 non-target indication studies in Crohn's disease (CD) patients and additional bioequivalence data from clinical and commercial products. Dosing in relapsing MS and active CD was intravenous (IV) every 4 weeks at doses up to 6 mg/kg or at a fixed dose of 300 mg. In two relapsing MS studies natalizumab was combined with Avonex or Copaxone. The fixed dose of 300 mg is the intended dose for marketing natalizumab.

- Safety in clinical trials

In general the incidence of commonly occurring adverse events was balanced between the verum and placebo groups. The most common events in the pooled MS study populations were headache, MS relapse, nasopharyngitis, fatigue, back pain, arthralgia, pain in extremity, depression, upper respiratory tract infection NOS, and urinary tract infection NOS. In the placebo-controlled MS studies, the incidence of depressive disorders was similar in natalizumab-treated subjects (15.6%) compared to placebo-treated subjects (15.1%). Hypersensitivity reactions were the most common cause of discontinuation in the natalizumab treatment group. Adverse events of pharyngitis (7.7% natalizumab, 5.2% placebo), gastroenteritis NOS (3.5%, 1.9%), peripheral oedema (3.8%, 2.2%), and rigors (3.4%, 1.1%) occurred at an incidence at least 1.5% higher in the natalizumab treatment group. Events being judged by investigators as related to natalizumab which occurred at a 0.5% incidence higher than placebo in the two longest trials were: urinary tract infection, nasopharyngitis, urticaria, hypersensitivity, headache, dizziness, vomiting, nausea, arthralgia, pain in extremity, rigors, and pyrexia. Of these, dizziness, nausea, urticaria, and rigors were infusion-related events. When looking at the development of adverse events over time (the short- and long-term dosing experience in CD, 6-month dosing intervals in MS) there is a tendency for the AEs to peak with the first 3 infusions and then decrease over time. Headache, the most common adverse event, was seen at a similar incidence in both placebo and verum groups. Adverse events more common in natalizumab-treated subjects included dizziness, pruritus, urticaria and hypersensitivity.

Aside from hypersensitivity reactions, the infusions were generally well tolerated. In the phase 3 trials, infusion-related events were conservatively defined and solicited from patients during the 1-hour infusion and the 1-hour post-infusion observation period. Infusion-related reactions in the pooled MS and CD studies were 24 % in the verum group (compared to 18% in the placebo group), the most common being headache, which occurred in 5% of natalizumab-treated subjects and 3% of placebo-treated subjects. Acute hypersensitivity reactions occurred in approximately 4% of subjects receiving natalizumab as monotherapy, whereas approximately 2% of subjects who added natalizumab to Avonex experienced such reactions. These reactions, which were usually characterized by urticaria in isolation or with associated symptoms, were mostly mild to moderate and the majority were reported within the first 7 infusions, although they were most common during the second infusion. In the MS placebo-controlled trials, 6 (0.4%) natalizumab-treated subjects had anaphylactic or anaphylactoid reactions compared to 2 (0.2%) placebo-treated subjects. Delayed hypersensitivity reactions were very rare; a clear relationship to antibody development could not be established.
In the C-1801 and C-1802 studies about 6% of subjects became persistently positive for antibodies to natalizumab. These patients show an incidence of important adverse events higher than the placebo groups. Moreover, in the C-1801 study the patients testing persistently antibody positive were less likely than the placebo group to remain relapse free and more likely to develop T2 hyperintense and gadolinium enhancing lesions on MRI.Patients who were persistently positive for anti-natalizumab antibodies showed a higher incidence of MS relapse which could reflect diminished effectiveness of natalizumab. Correspondingly, patients persistently positive for anti-natalizumab, also showed a higher incidence of Crohn's exacerbation in the CD studies. Data from chronic intermittent dosing CD

studies indicate that antibodies are more frequent with intermittent dosing compared with continuous dosing.

- Progressive multifocal leukencephalopathy (PML)

Two cases of PML, one fatal, were reported by the Applicant in two patients with RRMS, both treated with a combination of natalizumab and beta-interferon (Avonex) for more than 2 years. A third case of PML was later discovered upon re-evaluation of the Crohn's Disease safety database in a subject that was originally presumed to have died of a malignant astrocytoma.

PML is a frequently fatal re-infection of the central nervous system by JC virus, a polyoma virus. Approximately 85% of the healthy population carries this virus, but PML is very rarely described among healthy subjects. The causality of JC virus infection of the brain and PML is well studied in patients with HIV infection and occurs with an incidence of approximately 5 % in this population. PML is an AIDS defining disease. Systemic anti-JCV titres are seen in almost all JCV infected patients, but the titres do not correlate with risk to develop PML or with the disease progression, whereas the virus load in CSF correlates well with progression of PML. The immunity against JCV is performed by CD8+ T-Lymphocytes against JC virus. The route of primary infections with JCV is via the tonsils, appearing before the age of 6 years in most cases. After primary infection the JCV can persist for an unknown time in a latent phase either in bone marrow or kidney or both. If reactivated JCV can be detected in B cells from which it possibly infects the brain parenchyma. Once this compartment is infected the JCV starts to replicate. The highest replication measured in vitro has been seen in astrocytes and glial cells. Factors, which trigger the reactivation of JCV from latent phase, are not known. Once the JCV has entered the brain parenchyma the self-defence with cytotoxic T-Lymphocytes is very effective in controlling the spread of the virus between the glial cells.

Extensive studies of patients with HIV infection suffering from PML have shown that symptoms in the initial phase are similar to MS symptoms, e.g. visual symptoms, motor dysfunctions, headache, epileptic seizures. The time from reactivation and infection of the glial cells to visible MRI lesions is not known.

At present there are no accepted surrogate markers to describe the regulation or progression of JCV latency to reactivation causing PML.

Special therapeutic options for active PML in MS patients are limited. At the first signs or symptoms for PML raised either from laboratory results or imaging, stopping the medication with natalizumab and immediate performing of plasmapheresis in order to eliminate natalizumab in combination with supportive intravenous immunoglobulin treatment is the therapy proposed as the first choice although there is no experience with this treatment so far.

As natalizumab disrupts the transmigration of leucocytes across the endothelium into inflamed parenchymal tissue, the traffic across the blood-brain barrier of anti-JCV cytotoxic T- Lymphocytes might be inhibited. Obviously the interaction of α4-integrins with their receptors is a major contributor of extravasation of T cells into the CNS tissue for normal immunosurveillance. Disturbance by drugs like natalizumab, potentially enhanced by concomitant immunomodulators or immunosuppressant, may lead to reduced T cell surveillance of CNS in particular and therefore to uncontrolled reactivation of this virus (or potentially also other pathogens).

- Other infections including opportunistic infections

Infections prevailed at a rate of 1.54 per person year in the natalizumab-treated group compared to 1.5 in the placebo-treated patients. Few subjects experienced serious infections in either treatment group. Appendicitis and urinary tract infection NOS were the most common serious infections (0.4% natalizumab vs. 0.3% placebo for appendicitis; 0.4 vs. 0.2% for urinary tract infection NOS). Pneumonias, including bronchopneumonia, lobar pneumonia and atypical pneumonia, represent 6 (0.4%) serious infections in the natalizumab-treated and 2 (0.2%) infections in the placebo-treated subjects. All subjects responded to antibiotic therapy. The absence of increased bacterial infections is consistent with the knowledge that neutrophils are not affected by α-4 integrin blockade; neutrophils

primarily use the CD11b system for adhesion and trafficking to sites of inflammation rather than the α-4 integren system. One case of mycobacterial infection (patient was under steroid treatment) and one case of cryptosporidiosis occurred. In the placebo-controlled CD studies, a greater proportion of subjects receiving natalizumab experienced an infection compared to placebo (40.4% vs. 35.8%, respectively). The rates of infections were also somewhat higher in natalizumab-treated CD subjects, 1.66 per person per year, vs. 1.44 per person per year in the placebo group. The incidence of serious infections was comparable in the two treatment groups, 2.5% and 2.6% (natalizumab vs. placebo, respectively). The most frequently reported type of serious infection was an abscess within the gastrointestinal tract, e.g., perianal (0.6% vs. 0.6%), abdominal (0.3% vs. 0.2%) occurring in both treatment groups and abscess NOS, abscess intestinal, appendiceal, psoas, peritoneal, and rectal occurring in <0.1% to 0.4% in either treatment group.

Herpetic viral infections occur commonly in the general population. An analysis of herpes infections, both simplex and zoster, was conducted to assess a potential risk for these infections. In the placebo-controlled MS and CD studies, the incidence of herpes-like symptoms was slightly higher in natalizumab-treated subjects than placebo-treated subjects (natalizumab vs. placebo: 7.1% vs. 6.0% for MS and 1.6% vs. 1.0% for CD). Four subjects receiving natalizumab experienced serious infections, with 3 subjects receiving intravenous acyclovir for varicella infection.

Several uncommon presentations of infections known to occur in immunocompromised individuals were observed with natalizumab treatment, primarily in the CD clinical studies. Cytomegalovirus (CMV) infection occurred in 2 subjects with CD. One subject had asymptomatic elevation in liver function tests (LFTs) that resolved and the second subject had symptomatic involvement of the colon. Other infections in CD subjects include single cases of Mycobacterium avium intracellulare (MAI) pneumonia, pneumocystic carinii pneumonia (PCP), and bronchopulmonary aspergillosis. All of these subjects had significant co-morbidities or use of immunosuppressant that could contribute to the risk for these infections. A single case of cryptosporidiosis, which resolved following hospitalization for hydration, occurred in a subject with MS with no obvious source for infection. Ongoing vigilance with natalizumab will provide a more complete understanding as to the risk of infections with prolonged exposures.

Most of the infections reported in the post-marketing setting were consistent with typical community-acquired pneumonia. There was one case of herpes encephalitis, which resulted in death, and one case of herpes meningitis with full recovery. Both subjects had received only one dose of natalizumab. Herpes encephalitis is the most common cause of sporadic viral encephalitis in the USA and typically occurs in immune-competent individuals. In the integrated natalizumab clinical trial safety database, there were no cases of encephalitis and there was no safety signal when evaluating CNS herpetic infections

However, the herpes viral infections and possible risk for severe courses of encephalitis have to be considered in the post marketing surveillance and pharmacovigilance planning. In addition, it should be mentioned, that a suspected case of tuberculosis in a 20-year-old male with CD from New Zealand has to be considered as possibly related to Natalizumab.

- Malignancies

For neoplasms, no distinct differences in the incidence rates emerge as yet from the provided data set. Only more data from future PSURs will be able to satisfactorily answer the question of a possible increase in tumour formation in natalizumab treated patients. The incidence of breast cancer and basal cell carcinoma seen in this study is within keeping of the rates commonly seen in the population at large. For melanoma there are insufficient data to clarify whether and how natalizumab affects its development. In the cases of lung carcinoma, the patients had a history of nicotine abuse. The cases of uterine cancer were balanced between verum and placebo groups.

- Laboratory findings

The laboratory evaluation confirmed the pharmacodynamic effects of natalizumab on the leukocytes (except neutrophils), which increased in the peripheral blood. This effect seems to be reversible after

approximately 16 weeks after the last dose. Due to the increases in circulating white blood cells and given the preclinical findings of increased spleen size in practically all animals responding to the treatment, there is a lack of specific data on spleen examination in the clinical trials. Liver function tests for abnormalities did not reveal any distinct safety signals or trends. Transient elevations in liver enzymes or elevated liver enzyme elevations at screening were not accompanied by bilirubin elevations. Liver related SAEs in the natalizumab-treated subjects were attributable to other causes such as cholelithiasis or other medications (including Avonex). However, one patient in the phase I study in healthy volunteers did develop an unexplained hepatitis. The mild decrease in haemoglobin levels was not of clinical significance during the trials, and was readily reversible on natalizumab withdrawal.

- Safety in special populations

No distinct gender differences in adverse events evolved. In general, there was not sufficient data on natalizumab in geriatric or juvenile patients to make precise evaluations on safety. Race did not seem to affect the pattern of adverse events. Also, there were no concomitant diseases that increased risk of more serious events such as hypersensitivity-like reactions, including a history of immunological disease. Natalizumab was not studied adequately in subjects over age 65 and in subjects with renal and hepatic impairment. The efficacy, safety, and appropriate dosing in these populations are not known.

- Post-marketing experience

The marketing of the product on the US market, where it had been approved by fast track procedure on 23 November 2004, was voluntarily suspended following the reporting of PML cases (February 2005).

Between the approval of Tysabri in the US and the time of voluntary suspension of marketing, it is estimated that approximately 7000 subjects had been treated with Tysabri in the commercial setting, the majority of whom received only 1 or 2 doses. Comparing clinical and commercial products, the safety profile of Tysabri observed in the post-marketing setting is generally consistent with the adverse event profile observed in the clinical trial safety database and is consistent with the proposed Tysabri product labelling. Many of the adverse reactions were hypersensitivity-like in nature. Reports of allergic reactions, mainly involving a rash that occurred with the second infusion, are consistent with adverse events seen in the integrated clinical trial safety database. No confirmed cases of PML have been identified in the post-marketing setting.

1.5 Pharmacovigilance

Detailed description of the Pharmacovigilance system

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

Risk Management Plan

The MAA submitted a risk management plan.

The applicant has designed a pharmacovigilance plan, including risk mitigation activities, using a number of different approaches to further clarify and characterize unresolved safety issues, and at the same time allow ongoing safe use of the drug and monitoring of the emerging safety profile with use of natalizumab in the market. A clear structure of its pharmacovigilance systems has been provided with a number of proactive commitments regarding the collection, assessment and further investigation of adverse events arising from patients treated with natalizumab. The safety specification and the summary of the identified risks that require further evaluation are appropriately discussed with regard to PML. The relevant epidemiology facts of MS are addressed in the Plan with valid information concerning complications arising in the course of disease including PML.

The commitments for the planned post-marketing safety investigations and studies are based on the three important safety issues (Infusion/ hypersensitivity reactions, the antibody formation and PML). The applicant has tried to cover these commitments with a wide range of studies and clinical trials as well as preclinical studies.

The main purpose of the "Pharmacovigilance Plan" is to define the safety specification and summarise the identified and potential risks of the product including a sophisticated diagnostic pathway of PML assessment. Concerning risk minimization, the applicant proposed the establishment of a surveillance programme in the US concerning PML and an observational study in app. 5000 patients to determine the nature and incidence of infections that require hospitalisation or medically significant adverse events which may occur in a frequency of ≥1: 1500 (with a 95 % probability). The description of both, the surveillance programme and the observational study is fairly vague with regard to the routine follow-up in the usual standard of care, which may be significantly different in the countries the study will be conducted and concerning the proper case definition. The risk of opportunistic infections (e.g. mycobacterium Tuberculosis/ Avium, cryptosporidium, Herpes virus group, fungal infection) has been included on request by the CHMP, as has been the potential concern of malignancies occurring in long-term treatment due to reduced lymphocyte surveillance.

Summary of Activities in the EU - Risk Management Plan

Safety Concern	Proposed Pharmacovigilance activities	Proposed Risk Minimisation
Known Risks		
1. Hypersensitivity reactions	Routine Pharmacovigilance Incidence and nature from: o Re-dosing study o Clinical trials o TOUCH Surveillance Programme o TYGRIS Observational Cohort Study	Recommendation for management of hypersensitivity in section 4.2 of the SPC. Contraindication in section 4.3 of the SPC. Warning in Section 4.4 of SPC. Listed as ADR in Section 4.8 of SPC.
2. Antibody formation	Routine Pharmacovigilance Re-dosing study Have antibody assay for use in the marketplace available by time of launch.	Recommendation that therapy be carefully reconsidered in patients showing no evidence of therapeutic benefit beyond 6-months in section 4.2 of the SPC. Warning in Section 4.4 of SPC. Listed as ADR in Section 4.8 of SPC.
3.PML	Routine Pharmacovigilance Follow-up of spontaneous events with specific questionnaire. Pre-clinical/Clinical pharmacology studies to investigate effects on immune system. Incidence rate and risk factors for development of PML from: o Re-dosing protocol o Clinical trials o TOUCH Surveillance Programme o TYGRIS Observational Cohort Study	Contraindication for use in patients with PML in section 4.3 of the SPC. Warning in Section 4.4 of SPC. Listed as ADR in Section 4.8 of SPC. Educational materials including physician Prescribing Guidelines and Patient Alert Card. Diagnostic algorithms for PML.
4. Opportunistic Infections	Routine Pharmacovigilance Follow-up of all spontaneous events with specific questionnaire to characterize infecting pathogen. Incidence rate from: o Re-dosing protocol o Clinical trials o TOUCH Surveillance Programme o TYGRIS Observational Cohort Study	Contraindication in patients with increased risk of opportunistic infections in section 4.3 of the SPC. Warning in Section 4.4 of SPC. Listed as ADR in Section 4.8 of SPC. Educational materials including physician prescribing guidelines and patient alert card. All reported serious opportunistic infections will be expedited
Potential Risks		
5. Malignancy	Routine Pharmacovigilance Incidence rate from following studies with	Contraindication in patients with known active malignancies (except for patients with

Safety Concern	Proposed Pharmacovigilance activities	Proposed Risk Minimisation
	comparison to background rates from National and International databases: o Re-dosing protocol o Clinical trials o TYGRIS Observational Cohort Study	cutaneous basal cell carcinoma) in section 4.3 of the SPC.
6. Re-activation of Tuberculosis	Routine Pharmacovigilance Clinical pharmacology studies to investigate effects on immune system	
7. Immunisation Response	Specifically designed clinical trials to investigate effect on immunisation response.	
8. Cardiac profile	Routine Pharmacovigilance. Incidence rate from: o Re-dosing protocol o Clinical trials o TYGRIS Observational Cohort Study	
Unknown Information		
9. Pregnancy and Pregnancy Outcome	Routine Pharmacovigilance Pregnancies from clinical trials, registries and spontaneous events followed to outcome. Pregnancy registry of 300 patients.	Recommendations for discontinuation of Tysabri with occurrence of pregnancy as listed in section 4.6 of the SPC.
10. Special Populations	Routine Pharmacovigilance	Information on use of drugs in elderly, and patients with renal and hepatic impairment in section 4.2 of SmPC Information on posology in children and adolescents in section 4.2 of SmPC Contraindication for children and adolescents in section 4.3 of SmPC

1.6 Overall conclusions, risk/benefit assessment and recommendation

Quality

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way. Data has been presented to give reassurance on viral/TSE safety. Minor unresolved quality issues having no impact on the Risk-benefit balance of the product will be addressed as part of post-authorisation commitments undertaken by the Company within an agreed timeframe.

Non-clinical pharmacology and toxicology

Natalizumab is a selective adhesion-molecule inhibitor and binds to the α4-subunit of human integrins, which is highly expressed on the surface of all leukocytes, with the exception of neutrophils. A further mechanism of action of natalizumab may be to suppress ongoing inflammatory reactions in diseased tissues by inhibiting the interaction of α4-expressing leukocytes with their ligands in the extracellular matrix and on parenchymal cells.

Preclinical data revealed no special hazard for humans based on conventional studies of safety pharmacology, repeated dose toxicity and genotoxicity. There are no studies on the carcinogenic potential of natalizumab. However, as regards reproductive potential adverse effects, pre-clinical reproductive studies of natalizumab suggest the possibility of an increase in abortion rate. The effect is consistent with the known role of α4-integrins in fertilization and implantation. An appropriate warning has been added to the SPC, discouraging the use of Tysabri during pregnancy. Natalizumab is immunogenic in almost all animals within 2-4 weeks depending on the administered dose of natalizumab.

Efficacy

Efficacy has convincingly been demonstrated at the pre-specified 2-year clinical endpoints, including the clinically highly relevant impact on disability progression, which is the major goal of treatment of RRMS. Treatment with 300 mg natalizumab every 4 weeks resulted in a highly statistically significant 42% decrease in the risk of disability progression, as measured by sustained changes on EDSS, when compared to placebo over a 2-year period, and a 68% decrease in the annualized relapse rate versus placebo over both 1 and 2 years. Through year 2, there was a highly statistically significant 83% reduction in the number of new or newly enlarging T2-hyperintense lesions in natalizumab-treated subjects compared to placebo. 96% of subjects in the natalizumab group were free of Gd-enhancing lesions after 1 year of treatment, and 97% after 2 years.

Safety

Reduced lymphocyte surveillance as induced by alpha4-integrin antagonism by natalizumab might have been causative to the occurrence of 2 cases of progressive multifocal leukencephalopathy in patients with MS, and a further case in a patient with Crohn's disease. Two of the cases (one in the MS trials and the CD patient) were fatal. The current safety database does not yet allow for a clear estimation of the risk of serious and/or fatal adverse events, like PML or other serious infections. Since MS is a disease of a typically young patient population with usually no reduced lifespan, and since there are therapeutic alternatives with an established safety profile, Tysabri should be clearly restricted to patients that are really in need of such a therapy. Expert view, also from Patient Representatives, agreed to this principle.

Having considered the safety concerns in the risk management plan, the CHMP agreed that the proposed activities described in section 2.5 adequately address these.

Risk-benefit assessment

In clinical practice, there is a patient population that has a clear unmet need for an active treatment due to sustained severe disease activity, defined by a high load of active lesions and frequent relapses. These patients are very likely to progress to sustained disability with all related complications and therefore eventually a reduced life expectancy. If these patients show inadequate response to (or contraindications for) beta-interferons or glatiramer acetate, the gap to the next step of escalation therapy is rather large: Ignoring intravenous immunoglobulins, which are not licensed in this indication, patients can only be offered mitoxanthrone, immunosuppressive chemotherapy or stem cell transplantation. These therapies have a known high risk for severe and potentially fatal adverse events (cardiotoxicity, leukaemia and other malignancies, serious systemic infections etc.). Leaving the patients untreated would render patients progressing to wheelchair rapidly. For these patients, Tysabri is considered to be a viable treatment option.

Indications

Two different patient populations for whom a particularly high unmet medical need exists:

(*1) Patients who have failed to respond to a full and adequate course of a beta-interferon. Patients should have had at least 1 relapse in the previous year while on therapy, and have at least 9 T2-hyperintense lesions in cranial MRI or at least 1 Gadolinium-enhancing lesion.*

For this patient population relevant data could be derived from study C-1802. Patients had to be on Avonex treatment for at least one year (which can be considered a "full and adequate course") and to show active disease despite this active treatment with a beta-interferon. Unfortunately, there is no data on the efficacy of natalizumab monotherapy in these patients due to the design of the trial (add-on). However, the overall efficacy data suggest that efficacy in C-1802 is mainly driven by natalizumab and not by Avonex, since Avonex by definition was not sufficiently active. Therefore, the efficacy database is considered sufficient to support efficacy in patients being treated in case of failure of beta-interferon. The other potential alternatives in the indication wording (e.g. failure of glatiramer acetate) for the SPC are not represented in this C-1802 population, however, are relevant from a clinical perspective, and it can be assumed that natalizumab will be efficacious.

It is considered that a requirement for the use of (nearly) all of the available treatments for the treatment of RRMS before Tysabri can be given would be problematic, since all available therapies only show modest activity, and a considerable time span for adequate treatment would be lost. During that time, patients as defined above might considerably progress, which is currently an irreversible process.

(2) Patients with rapidly evolving severe relapsing remitting multiple sclerosis, defined by 2 or more disabling relapses in one year, and with 1 or more Gadolinium enhancing lesions on brain MRI or a significant increase in T2 lesion load as compared to a previous recent MRI.

In addition to the data from the ITT population, a post-hoc analysis of data from a sub-group of patients with rapidly evolving MS and naïve to treatment were submitted by the sponsor.

Table: AFFIRM Major Efficacy Endpoints (Patients with ≥ 2 relapses and ≥ 1 Gd-enhancing lesion)
(All 2-year results were evaluated at 24 months, except disability, which was assessed at 25 months.)

	TYSABRI (n=148)	Placebo (n=61)	p-value	
Disability endpoint				Reduction in risk
Sustained progression of disability (increase in EDSS sustained for 12 weeks)	Hazard ratio 0.47 (95% CI 0.24, 0.93)		p= 0.029	53%
Sustained progression of disability (increase in EDSS sustained for 24 weeks)	Hazard ratio 0.36 (95% CI 0.17, 0.76)		p= 0.008	64%
Relapse endpoints				Percent reduction compared with placebo
Annualised relapse rate	0.282	1.455	p< 0.001	81%
Percentage of patients relapse-free	68%	23%	p< 0.001	-
Annualised rate of relapses requiring steroids	0.154	0.765	p< 0.001	80%
Percentage of patients requiring steroid treatment for relapse	20%	59%		66%
Annualised rate of MS-related hospitalisations	0.015	0.137	p< 0.001	89%
MRI end points				
Median % change in T2-hyperintense lesion volume	-16.6%	+10.8%	p< 0.001	-
Mean number of new or newly-enlarging T2-hyperintense lesions	4.2	19.1	p< 0.001	78%
Mean number of T1-hypointense lesions	2.2	7.0	P< 0.001	69%
Mean number of Gd-enhancing lesions	0.5	3.2	p< 0.001	84%

In a sub-group of patients with 2 or more relapses and 1 or more Gd^+ lesion natalizumab resulted in a 81% decrease in the annualized relapse rate (ARR) versus placebo over 2 years, and a 64% decrease in the risk of disability progression. ARR was 0.282 in the natalizumab treated group (n= 148) and 1.455 in the placebo group (n= 61) (p <0.001). Hazard ratio for disability progression was 0.36 (95% CI : 0.17, 0.76) p=0.008. These data confirmed the treatment effect of natalizumab in this group of patients and the effect size supports the additional indication for treatment of patients with rapidly evolving relapsing remitting MS.

This subgroup analysis should be treated with caution since no documentation on the severity of relapses, neither by their clinical course (leaving a neurological deficit) nor by their duration, was collected through the entry criteria of the pivotal trials. However, given the demonstrated efficacy of natalizumab, one can assume that natalizumab will be efficacious. From a clinical perspective, Tysabri might indeed be used in a post marketing setting in these patients as an alternative for example to high-dose beta-interferon in order not to lose time and prevent accumulation of disability.
A risk management plan was submitted. The CHMP, having considered the data submitted, was of the opinion that additional risk minimisation activities were required:

Active safety measures for risk minimization

A number of significant measures were agreed for implementation to minimize the risks associated with Tysabri treatment as regards opportunistic infections including PML, which are the major known safety issues. These measures relate to the following principles:

- clear-cut definition of the target population, i.e. restricted use only for patients with highly active disease without reasonable alternatives
- Requirement for established MS
- Escape rule for non-responders to avoid unnecessary exposure
- Administration only in specialized centers by experienced physicians
- Clear contraindications including a contraindication for combination with other immunomodulators
- Patient alert card
- Educational program for physicians

One of the mainstays of this programme is the information provided in the SPC, patient alert card and patient information leaflet. The SPC and patient information leaflet should adequately inform physicians and patients on the risks of Tysabri treatment and of the measures to be undertaken in case treatment with the drug is jointly decided (by physician and patient). Close monitoring of patients treated with Tysabri would be of major importance in case of a marketing authorization, since the first signs and symptoms of PML are often similar to an MS relapse. An algorithm has been developed, focussing on suggestive clinical signs and symptoms as primary detector, followed by confirmation by MRI and JC virus detection by PCR in cerebrospinal fluid (CSF).

Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered by consensus that the risk-benefit balance of Tysabri indicated as single disease modifying therapy in highly active relapsing remitting multiple sclerosis for the following patient groups:

- Patients with high disease activity despite treatment with a beta-interferon (SPC: see 5.1);

or

- Patients with rapidly evolving severe relapsing remitting multiple sclerosis (SPC: see 5.1).

was favourable and therefore recommended the granting of the marketing authorisation.