European Medicines Agency
Evaluation of Medicines for Human Use

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REFUSAL CHMP ASSESSMENT REPORT FOR NATALIZUMAB ELAN PHARMA

International Nonproprietary Name: natalizumab

Procedure No. EMEA/H/C/000624

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

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PRODUCT INFORMATION

Name of the medicinal product:	Natalizumab Elan Pharma
Applicant:	Elan Pharma International Ltd. Six Hills Court Norton Green Road Stevenage, Herts SG1 2BA United Kingdom
Active substance:	natalizumab
International Nonproprietary Name/Common Name:	natalizumab
Pharmaco-therapeutic group (ATC Code):	Selective Immunosuppressive agents (L04AA23)
Therapeutic indication:	Treatment of moderately to severely active Crohn's disease for the reduction of signs and symptoms, and the induction and maintenance of sustained response and remission, in patients who have not responded despite a full and adequate course of therapy with a corticosteroid and an immunosuppressant; or are intolerant to or have medical contraindications to such therapies.
Pharmaceutical form:	Concentrate for solution for infusion
Strength:	300 mg (20 mg/ml)
Route of administration:	Intravenous use
Packaging:	Vial (glass)
Package size:	1 vial

TABLE OF CONTENTS

1. BACKGROUND INFORMATION ON THE PROCEDURE

1.1 Submission of the dossier

The applicant Elan Pharma International Ltd. submitted on 28 September 2004 an application for Marketing Authorisation to the European Medicines Agency (EMEA) for Natalizumab Elan Pharma, through the centralised procedure falling within the Article 3(1) and point 1 of Annex of Regulation (EC) No 726/2004 (Part A according to Council Regulation (EEC) No 2309/93).

The legal basis for this application refers to:
Article 8.3 of Directive 2001/83/EC, as amended - complete and independent application

The applicant applied for the following indication treatment of Crohn's disease.

Scientific Advice:
The applicant received Scientific Advice from the CHMP on 17 October 2002. The Scientific Advice pertained to quality and clinical aspects of the dossier.

The Rapporteur and Co-Rapporteur appointed by the CHMP and the evaluation teams were:
Rapporteur: Manfred Haase
Co-Rapporteur: Pasqualino Rossi

1.2 Steps taken for the assessment of the product

- The application was received by the EMEA on 28 September 2004.
- The procedure started on 18 October 2004.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 30 December 2004 (Annex 1). The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 27 December 2004 (Annex 2).
- During the meeting on 14-17 February 2005, the CHMP agreed on the consolidated List of Questions to be sent to the applicant. The final consolidated List of Questions was sent to the applicant on 17 February 2005 (Annex 3).
- The CHMP agreed in a letter submitted to the company on 5th of January 2006 on an extension of the clock-stop.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 5 September 2006.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 30 October 2006 (Annex 4).
- During the CHMP meeting on 13-16 November 2006, the CHMP agreed on a list of outstanding issues to be addressed in writing and in an oral explanation by the applicant (Annex 5).
- The applicant submitted the responses to the CHMP consolidated List of Outstanding Issues on 23 February 2007.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Outstanding Issues to all CHMP members on 3 April 2007 (Annex 6).
- During the CHMP meeting on 23-26 April 2007, the CHMP agreed on a 2nd List of Outstanding Issues to be addressed in an oral explanation by the applicant (Annex 7)
- During the CHMP meeting 18-21 June 2007, outstanding issues were addressed by the applicant during an oral explanation before the CHMP.
- During the meeting on 16-19 July 2007, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a negative opinion for granting a Marketing Authorisation to Natalizumab Elan Pharma on 19 July 2007.

1.3 Steps taken for the re-examination procedure

The Rapporteur and Co-Rapporteur appointed by the CHMP and the evaluation teams for the re-examination were:
Rapporteur: Dr. S.Thirstrup
Co-Rapporteur: Dr. G. Calvo Rojas

- The applicant submitted written notice to the EMEA on 07 August 2007 to request a re-examination of the Natalizumab Elan Pharma CHMP opinion of 19 July 2007.
- During its meeting on 17-20 September 2007, the CHMP appointed Dr. S.Thirstrup as Rapporteur and Dr G. Calvo Rojas as Co-Rapporteur.
- The applicant submitted written notice to the EMEA on 07 August 2007 to request a re-examination of the Natalizumab Elan Pharma CHMP opinion of 19 July 2007.
- During its meeting on 17-20 September 2007, the CHMP appointed Dr. S. Thirstrup as Rapporteur and Dr. G. Calvo Rojas as Co-Rapporteur.
- The detailed grounds for the re-examination request were submitted by the applicant on 21 September 2007 (Appendix 2 of Final Opinion). The re-examination procedure started on 22 September 2007.
- The Rapporteur's Assessment Report was circulated on 22 October 2007 (Annex 4.8). The Co-Rapporteur's Assessment Report was circulated on 22 October 2007 (Annex 4.9).
- The CHMP adopted a List of Question to the Experts (4.10), List of Participants to the Ad-hoc Expert meeting to be held on 5 November 2007 and the Agenda through written procedure 31 October 2007.
- During a meeting of the CHMP Ad-hoc Expert meeting on 05 November 2007, experts were convened to consider the grounds for re-examination. During this meeting the applicant presented an oral explanation.
- The Rapporteurs' Joint Assessment Report was circulated to all CHMP members on 9 November 2007 (Annex 4.10)
- During the meeting on 12-15 November 2007, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a final Opinion recommending the refusal of granting a Marketing Authorisation for Natalizumab Elan Pharma.
- The CHMP opinions were forwarded, in all official languages of the European Union, to the European Commission, which adopted the corresponding Decisions 11 January 2008.

2 SCIENTIFIC DISCUSSION

2.1 Introduction

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 Crohn's disease. The rationale for use in Crohn's disease is based on the fact that natalizumab blocks the interaction of α4β7-integrin with MadCAM-1, which is expressed prominently on endothelial cells in the gut, and thus inhibits T cell transition into the gastrointestinal tract. Natalizumab also suppresses ongoing inflammatory reactions by inhibiting the interaction of α4-integrin-expressing leucocytes with ligands like osteopontin and epitopes of fibronectin that play a key role in supporting immune cell activation and survival. Thus the inflammatory process of Crohn's disease may be disrupted and/or even prevented.

The *originally claimed indication* was "for the treatment of moderately to severely active Crohn's disease for the reduction of signs and symptoms, and the induction and maintenance of sustained response and remission, in patients who have not responded despite a full and adequate course of therapy with a corticosteroid and an immunosuppressant; or are intolerant to or have medical contraindications to such therapies."

The recommended dose of natalizumab is 300 mg IV once a month.

After review of the dossier, the applicant proposed a revised indication as follows:

"Natalizumab Elan Pharma is indicated for the treatment of severe, active Crohn's disease, in patients who have:

- had an inadequate response to conventional therapy including a TNF-α inhibitor, or who are intolerant to or have medical contraindications to such therapies.

- evidence of active inflammation defined by raised serum levels of C - reactive protein.

Natalizumab Elan Pharma can be used alone or in combination with aminosalicylicate products or antibiotics. Brief courses of corticosteroids can be used with natalizumab to treat acute exacerbations of Crohn's disease."

2.2 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 kilo Daltons. 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 approximately 1 hour.

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).

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.

Characterization
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 characterization 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 characterization 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. Ongoing stability monitoring for these batches is 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 by affinity chromatography 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 characterization 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.

The drug substance and drug product are identical to those of the Centrally Authorised product Tysabri and the quality documentation that have been reviewed were identical. 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.

2.3 Non-clinical aspects

Introduction

The non-clinical evaluation programme addresses in various degrees issues around the indications multiple sclerosis (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 pharmacology section gave sufficient information in the guinea pig EAE model on the efficacy of natalizumab. 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.

- 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 natalizumab and of antibodies to natalizumab was measured using ELISA assays. The limit of quantitation (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 company itself states 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 quantitation (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 97hr 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 for natalizumab are as stated in the Tysabri Multiple Sclerosis EPAR and SPC.

Higher dose levels were demonstrated to provide adequate exposure in sufficient animals for evaluation of toxicity with the exception of the early embryo foetal reproductive study in guinea pig.

- 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. The evaluation of the blood and/or tissues of animals on these studies specifically for changes in SV40 has not yet been started. The Applicant commits to submit the data from this study as soon as it becomes available.

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 immunogenicity

of humanized monoclonal antibodies in cynomolgus monkeys can be highly variable; an antibody incidence of up to 100% in this species is not unusual for these types of products.

The mechanism of the sensitation of the male guineas pig remains unclear. The most obvious explanation (misdosing) is excluded by the applicant since the administration of natalizumab in a high number of animals in this non-GLP study should have been noticed by the personnel and the test article consumption protocols. Therefore the mechanism leading to the deaths of the animal remains open.

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.

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.

Prenatal and postnatal development, including maternal function

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.

- Local tolerance

Histopathological evaluation of injection sites, was performed in the repeated dose studies in accordance with ICH S6 guidelines. This approach is considered as acceptable.

- 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.

Effects on the spleen and thymus sizes as well as elevated reticulocyte counts and dose related, significantly increased counts of WBC even at the end of recovery periods were described in most of the studies in the toxicology section.

Since the induction and the effects of anti-natalizumab antibodies are described in many of the non-clinical studies especially in monkeys, further non-clinical studies are not considered to add additional information. Further evaluations of anti-natalizumab antibody induction should be performed in clinical trials after the issuing of the marketing authorisation. The applicant provided an acceptable explanation: The immunogenicity of humanized monoclonal antibodies in cynomolgus monkeys can be highly variable; an antibody incidence of up to 100% in this species is not unusual for these types of products. The immunogenicity of natalizumab in cynomolgus monkeys is not dissimilar with what has been observed with some other humanized monoclonal antibodies in this species.

Study P00002-04-02 was initialized to determine if six weekly intravenous infusions of natalizumab affect humoral response to T cell-dependent antigens in cynomolgus monkeys, and to evaluate recovery from natalizumab effects during a treatment-free period of at least 8 weeks.
Treatment of dams with natalizumab resulted in increases in WBC, lymphocyte and NRBC counts in dams and in infants born to dams treated up to delivery. These increases are an expected pharmacologic effect of natalizumab treatment and were associated with the presence of serum natalizumab. Clearance of natalizumab from circulation resulted in a return to normal control levels for these parameters in both dams and infants.

The effects of natalizumab treatment on the ability to generate humoral responses to T cell dependent antigens have been evaluated in an immunotoxicity study in cynomolgus monkeys by challenging with keyhole limpet hemocyanin (KLH). There appeared to be a natalizumab-related effect on the early immune response (increase) and late (decrease) response to KLH, although the high variability makes an evaluation of the data extremely difficult. Additional experiments are proposed to address the issue of the immune response.

The objective of one major study in cynomolgus monkeys was to provide an evaluation of the potential for effects on embryo/fetal development in cynomolgus monkeys treated with natalizumab during organogenesis (GD20-GD70).

All fetuses removed by caesarean section on GD100 were alive. There were no treatment- related external abnormalities. No differences were found in fetal weight, placental morphology or weight, amniotic fluid volume, or fetal external measurements. No visceral abnormalities or variations were seen. Significantly low or trends towards low liver and thymus weights (absolute and relative) and significantly high spleen weights (absolute and relative) were noted in the 10 and 30 mg/kg groups. No skeletal abnormalities were seen that were considered to be test article-related.

WBC counts were significantly increased in GD100 foetuses from dams in the 10 and 30 mg/kg treatment groups. WBC counts were 6.8-7.5-fold that of control fetuses. Fetal serum natalizumab concentrations levels ranged from 8-79% of the dam's serum levels.

No abortifacient effects, fetotoxicity, or teratogenicity was observed in the study following treatment with natalizumab through the period of organogenesis. Other findings, seen at dose levels of > 10 mg/kg, were trends toward low liver and thymus weights with decreased extramedullary haematopoiesis, increased spleen weights with increased extramedullary hematopoiesis, decreases in $CD3^+$ and $CD20^+$ cells in some lymphoid organs, mild anemia, increases in circulating NRBC, and increases in background proliferation in response to PHA stimulation.

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 aspects

Data on the effects of α4-integrin blockade on immune function in normal animals (primarily in cynomolgus monkey) and isolated human PBMC 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 PML cases, the applicant provided a list of proposed studies to address this issue.

2.4 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, 2 PK studies with Crohn's disease patients were conducted. These data provided evidence that the pharmacokinetics of natalizumab is non-linear. In this condition, weight-based dosing would be inappropriate since it could expose overweight subjects to an excessive dose of natalizumab. The alternatives to body weight criterion are ideal body weight, body surface area, and a fixed dose. The

applicant chose the simplest of them, i.e. a fixed dose (300 mg). The pharmacokinetic and pharmacodynamic data obtained with this fixed dose are sufficiently valid. 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. According to this analysis, age, serum creatinine, ALT, AST, bilirubin and creatinine clearance had no relevant influence on the PK of natalizumab. However, body weight as well as the presence of persistent anti-natalizumab antibodies were shown to influence the clearance of natalizumab. There was no significant impact of IFNβ-1a on natalizumab clearance.

The findings of the population PK in CD patients are similar as those for MS patients. In both patient populations an (unexplained) time dependency of CL was found (in the recent PPK analysis this was accounted for by proper modeling). However, an explanation for this finding including its clinical relevance is not given. Therefore, further investigations seem necessary.

- 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 PD 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 grant activity. However, one study showed 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 is too high in certain subjects. A possible suggestion would be to use body weight dosing in subjects with ideal body weight ≤60 kg.
The 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 the overall observed incidence of HAHAs after one single dose of natalizumab was 21% with a wide inter-trial-range and slightly higher than in healthy volunteers who had received a single dose of the commercial material (12-16%). The response was visible within 5 weeks. After repeat infusions the HAHA incidence was reduced to 12%, and in the Phase 3 studies to 10%. In the phase III studies antibodies were persistently detectable in 6% of patients. The HAHA test was positive at median week 12, a time point correlated with reduced serum levels of natalizumab. However, serum concentrations increased again and recovered as antibody levels diminished in these patients. Despite the transient reduction of the natalizumab concentrations, α4-integrin saturation was maintained stable throughout the dosing interval in transiently antibody positive patients. A respective wording has been proposed for inclusion in the SPC, giving guidance to the prescriber when to measure anti-natalizumab antibodies and how to proceed in case of persistent positivity.

Based on CHMP request in the day 120 LoQ, the Applicant performed a rather extensive immunological programme in humans to measure potential impacts on immune function, although only in single-dose studies. Concerning cellular immunity, no particular change in T cell subsets was measured, which might also be expected. Interestingly, the mean absolute B cell count increased almost four-fold. Since B cells are discussed to be potential vectors for JC virus, which is the causative agent of PML, this is an interesting finding. Besides this finding, no other particular finding was observed that would further elucidate the immunocompromising effect of natalizumab besides the inhibition of leukocyte trafficking.

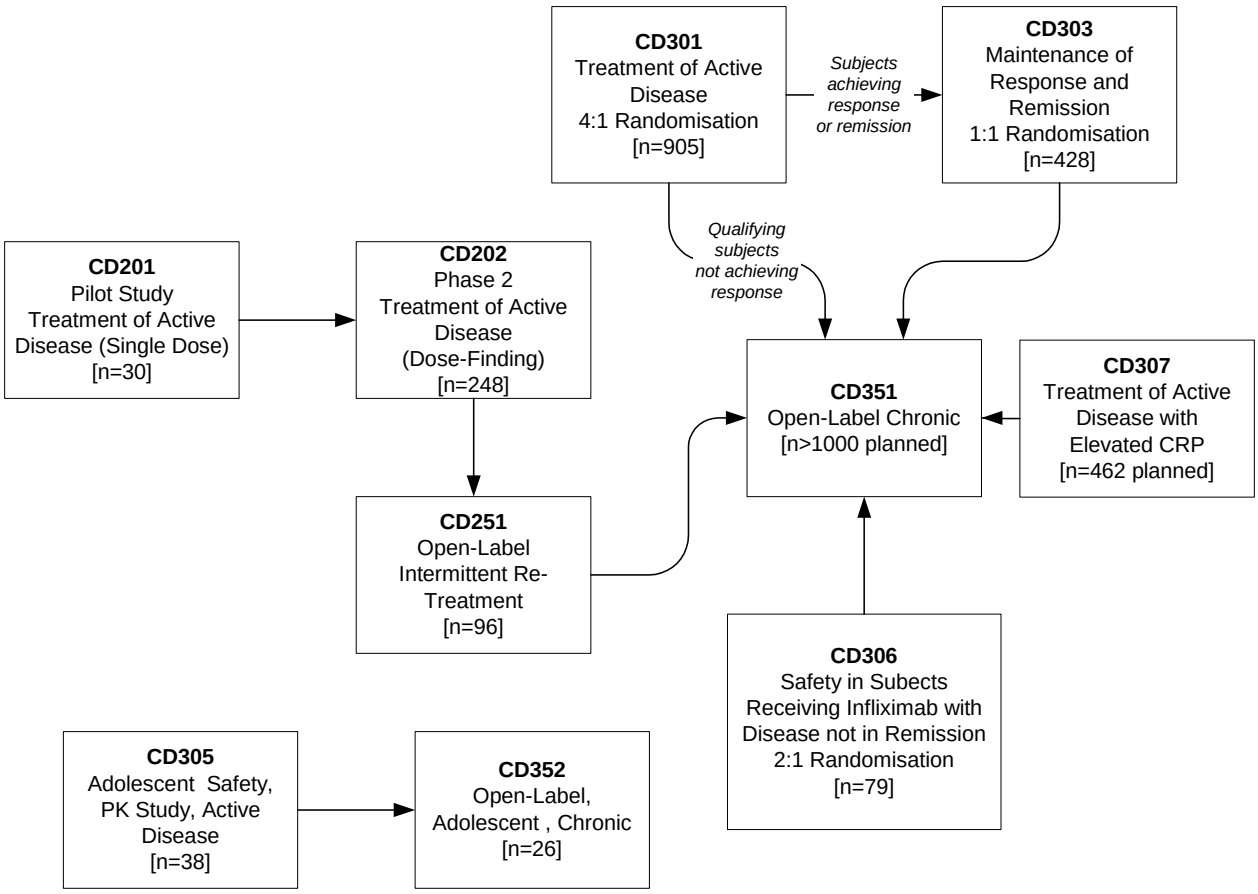
Clinical efficacy

The efficacy of natalizumab in active Crohn's Disease (CD) had initially been examined in a total of 1295 patients enrolled in 9 clinical trials and dosed up to 15 months. The clinical study programme for natalizumab in CD was designed to demonstrate that natalizumab is efficacious (1) in the induction of clinical response and remission and (2) in the maintenance of this response or remission in patients with moderately to severely active CD in patients who have not responded despite a full and adequate course of therapy with a corticosteroid and an immunosuppressant, or are intolerant to or have medical contraindications to such therapies. The clinical development programme included 2 pivotal studies **CD301** (as induction study) and **CD303** (as maintenance study). Both studies were conducted in tandem, as only patients with a response or remission in CD301 were allowed to participate in CD303.

The efficacy results are supported by the data from Study **CD202**. The remaining six studies are also supportive and assessed the safety of natalizumab (**CD201**), safety with chronic retreatment in adults (**CD351**), safety with intermittent natalizumab retreatment (**CD251**), safety of natalizumab in combination with infliximab (**CD306**), and safety in adolescent patients (**CD305** and **CD352** [retreatment]).

Three of these studies were long-term, open-label safety studies: Studies CD251, CD351 and CD352.

Figure 2: Clinical Development of Natalizumab in Crohn's Disease



The Applicant meanwhile performed an additional study, **CD307,** in subjects with moderately to severely active CD and elevated CRP, to confirm the findings of trial CD 301.

- Dose finding studies

The initial dose of natalizumab for CD therapy was selected according to pre-clinical data in the guinea pig EAE (experimental autoimmune encephalomyelitis) model, where a dose of 3mg/kg was defined as minimum effective dose. Based on further in vitro binding studies, a single dose of 3mg/kg was estimated to provide α4-integrin receptor blockade for 3 to 4 weeks, whereas 6mg/kg were estimated to block α4-integrin receptors for approximately 6 weeks. This was confirmed by phase I single dose PK studies in healthy volunteers, and in patients with CD and MS which also showed that a 3mg /kg infusion of natalizumab maintains serum concentrations of at least 2.5-3.0 µg/ml for around 4 weeks.

Pharmacokinetic, pharmacodynamic and efficacy data from two dose-ranging Phase 2 studies, one Crohn's disease (CD) (CD202) and one MS study (MS 231) confirmed these PK/PD data and provided the basis for the Applicant's justification for fixed dosing in Phase 3.

Trial **CD202** was a Phase II, multicentre, double-blind, placebo-controlled, parallel-group, dose finding efficacy study comparing dosages related to body weight. At week 0, a total of 248 subjects with moderately to severely active CD (CDAI score ≥220) were randomised to one of four treatment

groups (1:1:1:1): one infusion of 3 mg/kg natalizumab (n=68), two infusions of 3 mg/kg natalizumab four weeks apart (n=66), or two infusions of 6 mg/kg natalizumab four weeks apart (n=51) or placebo (n=63). Eligible patients had to have a minimum of 6 months history of CD. The evidence of chronic active CD had to be confirmed by radiologic or endoscopic findings. The concomitant use of 5-ASA compounds, oral steroids, antibiotics, azathioprine, and 6-mercaptopurine was permitted providing that doses were stable in the period leading up to enrolment and during the study. The main study phase of 12 weeks was followed by a long-term safety monitoring phase consisting of a follow-up period of 6 months and an additional phone follow-up of 68 weeks. Alternatively, subjects were eligible to enter the open-label, intermittent retreatment study, CD251.

Clinical efficacy was evaluated in the ITT population by assessing remission (CDAI score <150) and response (using a definition of either ≥70 or ≥100-point reduction in CDAI score) as well as quality of life (IBDQ) at baseline and Weeks 2, 4, 6, 8, 12, and 16.

The primary endpoint was the proportion of subjects in the 6+6mg/kg natalizumab treatment group in clinical remission (CDAI score <150) at week 6 compared to placebo. Other treatment groups were compared to placebo as secondary endpoints.

The study did not meet its primary endpoint, because difference in the proportion of patients in clinical remission at week 6 was not significant (p=0.533) between the 6+6m/kg natalizumab group and the placebo group. However, the secondary comparison showed that in the 3+3mg/kg group a higher remission rate (p=0.030) was reached than in the placebo group; and this rate was also achieved at week 12 and 16. Although time to response was only shorter in the 3+3mg/kg group compared to placebo (p=<001), natalizumab induced response rates in all natalizumab groups at week 6. Only for the dosage groups receiving two natalizumab infusions (3+3 and 6+6 mg/kg) significant response rates were maintained at week 12 and 16.

The elevated baseline CRP in patients treated with natalizumab decreased at week 6 for both groups with two natalizumab infusions. In these groups quality of life (reduction in IBDQ) improved significantly compared to placebo at week 6 and could also be maintained at week 12.

In general, the Applicant based the fixed dose regimen of 300mg per infusion on three arguments: (1) 3mg/kg was efficacious in both CD and MS trials, (2) 6mg/kg resulted in no dose-limiting toxicity, and (3) there was no superior benefit of 6mg/kg as compared to 3mg/kg. The calculation of 300mg was based on the 99th percentile in weight from the aforementioned phase II trials (100kg), with a resulting fixed dose of 3mg/kg x 100kg.

- Main studies

Two studies were conducted as pivotal trials for the approved indication. They have the following titles:

- *A Phase III, international, multicenter, double-blind, placebo-controlled study of the safety, efficacy and tolerability of intravenous Antegren (natalizumab) in subjects with moderately to severely active Crohn's Disease (**CD301**)*
- *A Phase III, international, multicenter, double-blind, placebo-controlled study of the safety, efficacy and tolerability of intravenous Antegren (natalizumab)(300mg monthly) in maintaining clinical response and remission in subjects with Crohn's Disease (**CD303**)*

These studies were conducted in tandem in order to demonstrate that a fixed, monthly dose of 300mg natalizumab is efficacious to induce and to maintain clinical response and remission in patients with active CD. Therefore study CD303 was designed as an add-on study to CD301.

METHODS

Study Participants

Patients in CD301 and CD303 were enrolled at 142 and 123 centres in North America, Europe and selected countries from the rest of the world.

To enter Study CD301 eligible patients had to have a diagnosis of active of moderately to severely active CD (based on clinical evaluation and CDAI score ≥220 and ≤450). Eligible to enter the maintenance Study CD303 were only patients who responded to treatment (natalizumab or placebo) in CD301 and had a mildly active CD or disease in remission at both week 10 and week 12 of Study CD301.

Key inclusion and exclusion criteria were:

Inclusion criteria **(Table 3)**

	CD301	CD303
Age ≥18 years	yes	yes
≥ 6 month history of CD	yes	yes[1]
CD history confirmed by radiologic or endosopic findings	yes	yes[1]
CDAI score of ≥ 220 and ≤ 450	YES	YES
Response/remission to Study CD301[2] at both weeks 10 and 12	N/A	yes

[1] Subjects met this criteria at enrolment in Study CD301 (i.e., this was not an entry criterion for CD303)

[2] Response defined as ≥ 70-point reduction in CDAI score, CDAI score < 220, remission defined as CDAI<150 and no use of rescue intervention

Main exclusion criteria **(Table 4)**

CD301	CD303
Women of childbearing potential had to practise adequate contraception	Women of childbearing potential had to practise adequate contraception
Concomitant use of 5-ASA, antibiotics, oral steroids or immunosuppressants had to be stable for a period of time prior and throughout the study	Change of the concomitant CD medication during Study CD301 with the exception of immunosuppressants (due to toxicity)
Concomitant use of anti-TNF	Concomitant use of anti-TNF
Short bowel syndrome, active or draining fistulae or neoplastic disease	
	Criteria for discontinuation of study drug during CD301

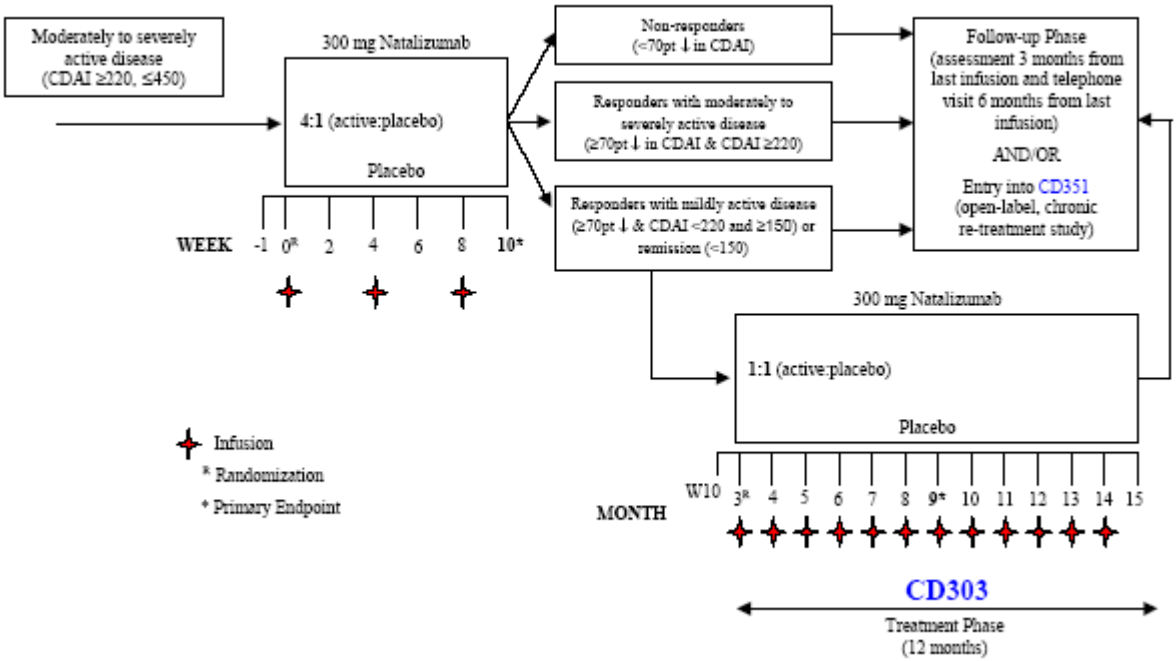
Patients receiving oral steroids in CD301 were to have been limited to 25 mg prednisolone or equivalent at enrolment. Those patients intending to participate in CD303 were required to taper the use of oral steroids with the beginning of week 10 using a protocol-defined algorithm.

Treatments

Natalizumab was to be administered as a fixed dose of 300mg as an intravenous infusion every four weeks.

Before the first infusion in CD301, patients were randomly assigned in a 4:1 ratio to natalizumab 300mg fixed dose (3 IV infusions every 4 weeks) or placebo. The randomisation was stratified based on disease severity (CDAI score <330 or >330) and the concomitant use of oral steroids. Infusions were administered on week 0, 4 and 8. The efficacy was evaluated at week 10 and 12. Those patients who met the criteria of a response/remission and had a CDAI score <220 were re-randomised to receive monthly intravenous (IV) infusions of natalizumab 300 mg (n=214) or placebo (n=214) for up to 12 consecutive months in Study CD303 (Figure 2). Randomisation was central and stratified according to three factors: disease status at Week 12 (remission versus no remission [i.e., a CDAI score <150 or ≥150]), use of oral steroids at entry in Study CD301, and use of immunosuppressants at entry in Study CD301. In case of a concomitant use of oral steroids patients began a steroid taper according to a fixed algorithm starting at Week 10.

Figure 3: Study Flowchart for Study CD301 and CD303



Outcomes/endpoints

Table 5 : Endpoints

	Induction Study CD301	Maintenance Study CD303
Primary endpoint	• Proportion (%) with a clinical response (defined as >70%-point decrease from baseline in CDAI) at week 10 • Proportion (%) achieving clinical remission (defined as CDAI score <150) at week 10	• Proportion (%) maintaining a clinical response through month 9 • Proportion (%) maintaining a clinical remission through month 9
Secondary endpoints	• Proportion (%)achieving clinical remission (defined as CDAI score <150) at week 4 • Proportion (%)achieving clinical response (defined as >70-point decrease from baseline in CDAI) at week 2 • Proportion (%)achieving a >100-point decrease from baseline CDAI score at week 10 • Mean change from IBDQ from baseline at week 10	• Proportion (%) maintaining a clinical response through month 15 • Proportion (%) maintaining a clinical remission through month 15 • Time to loss of clinical response • Time to loss of clinical remission • Mean change of IBDQ score from CD301 baseline to month 9 of CD303 • Number (%) not taking oral steroids at month 9 of study CD303 • Number (%) in remission and not taking oral steroids at month 9 of study CD303
Tertiary endpoints	• Clinical response or remission at other time points and at any time (i.e. through week 12) • Quality of life • Need for rescue intervention • Effects on inflammatory markers	• Clinical response or remission at other time points and at any time • Quality of life • Need for rescue intervention • Effects on inflammatory markers • Effects on withdrawal of oral steroids

Sample size

Sample size calculations for study CD301 were based on estimates of response rates (defined as ≥70-point decrease from baseline [Week 0] CDAI score at Week 10). Based on data from study CD202 a 40% response rate was expected in the placebo group and a 55% response rate in the natalizumab treatment arm. Subjects were randomized in a 4:1 ratio of active to placebo. The sample size calculation was performed based on a continuity-corrected chi-square test. Anticipating a dropout rate of 10% 845 subjects (676 natalizumab, 169 placebo) were to be randomised in order to achieve 90% power in a two-sided test with a Type I error of 5%.
It was expected that 285 subjects who responded in study CD301 would be randomized into study CD303. Anticipating a 65% response rate for natalizumab and a 44% response rate for placebo and allowing for a 10% dropout rate, a sample size of 285 subjects (142 per treatment group, 1:1 ratio) provided a power of 90% at the 5% significance level (two-sided Fisher's exact test) to detect a difference between the natalizumab-treated group and the placebo group in maintenance of response rates, where loss of response is defined as either 1) a CDAI score ≥220 and a ≥ 70-point increase from the baseline (Week 12) visit, or 2) use of rescue intervention.

Randomisation

In both studies patients were centrally randomised using an interactive voice recording system.

In study CD301 subjects were randomized within strata defined by disease severity (CDAI score <330 or ≥330) and whether or not the subject was taking steroids. The randomization within each of the four strata was conducted using a permuted fixed blocks of length 5. For each complete block there was a 4:1 ratio of natalizumab-treated subjects versus placebo-treated subjects.

Randomisation in study CD303 was stratified by disease severity (CDAI score <150 or ≥150), concomitant use of oral steroids at baseline of study CD301 and concomitant use of immunosuppresants (AZA, 6MP, or MTX) at baseline of study CD301. The randomization within each stratum was conducted using a permuted fixed blocks of length 4. For each complete block there was a 1:1 ratio of natalizumab-treated subjects versus placebo-treated subjects.

Blinding (masking)

Both, the initial study CD301 as well as the maintenance study CD303 was performed as double-blind, randomized, placebo-controlled studies using vials of study drug containing natalizumab or placebo identical in appearance.
As CRP levels might be influenced by natalizumab and these changes have the potential to unblind the investigator, CRP results should not be supplied to investigators to prevent potential unblinding. In the event of a clinically significant CRP result, the investigator was contacted by an independent medical monitor (knowing the unblinded CRP results) to discuss the subject's medical history and current clinical status and how they might relate to the observed result. A similar procedure was applied to WBC and WBC differential counts as they also had the potential to unblind the study.

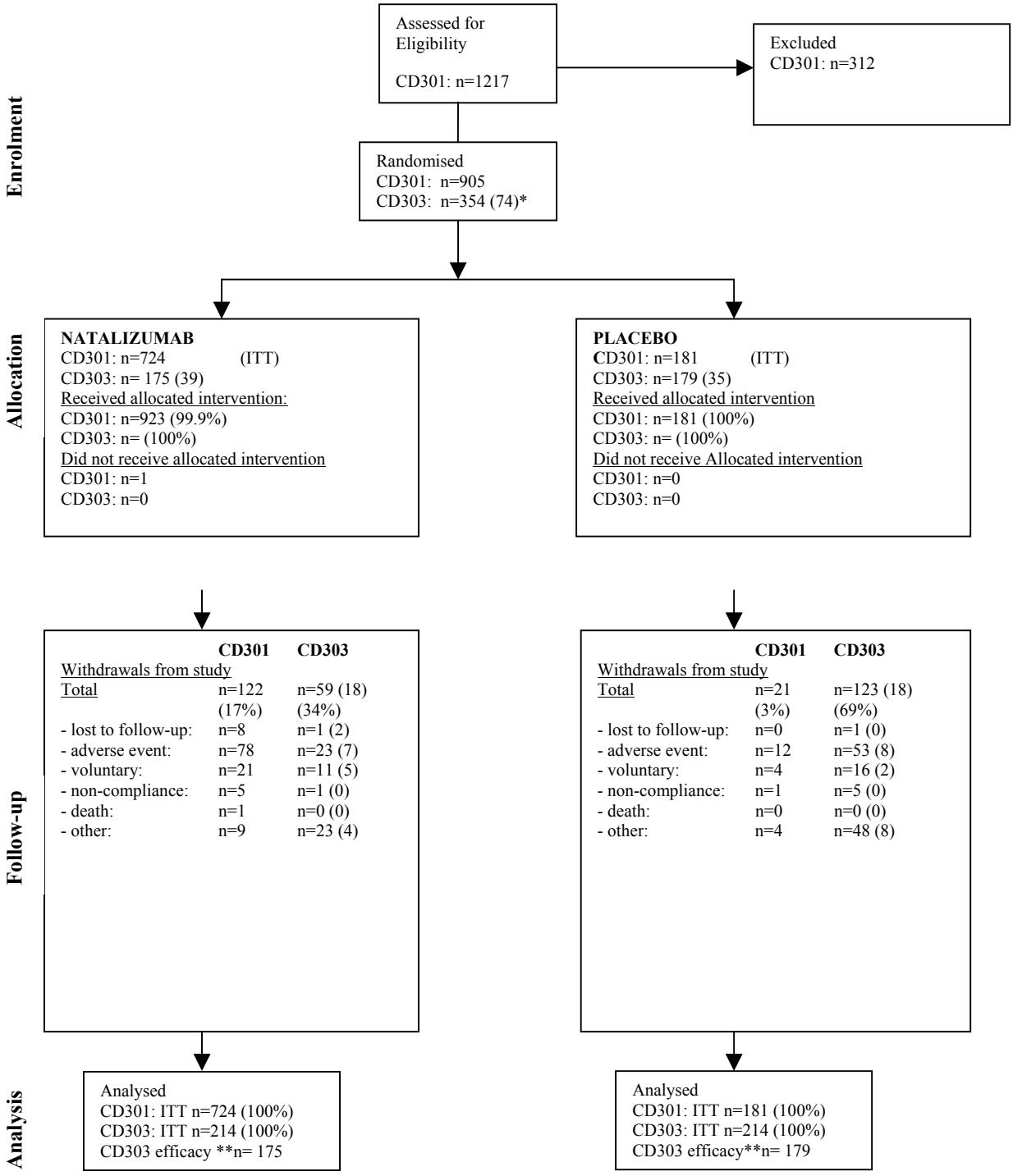
Statistical methods

Similar statistical methods were applied in studies CD301 and CD303. In general, summary data were presented for each treatment group by type of variable. Categorical data were presented by counts and percentages. Continuous data were presented by number of subjects, mean, standard deviation, median, minimum, and maximum. As a rule dichotomous data were analysed using the same model as for the primary and contingent primary endpoint respectively (see below). Quantitative data were analysed by means of ANCOVA models adjusting for the stratification factors used in randomisation. Time-to-event data were graphically assessed using Kaplan-Meier-plots while treatment comparisons were made using the Cox proportional hazards model, adjusting for randomization strata.

All statistical comparisons were conducted by means of two-tailed tests applying an alpha level of 0.05. When appropriate, the difference between the treatment groups and the associated 95% confidence interval were estimated from the pertinent analysis model.

RESULTS

Participant flow



* in brackets: patients in response or remission and receiving placebo in CD301
** efficacy population consisting of those patients who achieved response or remission in CD301

Recruitment

In study CD301 the first subject was enrolled on 4 December 2001; the last visit was completed on 3 September 2003. For study CD303, enrolment started on 23 March 2002, the last subject completed month 15 visit on 25 March 2004.

Baseline data

In Study CD301 and CD303 approximately 60% of the patients were female, 94% were Caucasian with a median age of 36-37 years and around one quarter was smoking more than 10 cigarettes a day. In total 73% in CD301 had an elevated baseline serum CRP (>2.87 mg/l). Overall, in both studies no differences between the two treatment groups were observed in the demographic characteristics as well as in the main disease characteristics, which are summarised in the following tables:

Although patients in the CD301 had to have moderately to severely active CD to be eligible to enter the study, the median CDAI only represents a patient population with moderate disease. Patients with severe active CD (CDAI> 450- 600) are not covered in this study.

Table 6: Baseline characteristics of CD induction (CD301) and maintenance (CD303) study

	CD301		CD303[1]	
	Placebo	**Natalizumab**	**Placebo**	**Natalizumab**
Number of patients	181	724	171	168
(%females/males)	(60% / 40%)	(57% / 43%)	(65% / 35%)	(54% / 46%)
Age (median) (min, max)	37 (18 – 83)	36 (18 – 82)	36 (18 – 74)	35.5 (18 – 74)
Race (white)	94%	94%	95%	92%
Weight (kg) median (min, max)	68.0 (36 – 151)	69.0 (38 – 168)	69.3 (38 – 123)	71.5 (43 – 166)
>10 cigarettes per day N (%)	44 (24%)	164 (23%)	45 (26%)	27 (16%)
Median CD duration (months)	76.5 (0 – 396)	97.7 (0 – 673)		
Ileocolonic site of disease N(%)	84 (46%)	373 (52)		
Baseline CDAI score CD301 (min, max)	287 (165 – 518)	292 (171 – 496)	292 (208 – 468)	283 (185 – 461)
Baseline CDAI at CD303				
CDAI ≥150			51 (30%)	38 (23%)
CDAI <150			120 (70%)	130 (77%)
Baseline CRP (mg/l) CD301 (min, max)	12.2 (0 – 127)	8.7 (0 – 370)	11.3 (0 – 236)	8.3 (0 – 145)
Baseline CRP (mg/l) CD303 (min, max)			3.9 (0 – 120)	4.3 (0 – 97)
Elevated baseline CRP N (%) CD301	134 (74%)	526 (73%)	129 (75%)	129 (77%)
Elevated baseline CRP N (%) CD303			100 (58%)	102 (61%)

[1] only patients with a response/ remission in CD301 were eligible to participate in CD303

Medication history for patients in CD301 and CD303 was similar. Nearly all subjects (99%) had previously been treated for CD, most of them with 5-ASA compounds, steroids and/or immunosuppressants. Approximately 40% of subjects had received treatment with an anti-TNFα agent. However, a high number of patients became either unresponsive or intolerant to the chosen

treatment: approximately two-thirds of those who had previously received 5-ASA compounds, oral steroids, immunosuppressants, or anti-TNFα therapy.

Ninety-three percent (279/300) of immunosuppressant-treated subjects in Study CD301 had been previously treated with steroids for their CD, and 70% (195/279) of these subjects were considered by their physicians to be unresponsive, intolerant or dependent upon steroids.

The pattern of concomitant medications was similar among treatment groups at baseline (table 8) with the only exception that in the placebo maintenance group more patients received immunosuppressants than in the infliximab maintenance groups (55% vs. 45%). The majority of patients (96%) were receiving some kind of concomitant medication at baseline, predominantly 5-ASA compounds and oral steroids, followed by immunosuppressants. Less than 10% were treated with antibiotics.

Table 7: Concomitant Medications for Crohn's Disease – Study 301 and Study 303

	Concomitant CD medication Placebo		**Concomitant CD medication Natalizumab**	
	CD301 (N=181) n (%)	**CD303 (N=171) n (%)**	**CD301 (N=724) n (%)**	**CD303 (N=168) n (%)**
≥1 CD medication at baseline	173 (96)		693 (96)	
5-ASA Compounds	80 (44)	94 (55)	345 (48)	76 (45)
Oral steroids	71(39)	76 (44)	283 (39)	67 (40)
Immunosuppressants	53 (29)	60 (35)	247 (34)	62 (37)
Antibiotics	12 (7)	10 (6)	43 (6)	15 (9)

Numbers analysed

In the phase III programme, study CD301 randomized 905 subjects, 724 subjects were randomized to receive 300 mg natalizumab, 181 received placebo. A total of 904 subjects were dosed (1 subject withdrew without dosing from the verum group). In study CD303, 428 patients were enrolled and randomized (214 to natalizumab, 214 to placebo); all of them received the study drug. However, the efficacy population in CD303 only included those patients who achieved response or remission with natalizumab treatment in CD301 and therefore consists of 354 patients, 175 randomised to natalizumab, and 179 randomised to placebo.

Outcomes and estimation

- **CD301:**

The results for the **induction** study **CD301** are presented in table 8. The **primary** endpoint "Clinical response" was evaluated at week 10 and was defined as >70%-point decrease from baseline in CDAI. The contigent primary endpoint "Clinical remission" was defined as CDAI score <150 at week 10. As the primary endpoint was not achieved the contingent primary endpoint was classified as secondary endpoint; however, the difference between the treatment groups did also not reach a significant difference. Nevertheless the treatment benefit was in favour of natalizumab and two weeks later at week 12, significantly more natalizumab treated patients achieved clinical response (61.3% vs. 50.8%, p=0.009) and remission (39.8% vs. 30.9%, p=0.037) compared to patients receiving placebo.

Secondary endpoints reaching significance were clinical response (defined as ≥100 point reduction in CDAI) at week 10, and the improvement in total IBDQ score. The treatment effect of natalizumab might not be apparent due to the unexpected great placebo response.

The results of the **tertiary** efficacy analyses show a more pronounced benefit of natalizumab: more patients in the natalizumab group achieved a significant clinical response (either ≥70 or ≥100 point decrease in CDAI) or remission at week 12 than in the placebo group; also the proportion of patients with clinical remission at any time through week 12 favours treatment with natalizumab, whereas there was no difference in the proportion of patients with a clinical response at any time through week

12 for both treatment groups. During treatment with natalizumab CRP and albumin as objective markers of acute active inflammation also changed, leading to significantly decreased serum CRP- and increased serum albumin-levels at week 10. The Quality of Life scores (IBDQ, SF-36 health survey, EQ-5D, Subject Global Assessment VAS) were similar at baseline in both groups. At week 10, the improvement for IBQD and VAS scores from baseline was significantly higher in the natalizumab group than in the placebo group; whereas no marked differences could be observed for SF-36 and EQ-5D.

Colonoscopy substudy:

A total of 57 patients (15 placebo, 42 natalizumab) with colonic involvement participated in this substudy and received a baseline colonoscopy at week 0. As objective changes in the appearance of the gut mucosa by colonoscopy at week 10 were evaluated using the CD Endoscopic Index of Severity (CDEIS). However, the results are not convincing, as only trends have been observed in favour of natalizumab: more patients with colonic or ileocolonic ulcers at baseline had a histological healing of gastrointestinal lesions and a reduction in the mucosal inflammatory infiltrate at week 10 if they were treated with natalizumab.

Table 8: Efficacy results from CD301

		Placebo N=181	Natalizumab N=724	p-value
PRIMARY ENDPOINTS				
Clinical Response (≥70) at week 10	ITT	88 (**48.6%**)	408 (**56.4%**)	0.051
	Per protocol	70 (48.6%)	341 (55%)	0.148
SECONDARY ENDPOINTS				
Clinical Remission at week 10	ITT	55 (**30.4%**)	267 (**36.9%**)	0.124
	Per protocol	46 (31.9%)	216 (34.8%)	0.597
Clinical Remission at week 4		34 (18.8%)	163 (22.5%)	0.351
Clinical Response at week 2		59 (32.6%)	287 (39.6%)	0.060
Clinical Response (≥100) at week 10		75 (**41.4%**)	357 (**49.3%**)	**0.046**
Change in IBDQ score from baseline at week 10		+28.3	+35	**0.037**
TERTIARY ENDPOINTS				
Clinical response *at other time points*	week 12	92 (50.8%)	444 (61.3%)	**0.009**
Clinical response (≥100) *at other time points*	week 12	77 (42.5%)	365 (50.4%)	**0.048**
Clinical remission *at other time points*	week 12	56 (30.9%)	288 (39.8%)	**0.037**
Clinical response *at any time*	(i.e. through week 12)	132 (72.9%)	555 (76.7%)	0.259
Clinical response (≥100) *at any time*	(i.e. through week 12)	111 (61.3%)	479 (66.2%)	0.194
Clinical remission *at any time*	(i.e. through week 12)	79 (43.6%)	391 (54%)	**0.017**
Change in CDAI score	week 10	-90	-113.4	**0.004**
	week 12	-88.7	-113.4	**0.003**
Change from baseline CRP at week 2, 4, 6, 8, 10, and 12		↔	↓	**<0.05**
Change from baseline albumin		↔	↑	**<0.001**
Need for rescue intervention		Comparable between groups		
Quality of life				
Change in SF-36 health survey	week 10	+109	+135	0.053
Change in EQ-5D	week 10	No significant differences		
Change in Subject Global Assessment VAS	week 10	+60.8	+65.4	**0.026**

The results for the **maintenance** study **CD303** are presented in table 9.

The efficacy population of this maintenance study consists of those patients who had received successfully natalizumab in CD301, i.e. who were in clinical response/remission at week 10/12. Due to the rerandomisation to either natalizumab or placebo, half of patients were withdrawn from active

treatment, so that the initial 6 months of treatment (through Month 9) in CD303 are suitable to demonstrate natalizumab's ability to maintain clinical response or remission over 9 months of treatment.

The **primary** and **contingent primary** endpoints were the proportion (%) of natalizumab treated patients in CD301 that maintained clinical response and maintained clinical remission (from CD301 Week 12 or use of rescue medication), respectively, at any time for an additional 6 months of treatment (i.e., Month 9) in CD303. Subjects needed to remain in response or remission at each study visit to meet this stringent endpoint.

Table 9: Efficacy results from CD303 (maintenance)

	Placebo N=170	**Natalizumab N=168**	**p-value**
PRIMARY ENDPOINTS			
Clinical Response through month 9	48 (28.2%)	103 (61.3%)	**<0.001**
Clinical Remission through month 9	31(25.8%)	57 (43.8%)	**0.003**
SECONDARY ENDPOINTS			
Clinical Response through month 15	34 (20%)	90 (53.6%)	**<0.001**
Clinical Remission through month 15	18 (15%)	51(39.2%)	**<0.001**
Time to loss of response (median days)	86	Not reached	**<0.001**
Time to loss of remission (median days)	59	137	**<0.001**
Change in IBDQ score from CD301 baseline to month 9	+39.4	+53.4	**<0.001**
Patients (%) no oral steroids at month 9 of CD303	21 (27.6%)	39 (58.2%)	**<0.001**
Patients (%) in remission and no oral steroids at month 9	17(22.4%)	30 (44.8%)	**<0.001**
TERTIARY ENDPOINTS			
Clinical Response and no oral steroids at month 9	19/76 (25%)	35/67 (**52.2**%)	**0.003**
Clinical remission and no oral steroids at month 9	17/76 (22.4%)	30/67 (**44.8**%)	**0.014**
Change from baseline CRP at months 6, 9, 12, 15	↓ 17%	↓ 60%	**<0.001**
Change from baseline albumin at months 6, 9, 12, 15	↔	↑	**<0.001**
Need for rescue intervention	20%	37%	
Quality of life		In favour of natalizumab	
Change in IBDQ at months 6, 12, 15			≤0.003
Change in SF-36 health survey at months 6, 9, 12, 15			≤0.004
Change in EQ-5D at months 9, 12, 15			≤0.007
Change in Subject Global Assessment VAS at month 9, 12, 15			**<0.001**

The results of the efficacy analysis show that natalizumab is able to maintain a clinical response/clinical remission in a higher proportion of patients receiving monthly infusions of natalizumab (response 61.3% vs. 28.2%; remission 43.8% vs. 25.8%) and is in favour at each time point through month 9. The primary endpoint results were confirmed by the secondary clinical endpoints response/remission through month 15. A significant difference favouring natalizumab treatment was not observed before month 5 (two months after re-randomisation), consistent with the half life of natalizumab. This is as expected because the placebo-treated patients in CD303 who had been treated and responded to natalizumab in CD301 would have effective blood levels of natalizumab at entry into CD303. As a measure of acute inflammation, serum levels of CRP and albumin were also improved significantly. Concerning the quality of life (QoL) measurements as secondary and tertiary endpoints, differences favouring natalizumab treatment were also seen.

Per protocol, with the beginning of week 10, a corticoid tapering was allowed until a patient was either completely withdrawn from steroids, was considered a treatment failure, or the steroid taper was medically contraindicated. As a result more patients in the natalizumab group who entered CD301 on baseline steroids had discontinued oral steroids and maintained response (52% vs. 25%) or remission (44.8% vs. 22.4%) at month 9. The steroid-free response/remission period was seen up to 15 months. Over time, the mean dose of steroids in natalizumab-treated subjects was low and stable, whereas in placebo subjects the mean steroid dose steadily increased over time.

Ancillary analyses

- Subgroup analysis

The Applicant performed an extensive set of pre-planned subgroup analyses in order to assess the efficacy of natalizumab in various CD patient subpopulations. The primary (clinical response) and contingent primary (clinical remission) endpoints were analysed to assess consistency with the overall trial results. The pre-defined subgroups are: gender, age (≤65, >65), CDAI score (<330,≥330) at baseline of study CD301; medical use (yes, no) at baseline of Study CD301 including steroids, 5-ASA, immunosuppressants, antibiotics, and previous anti-TNF therapy failure, geographical location, weight (<50kg, 50-90kg, >90kg) and by BMI (body mass index: ≤27, >27kg/m^2).

As a result it can be summarised that age, gender, severity of disease, or weight as well as the use of concomitant CD medication did not appear to markedly affect the efficacy of natalizumab to induce and maintain a clinical response or remission.

In order to characterise in more detail the patient group that benefits most from the natalizumab treatment additional post hoc analyses of response and remission were performed in the following subgroups (see figures below):

- elevated CRP (>2.87 mg/ml) at baseline
- elevated CRP as well as concomitant immunosuppressant and/or steroid

Based on these analyses the applicant identified retrospectively a group of 'refractory patients' as target population for the marketing application. This group is defined as follows:

- Patients with chronic, active CD, who are either unresponsive or intolerant to corticosteroids and immunosuppressive, characterized by an elevated CRP level and the use of at least one concomitant immunosuppressant at baseline.

The applicant provided extensive analyses for this patient population. Below these analyses are briefly summarised:

Baseline data

A total of 222 subjects (out of 905, 25%) in CD301 were included in the refractory population with an elevated serum CRP (above ULN) and a concomitant immunosuppressant therapy at baseline: 184 patients received natalizumab and 38 placebo. With a mean age of 34 years, slightly more females and more than 90% Caucasian, the baseline characteristics are comparable to those of the overall population. The same holds for the mean disease duration (slightly > 9 yrs) and mean CDAI score (~ 310). Of the 184 subjects treated with natalizumab in the refractory population, 137 (74%) were either on steroids at baseline or had used steroids previously, but were unresponsive, intolerant, or dependent upon steroids. Of the 38 placebo subjects, 30 (79%) were either on steroids at baseline or unresponsive, intolerant, or dependent upon steroids.

Of the 184 natalizumab-treated subjects in the refractory population, 98 subjects (53%) responded to natalizumab treatment and were enrolled in CD303. These subjects were re-randomised equally in Study CD303 to natalizumab (n=49) or placebo (n=49).

Efficacy results

The results are summarised in the tables below:

Table 10: Efficacy results - CD301 refractory population

	Placebo N=38	Natalizumab N=184	p-value
PRIMARY ENDPOINTS			
Clinical Response (≥70) at week 10	14 (**36.8%**)	114 (**62**%)	**0.005**
Clinical Remission at week 10	7 (**18.4%**)	76 (**41.3**%)	**0.011**

Table 11: Efficacy results - CD303 refractory population

	Placebo N=49	Natalizumab N=49	p-value
PRIMARY/SECONDARY ENDPOINTS			
Clinical Response through month 9	13 (26.5%)	30 (**61.2**%)	**<0.001**
Clinical Response through month 15	11(22.4%)	25 (**51**%)	**0.004**
	Placebo N=36	**Natalizumab N=39**	**p-value**
Clinical Remission through month 9	7(19.4%)	20 (**51.3**%)	**0.005**
Clinical Remission through month 15	5 (13.9%)	15 (**38.5**%)	**0.020**
SECONDARY ENDPOINTS			
	Placebo N=49	**Natalizumab N=49**	**p-value**
Time to loss of response (median days)	83	Not reached	**0.003**
Time to loss of remission (median days)	58	223	**0.007**
	Placebo N=26	**Natalizumab N=25**	**p-value**
Subgroup receiving steroids at baseline			
Clinical Response and **no steroids** at month 9	7 (27%)	15 (**60**%)	**0.028**

In study CD301 natalizumab treatment was shown to be superior to placebo in the refractory patient population. Significantly more patients in the natalizumab group were in clinical response (62% vs. 37%, p=0.005) or even remission (41% vs. 18%, p=0.011) at week 10 in comparison to patients receiving placebo.

Results of the maintenance study CD303 (recruitment of natalizumab responders at week 10 and week 12) were also in favour of the natalizumab treatment with more patients sustaining clinical response or remission through month 9 and 15, as well as a longer time to loss of response and remission. For the maintenance treatment the data gained from the refractory population confirm those of the efficacy population, but significant differences in favour of natalizumab were not seen before month 6.

The efficacy was also confirmed by positive results for CRP as a marker for inflammation, and the ability to discontinue oral steroids while maintaining response through month 15. However, in the refractory population the number of patients receiving steroids at study entry was very low, so that no conclusion can be drawn with regard to the tapering of oral steroids.

Following comments from the CHMP, the Applicant proposed a revised indication and submitted a post-hoc analysis in a subpopulation of patients who had previously failed therapy with an inhibitor of TNFα.

- **Study CD307**

This was a multicentre, randomised, double-blind, placebo-controlled, parallel-group study in adult subjects with moderately to severely active CD (based on clinical evaluation and CDAI score ≥220 to ≥450) and elevated CRP levels (defined as >2.87 mg/L, the upper limit of normal [ULN]) as assessed by the study central laboratory at the screening visit.

METHODS

Study Participants

To be eligible for **inclusion** in the study, each subject was to have met the following criteria:

-Male or female subjects, at least 18 years of age at the first visit;
-At least a 6–month history of CD;
-Clinical evidence of active (symptomatic) CD based on clinical history and radiological or endoscopic findings within the previous 36 months. Subjects with active disease following surgical resection were to have had radiological or endoscopic confirmation of CD after surgery;
-CDAI score of ≥220 and ≤450 at Week 0;
-CRP level above ULN (i.e., >2.87 mg/L) at the study's central laboratory at the screening visit.

Exclusion criteria were (shortened to most pertinent issues):
-Women of childbearing potential unless surgically sterile or adequate contraception
-Women who were pregnant or breastfeeding;
-Prior treatment with natalizumab;
-Subjects with symptoms suggestive of intestinal stricture or obstruction;
-Subjects with known active or draining fistulae;
-Subjects who did not meet any of the following criteria regarding baseline medications for CD:
-Any baseline 5-acetylsalicylic acid (5-ASA) compounds or antibiotics were to be administered at a stable dose for a minimum of 4 weeks prior to Week 0. If recently discontinued, were to be stopped at least 4 weeks prior to Week 0.
-Baseline use of rectal 5-ASA compounds was prohibited; these agents were to be discontinued by Week 0.
-Any baseline use of budesonide was not to exceed a daily dose of 6.0 mg. Budesonide was to be administered for a minimum of 6 weeks prior to Week 0 and at a stable dose for the last 4 weeks prior to Week 0. If recently discontinued, was to be stopped at least 4 weeks prior to Week 0.
-For oral steroids other than budesonide, subjects were not to exceed a daily dose of 20 mg prednisolone equivalent Subjects were to receive the drug for a minimum of 6 weeks prior to Week 0 and at a stable dose for the last 4 weeks prior to Week 0. If recently discontinued, were to be stopped at least 4 weeks prior to Week 0.
-Baseline use of rectal steroids was prohibited; these agents were to be discontinued by Week 0.
-Any baseline azathioprine (AZA), 6-mercaptopurine (6-MP), 6-thioguanine (6-TG), or methotrexate (MTX) was to be administered for a minimum of 4 months prior to Week 0 and at a stable dose for the last 8 weeks prior to Week 0. If recently discontinued, was to be stopped at least 4 weeks prior to Week 0.
-Baseline use of any other immunosuppressant (e.g., tacrolimus, cyclosporin, mycophenolate mofetil or leflunomide) was prohibited; these agents were to be discontinued at least 8 weeks prior to Week 0;
-Baseline use of anti-tumor necrosis factor (anti-TNF) therapy was prohibited; these agents were to be discontinued at least 12 weeks prior to Week 0.
-Experimental agents were to be discontinued at least 4 weeks prior to Week 0, or for a period equivalent to 5 half-lives (t½) of the agent (whichever was longer);

This study was conducted in 114 investigational sites in North America, Europe, and selected countries from the rest of the world (RoW).

Treatments

Subjects were screened for eligibility and randomized 7 to 14 days later at the baseline (Week 0) visit to receive monthly (defined as a 4-week period) intravenous (IV) infusions of natalizumab 300 mg or placebo (1:1 ratio) at Weeks 0, 4, and 8.

Objectives

Primary Objective was to compare the ability of natalizumab versus placebo to induce a clinical response in subjects with moderately to severely active Crohn's disease (CD) and elevated serum C-reactive protein (CRP)

Secondary Objectives were to compare the ability of natalizumab versus placebo to induce a clinical remission in subjects with moderately to severely active CD and elevated CRP and to compare the ability of natalizumab versus placebo to induce a clinical response or remission at Week 12.

Outcomes/Endpoints

Primary endpoint was the proportion (%) of subjects with a ≥70-point decrease from baseline (Week 0) in Crohn's Disease Activity Index (CDAI) score at both Weeks 8 and 12.

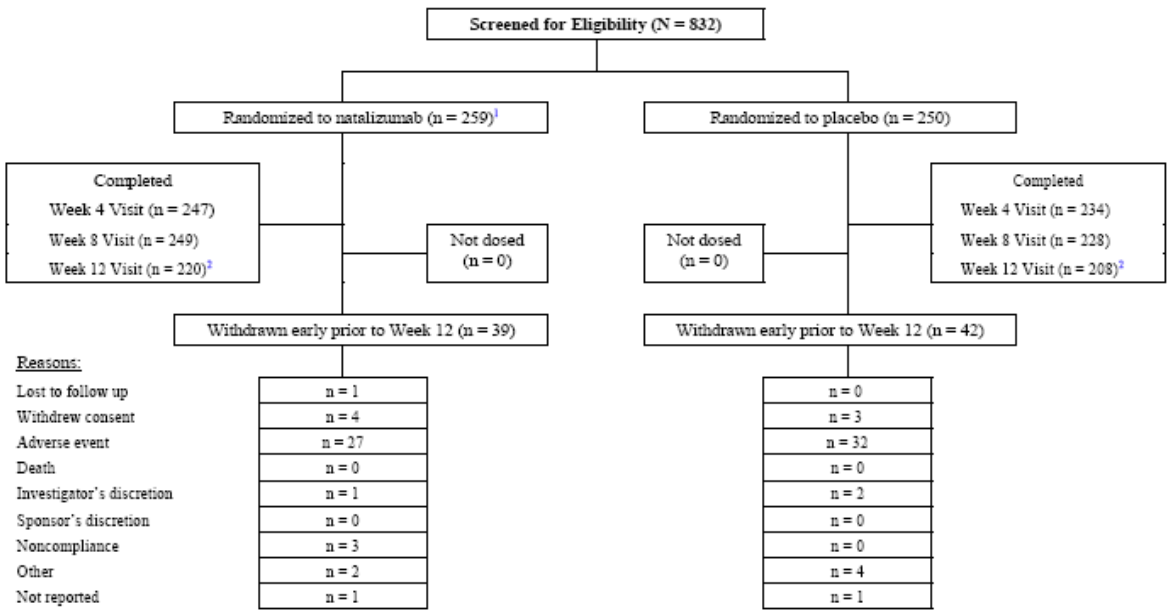
Secondary endpoints were
-the proportion (%) of subjects with a CDAI score <150 at both Weeks 8 and 12
-the proportion (%) of subjects with a clinical response (≥70-point decrease from baseline [Week 0] in CDAI score) at Week 12 and proportion (%) of subjects in clinical remission (CDAI score <150) at Week 12.

Sample size

Approximately 462 subjects with moderately to severely active CD and elevated CRP were to be randomized in the study in a 1:1 ratio of natalizumab to placebo.

RESULTS

Figure 4: Participant flow



Recruitment

The first subject was enrolled on 29 March 2004; the last Efficacy (Week 12) Visit was completed on 14 March 2005.

Baseline data

Table 12: Baseline data

Variable	Placebo (n=250)	Natalizumab (n=259)	Overall (n=509)
Age (yr)			
Mean	37.7	38.1	37.9
Median	35.0	6.0	36.0
Age Group (yr) N (%)			
<= 65	245 (98)	253 (98)	498 (98)
> 65	5 (2)	6 (2)	11 (2)
Gender N (%)			
Female	148 (59)	154 (59)	302 (59)
Male	102 (41)	105 (41)	207 (41)
Weight (kg)			
Mean	74.4	71.9	73.1
Median	71.1	68.0	69.5
Weight Group (kg) N (%)			
< 50	14 (6)	18 (7)	32 (6)
50-75	128 (51)	151 (58)	279 (55)
76-100	83 (33)	67 (26)	150 (29)
> 100	25 (10)	23 (9)	48 (9)
Height (cm)			
Mean	170.4	170.1	170.2
Median	171.5	170.0	170.2
Body Mass Index (kg/m^2)			
Mean	25.7	24.8	25.2
Median	24.1	23.5	23.9
Baseline CDAI Score			
Mean	299.5	303.9	301.7
S.D.	63.19	64.80	63.99
Median	287.0	286.0	286.0
Min., Max.	149,483	147,472	147,483
Baseline Disease Status N (%)			
CDAI < 330	178 (71)	174 (67)	352 (69)
CDAI >= 330	71 (28)	84 (32)	155 (30)
Baseline C-Reactive Protein (mg/L)			
Mean	23.4	23.0	23.2
Median	14.2	12.7	13.7
Baseline CRP (mg/L) N(%)			
<= 2.87	18 (7)	14 (5)	32 (6)
> 2.87	232 (93)	245(95)	477 (94)
Baseline Serum Albumin N (%)			
< LLN	39 (16)	52 (20)	91 (18)
>= LLN	210 (84)	206 (80)	416 (82)
Baseline Platelet Count N (%)			
<= ULN	168 (67)	160 (62)	328 (64)
> ULN	81 (32)	97 (37)	178 (35)

Smoking Status of More Than 10 Cigarettes per Day N (%)			
Yes	48 (19)	57 (22)	105 (21)
No	201 (80)	201 (78)	402 (79)
Duration of Disease (months)			
Mean	120.3	121.4	120.9
Median	84.0	92.5	89.5
Site of Disease N (%)			
Ileum	65 (26)	56 (22)	121 (24)
Colonic	65 (26)	69 (27)	134 (26)
Ileocolonic	120 (48)	134 (52)	254 (50)
Prior or concomitant medication N (%)			
Treatment Naive	2 (0.8)	3 (1.2)	5 (1.0)
5-ASA Compounds	232 (92.8)	241 (93.1)	473 (92.9)
Steroids	235 (94.0)	237 (91.5)	472 (92.7)
Immunosuppressants	182 (72.8)	194 (74.9)	376 (73.9)
Antibiotics	139 (55.6)	149 (57.5)	288 (56.6)
Prior Medication N (%)			
5-ASA Compounds	112 (44.8)	113 (43.6)	225 (44.2)
Steroids	140 (56.0)	127 (49.0)	267 (52.5)
Immunosuppressants	84 (33.6)	97 (37.5)	181 (35.6)
Antibiotics	123 (49.2)	130 (50.2)	253 (49.7)
Anti-TNF Agents	113 (45.2)	131 (50.6)	244 (47.9)

An overall analysis of treatment outcomes for prior use of medications for CD showed that 61% of subjects previously treated with oral steroids, other than budesonide, discontinued use of the medications: 20% discontinued treatment due to lack of response (unresponsive) and 12% due to dependency on the medication. Of those subjects treated previously with budesonide alone, approximately 62% of subjects in both treatment groups reported lack of response.

Subjects who were previously treated with azathioprine or 6-MP or 6-TG had the highest rate of discontinuation due to adverse events and/or intolerance (55% for both treatment groups). The rates of discontinuation due to adverse events and/or intolerance for other prior medications for CD in decreasing frequency were 43% (methotrexate), 29% (anti-TNF agents), 20% (other immunosuppressants, i.e., cyclosporine), 18% (oral steroids, other than budesonide), 17% (5-ASA compounds), 10% (budesonide alone), and 8% (antibiotics).

Numbers analysed

Table 13: Numbers analysed

	Placebo (N = 250)	**Natalizumab (N = 259)**	**Overall (N = 509)**
Number (%) of Subjects Randomized (ITT Population)	250 (100)	259 (100)	509 (100)
Number (%) of Subjects Treated with Study Drug (Safety Population)	250 (100)	260[2] (100)	510 (100)
Number (%) of Subjects with no Major Protocol Deviations (Per Protocol Population)	215 (86)	221 (85)	436 (86)
Number (%) of Subjects assessed for PK	246 (99)	257 (99)	503 (99)
Number (%) of Subjects assessed for PD	250 (100)	259 (100)	509 (100)

Outcomes

Primary endpoint: Proportion of Subjects with a Clinical Response (at least 70-point decrease from baseline in CDAI) – ITT Population

Table 14

Visit	**Placebo** (n=250) N (%)	**Natalizumab** (n=259) N (%)	**Odds Ratio** (a)	**95% CI of Odds Ratio** (a)	**p-value** (a)
Week 4	92 (36.8)	133 (51.4)	1.801	(1.262, 2.570)	0.001
Week 8	99 (39.6)	146 (56.4)	1.969	(1.382, 2.805)	<0.001
Week 12	109 (43.6)	155 (59.8)	1.953	(1.370, 2.783)	<0.001
Weeks 4 & 8	62 (24.8)	109 (42.1)	2.191	(1.500, 3.202)	<0.001
Weeks 8 & 12	**81 (32.4)**	**124 (47.9)**	**1.924**	**(1.341, 2.760)**	**<0.001**
Any Time (b)	146 (58.4)	192 (74.1)	2.049	(1.406, 2.987)	<0.001

(a) The adjusted odds ratio, 95% CI, and p-value are from logistic regression adjusting for disease severity (CDAI < 330 vs >= 330) at baseline.

Secondary endpoint: Proportion of Subjects in Clinical Remission (CDAI less than 150) –ITT Population

Table 15

Visit	Placebo (n=250) N (%)	Natalizumab (n=259) N (%)	Odds Ratio (a)	95% CI of Odds Ratio (a)	p-value (a)
Week 4	39 (15.6)	62 (23.9)	1.838	(1.164, 2.902)	0.009
Week 8	52 (20.8)	83 (32.0)	1.904	(1.264, 2.869)	0.002
Week 12	63 (25.2)	97 (37.5)	1.912	(1.292, 2.829)	0.001
Weeks 4 & 8	22 (8.8)	48 (18.5)	2.529	(1.464, 4.369)	<0.001
Weeks 8 & 12	**40 (16.0)**	**68 (26.3)**	**2.011**	**(1.285, 3.146)**	**0.002**
Any Time (b)	86 (34.4)	121 (46.7)	) 1.806	(1.247, 2.614)	0.002

Note: Subjects were classified as treatment failures at any visit after rescue, early withdrawal, or discontinuing use of study drug due to adverse event. If CDAI score was missing for a given time point, the subject was considered a treatment failure for that time point.
(a) The adjusted odds ratio, 95% CI, and p-value are from logistic regression adjusting for disease severity (CDAI < 330 vs >= 330) at baseline.
(b) Any time through Week 12.
Subgroup analysis

Analysis across subgroups shows mainly consistent results. The apparent absence of efficacy in CD of the disease of the ileum is curious and unexplained. Somewhat divergent effects are seen with respect to baseline immunosuppressants and baseline steroids. In both treated subgroups the effect of natalizumab is less pronounced.

- Other supportive studies

Study CD201= single-dose induction study

This was a Phase II, double-blind, placebo-controlled, randomised pilot study designed to evaluate safety and efficacy of a single dose of 3 mg/kg natalizumab. A total of 30 subjects with mildly to moderately active CD (CDAI score >150 and ≤450) were included, 18 received natalizumab, 12 placebo. The follow-up period was 12 weeks. The results of this pilot studies with positive efficacy trends initiated controlled, multi-dose studies.

Study CD306 = combination treatment with infliximab
This Phase II, randomised, multicentre, double-blind, placebo controlled pilot study assessed primarily the safety of natalizumab in patients with CD concurrently receiving infliximab and not in remission (CDAI score >150). The study was not sufficiently powered to demonstrate efficacy.

Study CD251 = intermittent retreatment
This phase II, multicentre, open-label study was conducted in extension to Study CD202 and evaluated safety, tolerability and effectiveness of intermittent administered natalizumab (6mg/kg) on an as needed basis. A total of 96 subjects with active CD (confirmed by CDAI score >150) who completed at least the week 16 visit of Study CD202 were enrolled for re-treatment. In the preceding study 22 subjects had received placebo, the other 30, 21, and 23 subjects had received a single 3mg/kg dose (3+0), two 3mg/kg doses (3+3) and two 6mg/kg doses of natalizumab 6+6), respectively. The drug holiday period from the last infusion in CD202 to the first natalizumab treatment in CD251 ranged from 43 weeks to 127.9 weeks. Due to methodological reasons, no conclusion regarding the efficacy could be drawn.

Study CD351 = long-term, open-label treatment

A Phase III, multicentre, open-label, study designed to evaluate long-term treatment with 300 mg natalizumab given monthly (every 4 weeks) for up to 24 infusions. It is the first study using commercial material. Only CD patients previously participating in Studies CD251, CD301, CD303, CD306, or CD307 can be enrolled. The primary objective is safety of natalizumab. A full efficacy analysis was not performed.

- Clinical studies in special populations

Two Phase II, multicentre, open-label, single-arm studies have been undertaken in adolescent patients (12-17 years of age) with moderately to severely active CD (PCDAI score >30). **Study CD305** (induction) and **CD352** (long-term) were conducted in tandem and were primarily designed for safety.

Eligible patients for CD305 received IV infusions of natalizumab (3 mg/kg) at Weeks 0, 4, and 8 and were assessed through Week 12 for the treatment phase and through Week 32 for the follow-up phase. At the Week 12 visit of Study CD305, eligible subjects may have elected to continue natalizumab treatment every 4 weeks by enrolling in CD352 and to receive up to 24 infusions of 3 mg/kg natalizumab every 4 weeks. During the study subjects were allowed to remain on stable doses of oral 5-ASA compounds, oral steroids (< 40 mg prednisolone or equivalent), antibiotics, and specified immunosuppressants, as well as nasogastric/nasoenteric tube feeding and elemental/polymeric diets. Other medication was prohibited.

Thirty-eight patients enrolled in CD305 with a mean age of 14.4 years (11 to 17), 71% were male and 84% white. The BMIs ranged from 13 to 32 kg/m^2. The mean baseline values for efficacy parameters were P(aediatric)CDAI score (38.3), CRP (15.9 mg/L), serum albumin (34.4 g/L), and ESR (32.2 mm/hr). Over two thirds of the subjects (74%) had elevated CRP levels (defined as >2.87 mg/L) at baseline. At week 12, twenty-four patients (24/38; 63%) decided to continue receiving monthly natalizumab infusions and to enter Study CD352.

The efficacy was assessed by evaluating clinical response (defined as ≥15-point decrease in PCDAI score from baseline and clinical remission (PCDAI score ≤10). During the 12-week treatment period 63% (24/38) of patients received a clinical response and 34% (13/38) received clinical remission at some time point. The mean PCDAI score was significantly ($p<0.001$) decreased from baseline at all time points. Quality of life was not improved. The decrease in CRP levels (4.0 mg/l decrease within the first two weeks of therapy) was similar in the overall population regardless of baseline CRP (elevated and non-elevated). They remained decreased throughout the treatment period.

The results for the clinical study 352, although preliminary, show an ongoing decrease in mean PCDAI from baseline to month 6, and more patients were in clinical remission at Month 6 compared to baseline.

- Effect of anti-natalizumab antibodies on efficacy

The antibody status was correlated with efficacy for the two pivotal studies (CD301 and CD303), the open-label study CD351, and the intermittent, retreatment study, CD251. Anti-natalizumab antibodies were defined to be persistent in case of either two positive tests at least 6 weeks apart, or a single positive test at the last measurement. A positive antibody response is defined transient with at least one positive screening test unless not classified as persistent positive.

Overall, 53/650 patients (8%) were positive (as concentration in mAb equivalents ≥0.5 µg/ml) for anti-natalizumab antibodies in study CD301; in study CD303 a similar percentage was observed (11/168 patients, representing 6.5%). An analysis of primary and secondary endpoints by antibody status was performed, considering both transiently and persistently positive subjects in CD303, but not in CD301, due to a small number of drug infusions and limited antibody testing. In CD301 less antibody-positive patients reached the primary endpoint clinical response [23/53 (43%)] at week 10, compared to antibody-negative patients [348/597 (58%)]. The improvement in CDAI scores over time was greater in a patient tested negative compared to someone tested positive.

Data from the efficacy population in CD303 suggest that a persistence of anti-natalizumab antibodies is associated with a decline in efficacy: clinical reponse or remission could be maintained through month 9 in 4/4 (100%) and 2/4 (50%) patients with transient positive antibody reponse, whereas in the population with persistent anti-natalizumab antibodies and clinical reponse (7/168; 4%) or clinical remission (3/130; 2%) only 1/7 (14%) could maintain response and none clinical remission through month 9. At month 15 no patient with persistent antibodies was in response or remission compared to 56% and 41% of patients without antibodies.

Accordingly, over time the mean CDAI score was: in patients with no antibodies < transient antibodies < persistent antibodies.

Table 16: Effect of anti-natalizumab antibodies on efficacy CD303: Efficacy population

MAINTAINING RESPONSE			**Natalizumab group (N=168)**	
Visit	**Antibody positive**	**Persistent positive**	**Transient positive**	**Antibody negative**
n (%)	**(N=11)**	**(N=7)**	**(N=4)**	**(N=156)**
Month 9	5 (45%)	1 (14%)	4 (100%)	98 (63%)
Month 15	3 (27%)	0 (0%)	3 (75%)	87 (56%)
MAINTAINING REMISSION			**Natalizumab group (N=130)**	
Visit	**Antibody positive**	**Persistent positive**	**Transient positive**	**Antibody negative**
n (%)	**(N=7)**	**(N=3)**	**(N=4)**	**(N=122)**
Month 9	2 (29%)	0 (0%)	2 (50%)	55 (45%)
Month 15	1 (14%)	0 (0%)	1 (25%)	50 (41%)

Note: 167 subjects had antibody status; one subject did not have antibody status (response)
Note: 129 subjects had antibody status; one subject did not have antibody status (remission)

- Discussion on clinical efficacy

Induction therapy

For the **induction therapy** study CD301, the overall target population, i.e. treatment in a second line setting, has been defined along definitions of already approved products in this indication, both with regard to the severity of the disease (refractory, moderately to severely active Crohn's disease) and with regard to the pre-treatment of the patients (i.e. oral steroids, immunosuppressant). The definitions are considered appropriate, as the studies thus included patients who are particularly resistant to therapy.

The chosen study design for CD301 is based on a 4:1 randomisation natalizumab vs placebo. During study CD301 all patients received a 3-dose induction regimen with either a fixed dose of 300mg natalizumab or placebo at week 0, 4 and 8. After assessing the response status at week 10 and 12, patients in response or remission were re-randomised to participate on CD303.

Study CD301 failed to reach the (contingent) primary (clinical response and remission at week 10) and most secondary endpoints. Although 56.4% (408/724) of natalizumab treated patients were in clinical response (>70%-point decrease from baseline in CDAI) at week 10, the percentage in the placebo group was also high, namely 48.6% (88/181), p=0.051. The contingent primary endpoint, clinical remission (CDAI score <150) at week 10, was achieved in 267 (36.9%) natalizumab treated patients compared to 55 (30.4%) patients in the placebo group. In order to create a subgroup with a positive outcome the protocol was amended retrospectively and a post hoc subgroup analysis was performed. The results revealed a statistically significant effect for a certain subgroup of patients ("refractory population"), characterized by an elevated CRP at baseline and at least one concomitant immunosuppressant at baseline. In this subgroup significantly more natalizumab treated patients achieved clinical response (62%; 114/184 vs. 37%; 14/38, respectively; p=0.005) or remission (41%; 76/184 vs 18%; 7/38, respectively; p=0.011) at week 10. The fact that this subgroup was identified post hoc, in the knowledge of the data, the CHMP had considered that efficacy was not sufficiently

proven for the "high CRP" population. While such definition per se was acknowledged of being reasonable from a clinical point of view (these patients show active, ongoing inflammation despite immunosuppression), such approach was not considered acceptable from a methodological point of view.

Using the outcomes from study CD301 and exploratory analyses, the applicant has generated a new hypothesis, performed a completely **new study for induction therapy (CD 307)** and submitted the appropriate documentation in order to answer the questions resulting from the assessment of the CD301 study. Study CD307 was a multicentre, randomised, double-blind, placebo-controlled, parallel-group study in adult subjects with moderately to severely active CD (based on clinical evaluation and CDAI score ≥220 to ≥450) and elevated CRP levels (defined as >2.87 mg/L, the upper limit of normal [ULN]) as assessed by the study central laboratory at the screening visit. No subsequent maintenance study was initiated for subjects from study CD307.

Study CD307 was designed to confirm the post-hoc results from CD301. Baseline demographic and disease characteristics were comparable. The primary endpoint (proportion (%) of subjects with a ≥70-point decrease from baseline (Week 0) in Crohn's Disease Activity Index (CDAI) score at both Weeks 8 and 12) was reached for this study in a statistically significant manner.

Secondary endpoints of the newly performed study CD307 were met as well. Subgroups with baseline use of corticosteroids or other immunosuppressants had a less pronounced effect of natalizumab.

Overall, study CD307 showed efficacy in the induction of remission in moderate to severe CD. Unfortunately, the inclusion criteria were not used to accurately define the required prior therapy, (e.g. a full and adequate course of steroids and immunosuppressants) which was considered important for placing this drug in the therapeutic cascade. Introducing CRP as a parameter for selecting patients with a therapeutic need presents an interesting concept and considering pathophysiology appears justified. It is currently unclear how this screening criterion can be translated into clinical practice, as different laboratories have different methodologies and cut-off values separating normal from elevated values. The effect size observed indicates clinically relevant although not outstanding efficacy for the induction of remission (differences in percentage of patients in remission are 10.3 % [16.0 vs 26.3]). Whilst the patients had to have active disease to be included in the study it could not be confirmed that all the patients included in the analysis who were defined as "immunosuppressant and steroid failures" were not on baseline immunosuppressant and steroids. The relevant population to investigate would be patients with adequate prior therapy and discontinuation prior to inclusion in the study as these would constitute true failures. In addition there remain concerns with respect to external validity of the study as approximately one quarter of the patients did not receive prior or concomitant therapy with immunosuppressants that are currently considered standard of care in patients with moderate to severe disease.

Maintenance therapy

Concerning maintenance therapy of patients who showed at least a response in the induction therapy study, the Applicant had presented data from one study (CD303). For the newly performed study, CD307, no subsequent maintenance study was performed as patients entered open label study CD351. In study CD303, statistically significant differences in favour of natalizumab as compared to placebo were detected. This was shown by a higher proportion of patients maintaining clinical response (103/168; 61%) or remission (57/130; 44%) through month 9 compared to placebo (48/170, 28% and 31/120, 26%, respectively. However, when assessing the overall clinical course of natalizumab therapy concerning onset of efficacy, beginning at the start of treatment (CD301), the time until an effect was seen for natalizumab was three months. The possibility that this amelioration of symptoms in patients treated could also be explainable by a chance effect, i.e. a spontaneous remission of symptoms in this disease which is known to exhibit a rather fluctuating clinical course, can therefore not be dismissed by the currently available database. It should be noted that these results were obtained in a much smaller patient population than initially recruited in CD301. In this "responder"

population efficacy in maintenance, at every monthly assessment, was only evident in around 50% after a further 12 months of active treatment.

Patients randomised into study CD303 were responder in study CD301 (and not necessarily in remission). Thus, study CD303 did primarily assess maintenance of response and not maintenance of remission as required by the Points to Consider on clinical investigation of medicinal products for the management of Crohn's disease (CPMP/EWP/2284/99). However, maintenance of remission, in patients who were in remission at the end of CD301, was assessed as a co-primary in study CD303.

As the population primarily studied in CD303 is not the population that will be eligible for treatment, as outlined by the applicant, the result from a single study are not considered sufficient to support this indication. Applicability of the results to the intended population has to be demonstrated.

Influence of anti-natalizumab antibodies on efficacy

Proposed management strategies for suspected antibody positivity seem appropriate, i.e. patients who develop clinical signs in the form of infusion reaction should discontinue treatment and not be re-exposed to treatment. However, there is remaining concern that antibody formation in the intended target population (no immunosuppressant) will be higher and impinge further on the not outstanding efficacy. The applicant demonstrates that antibody formation in the population without concomitant immunosuppression is approximately doubled in comparison to the population with concomitant immunsuppression. The incidence in this population is 5% which is considered acceptable and will not have a major impact on efficacy.
In addition there is an indication from these data that weight-based dosing might be preferable as the incidence of hypersensitivity reactions and anti-natalizumab antibodies was higher in the upper body weight quartile (see: fixed dose regimen).

Fixed dose regimen

Higher weight is associated with a decreased response and remission rate. Together with the presented data on immunogenicity and hypersensitivity reactions a point can be made for weight based dosing. This analysis further emphasises the need of additional data in the target population in order to substantiate efficacy and to be able to place natalizumab appropriately in the therapeutic cascade.

Conversely, population at the lower end of the weight spectrum might experience a substantially higher drug exposure in chronic treatment. If this might become an issue as regards accumulation and the risk for development of PML remains unknown.

Efficacy in anti-TNF failures

Subgroup analysis for the different studies shows that the impact of prior anti-TNF failure is not homogenous. Study CD301 shows reduced efficacy in anti-TNF failure compared to anti-TNF responders, study CD 307 did not allow for a direct comparison but shows efficacy in anti-TNF failures with respect to the effect of natalizumab. The Applicant provided an analysis of response and clinical remission restricted to comparable subgroups of CD301 and CD307 (elevated CRP, anti-TNF-α failure). Also in this analysis the response and the remission in the CD301 trial was considerably higher than in study CD307. This analysis emphasises the need of additional data in the target population in order to substantiate efficacy.

Clinical safety

- Patient exposure

The safety data to support these claims is mainly derived from:

(a) 9 target indication studies in Crohn's disease (CD) patients and

(b) 8 non-target indication studies in relapsing Multiple Sclerosis (including both RRMS and SPMS)

Dosing in CD and relapsing MS was 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 natalizumab.

Within the CD population the analysis of the patients' data is separated into short- and long-term dosing, to ascertain the effects of cumulative dosing of natalizumab. The short-term dosing interval was defined as the exposure of 1 to 3 infusions, with subsequent infusions grouped by 3. The analysis provided by the applicant included all CD patients who were exposed to natalizumab at any time.

In the relapsing MS studies natalizumab was administered in 79% of the patients at 300 mg either as a fixed dose alone, or in combination with Avonex or Copaxone.
In the assessment the target indication studies and non-target indication data is reviewed separately and pooled for common analysis.

Target indication studies: **Crohn's disease (CD)**

In the active CD placebo-controlled studies 922 patients were treated with natalizumab, of which 723 received 300 mg as a fixed dose. Sixty-seven percent (614 patients) of the 922 natalizumab-treated patients and 57% of the 256 placebo patients received 3 infusions, the maximum number allowed. Of the natalizumab-treated patients, 52% had been exposed for 12 to 16 weeks.

In the short- and long-term dosing CD studies 1098 patients were treated and 866 received at least 1 fixed dose of 300 mg natalizumab
In order to estimate incidence rates of serious and rare events and compare these to available rates, the company calculated total person-years using the date of first dose to the last date of contact. The calculation is based upon the summation of time exposed to natalizumab across patients expressed in person-years. For the 1,617 MS patients in placebo-controlled studies, exposure was 2,174 person-years. For the 1,089 CD patients who received natalizumab at any time, exposure to natalizumab amounted to 1,037 person-years.

Non-target indication studies: **Multiple Sclerosis**

In the MS placebo-controlled studies 1617 (100%) patients received at least one dose of natalizumab and 1135 (100%) patients were treated with at least one placebo infusion. 1326 (82%) of the 1,617 natalizumab-treated patients and 953 (84%) of the 1,135 placebo patients received at least 6 infusions, and 1035 (64%) and 738 (65%) respectively received at least 13 infusions. Of the natalizumab-treated patients, 1,155 (71%) have been exposed for at least 6 months and 1,015 (63%) for at least 1 year, implying that over a third had been exposed for under a year. The median exposure was 56 weeks (min - max = 4 - 92 weeks), the mean was 46 weeks (SD ± 23 weeks). According to the data submitted 1,617 patients received natalizumab; 627 received 300 mg as a fixed dose alone, 589 received 300 mg in combination with Avonex, and 55 received 300 mg in combination with Copaxone. The remaining 346 patients received either single or up to 6 infusions within phase one and phase II studies. Of the 1,135 patients who received placebo, 582 received AVONEX and 55 received Copaxone in addition.

Table 17: Exposure to Study Drug in Pivotal Studies in CD and MS

	CD301	**CD303**	**Study 1801**	**Study 1802** + Avonex
Patients randomised /dosed	723/723 (100%)	214 /214 (100%)	627/627 (100%)	589/589 (100%)
Median No. infusions	3	12*	14	15
Min-max	1-3	1-12	1-23	1-23

*note that 175 of these patients had already received 3 infusions in CD301 (39 had received placebo)

Other studies

1. Study AN 100226 -101 in healthy volunteers

This Phase I, double-blind, placebo controlled, ascending single dose study to investigate the safety, tolerability and pharmacokinetics of ANI00226 at five dose levels (0.03, 0.1, 0.3, 1.0 and 3.0 mg/kg) was conducted in a total of 35 volunteers. Nine volunteers received placebo, 4 volunteers each

received 0.03 and 0.1 mg/kg AN100226 and 6 volunteers each received 0.3, 1.0 and 3.0 mg/kg AN100226.

2. Study 1805 Lonza/Commercial Scale Bioequivalence Study

This bioequivalence study was conducted in 89 healthy volunteers. Subjects were randomized in a 1:1 ratio to 1 of 2 sequences of 2 preparations of natalizumab (LNZRSH* and BGNCOM**) and were to receive a 300 mg IV infusion of either preparation, followed 8 weeks later by a 300 mg IV infusion of the other preparation. All subjects were randomized and received at least 1 dose; 56 subjects received both doses.

* LNZRSH (AN100226, natalizumab drug product containing drug substance produced at a 2,000 L bioreactor scale at Lonza Biologics) and

** BGNCOM (BG00002-B, natalizumab drug product containing drug substance produced at a 15,000 L bioreactor scale at Biogen Idec),

3. Study 1806 Clinical Material /Commercial Scale Bioequivalence Study

This bioequivalence study was conducted in 86 healthy volunteers. Subjects were randomized in a 1:1 ratio to 1 of 2 sequences of 2 preparations of natalizumab (BGNRSH* and BGNCOM**) and were to receive a 300 mg IV infusion of either preparation, followed 8 weeks later by a 300 mg IV infusion of the other preparation. All subjects were randomized and received at least 1 dose; 66 subjects received both doses.

* BGNRSH (natalizumab clinical material produced by the Biogen 2,000 L bioreactor scale process; also referred to as BG00002-A in other reports within the application) and

** BGNCOM (BG00002-B, natalizumab drug product containing drug substance produced at a 15,000 L bioreactor scale at Biogen Idec),

<u>Study 307</u>

Subjects with moderately to severely active CD and elevated CRP were to be randomized in the study in a 1:1 ratio of natalizumab (n= 260) or placebo (n= 250).

- Adverse events

<u>AEs in pooled placebo-controlled studies of active CD</u>

Overall Incidence

In the placebo-controlled treatment studies of active CD 88% of patients in the natalizumab (811/922) and 88% in the placebo (224/256) groups experienced at least one adverse event.

As would be expected from the underlying illness, gastrointestinal disorders occurred more frequently in the active CD patients compared to the patients in the MS studies. Fatigue, nasopharyngitis, influenza-like illness, back pain was more prevalent in the MS patients; prevalence of headache, pyrexia and pharyngolaryngeal pain in CD and MS patients was similar.

At the level of preferred term, eight events in the natalizumab group, headache, fatigue, dizziness, pharyngolaryngeal pain, pyrexia, influenza-like illness, constipation and cough had incidence rates at least 1.5% higher than those in the placebo group. By comparison, abdominal pain NOS, Crohn's disease, abdominal pain upper and insomnia occurred with at least a 1.5% higher incidence in the placebo group.

Severity

In the placebo-controlled studies of active CD 20% of natalizumab-treated patients and 17% of placebo patients experienced severe events. The most common severe adverse events were Crohn's disease (4% natalizumab *vs* 6% placebo), abdominal pain NOS (3 *vs* 3%), and headache (3 *vs* 3%). All other severe events occurred with an incidence of 1% or less.

Short- and Long-term Dosing in CD
Patients were compared as to the development of AEs over time and divided into 6 groups (1^{st} – 4^{th} infusion, 4^{th} – 7^{th}, 7^{th} -10^{th}, 10^{th} -13^{th}, 13^{th} – 16^{th} and >16^{th}). The pattern and frequency of common AEs in the first time interval correspond to the placebo-controlled treatment studies of active CD. However, of the preferred term level events identified at a 2% higher incidence over placebo, the incidence of headache, fatigue, pharyngolaryngeal pain and cough tended to be 1% higher than in placebo-controlled studies. Most events decreased considerably in incidence over time, pharyngolaryngeal pain and influenza-like illness decreased only marginally (see table 8). Also the percentage of natalizumab treated-patients who experienced severe AEs decreased over time. Most commonly the following AEs were rated severe (% between infusions 1-3): CD 4.9%, headache 2.9%, abdominal pain 3.3%, and fatigue 1%.

AEs in pooled placebo-controlled studies of MS

Overall Incidence
In the placebo-controlled treatment studies of active MS 95% of patients in the natalizumab (1536/1617) and 97% in the placebo (1101/1135) groups experienced at least one adverse event.

Development of AEs in MS group over time

Natalizumab was administered every 4 weeks, so that 12 doses approximately represent one year of treatment. In the analysis the applicant divided the entire time (2 years for the two Phase III studies: 1801 and 1802) into 4x 6-month intervals in order to capture a) initially arising AEs, b) evolving AEs throughout a longer exposure period and the nature of these AEs (e.g. decrease or increase). Currently, the analysis can be regarded as preliminary as the resolution dates were unknown at the time of compilation, and the number of patients who received at least 19 infusions was small compared to those receiving 18 or less and was omitted by the applicant. Thus, the development of AEs over time comprises 18 month data.

Subjects with events that continued beyond one 6-month interval were counted once in the interval during which the event began and once for each interval through which the event persisted. To simplify the comparison the AEs that occurred at a 2% greater rate in the natalizumab group are cited below.

AEs arising within the first 6 months:

The event that was reported by at least 2% more of the natalizumab-treated subjects than those who received placebo and had not been previously identified was nausea.

AEs evolving throughout treatment

The events that were reported by at least 2% more of the natalizumab-treated subjects than those who received placebo and their change of incidence (including increases of at least 2%) over time are presented in the following table:

Table 18: Pivotal Studies in MS: Increases of Incidence of Adverse Events (≥2%) in Natalizumab-treated patients

Preferred term	Infusions 1-6 %	7-12 %	13-18 %	19-24 %
Fatigue	12	14	16	16
Nasopharyngitis	11	15	13	11
Arthralgia	7	8	9	9
Depression	7	9	10	10
Pain in extrimity	6	7	9	7
Asthenia	5	7	6	6

The following events decreased in incidence by approximately a half by the 12- to 18-month interval for natalizumab-treated subjects: headache, MS relapse, nasopharyngitis, nausea, back pain, influenza-

like illness, dizziness, diarrhoea, urinary tract infection, influenza, upper respiratory tract infection, sinusitis, upper respiratory tract infection viral, vomiting, and pyrexia.

Pooled Incidence in Common AEs in placebo-controlled active CD and MS studies

The combination of placebo-controlled treatment studies of active CD and placebo-controlled studies in MS involve 2,539 natalizumab-treated subjects (922 CD subjects and 1,617 MS subjects) and 1,391 who received placebo (256 CD subjects and 1,135 MS subjects). No new signals resulted from the pooling of the MS and CD adverse events data. The incidence of those events occurring in at least 5% of patients was either similar in incidence or higher in the placebo group. Abdominal pain was the only event with a 2% higher incidence in the natalizumab group. The incidence of severe adverse events was 22.1% vs. 23.8% in the natalizumab and placebo group, respectively. The most common events considered to be severe in natalizumab-treated subjects were headache (natalizumab vs placebo: 3.1 vs 2.9%), MS relapse (1.9 vs 6.0%), fatigue (2.0 vs 2.8%), abdominal pain (1.5 vs 0.8%), and Crohn's disease (1.5 vs 1.1%). The incidence of all other events rated severe occurred was less than 1.0%.
The applicant has provided AE data for patients treated up to 39 months. The incidence of all AE (and of serious AE) appears to be declining with increasing treatment duration. This trend is less clear in the infectious AE, in particular in the serious infectious AE which are more scattered although there appears to be peak from the the 7th to 13th infusion. The type of observed AEs give no additional safety concerns..

AEs for study 307

The overall incidence of treatment-emergent adverse events was comparable between subjects in the natalizumab (85%, 222/260) and placebo (82%, 206/250) treatment groups. The most common adverse events, reported by at least 10% of subjects in either treatment group, were headache, nausea, abdominal pain, nasopharyngitis, dizziness, fatigue, and CD exacerbation.
Most adverse events were mild to moderate in severity and the incidence of subjects reported to have severe adverse events was similar between treatment groups (7% natalizumab vs. 10% placebo). Exacerbation of CD (4 natalizumab vs. 11 placebo subjects) was the most commonly reported event assessed as severe by the Investigator; none was considered related to study drug.
The overall incidence of adverse events considered by the Investigator to be related to study drug was higher among subjects in the natalizumab group (27%) than those in the placebo group (20%). The events that led to discontinuation of study drug (9% natalizumab vs. 13% placebo) were similar between treatment groups. Exacerbation of CD was the most common cause of discontinuation of study drug reported in a lower percentage of natalizumab than placebo subjects (3% natalizumab vs. 10% placebo).

- Serious adverse event/deaths/other significant events

The combined population (original dossier) encompassed 2,539 natalizumab-treated patients and 1,391 placebo patients, thereof 362 (14%) and 270 (16%) respectively experienced SAEs. Serious adverse events occurred most commonly in the following system organ classes: nervous system disorders (natalizumab vs placebo: 4 vs 7%), gastrointestinal disorders (5 vs 3%), and infections and infestations (2 vs 2%). Two events contributed significantly to these incidences: Crohn's disease (2 vs 2%) and MS relapse (3 vs 6%). As for the majority of SAEs by preferred term there was only one patient difference (<1%) the pooled SAEs in the placebo-controlled MS and CD studies do not as yet give rise to any new signals.

With reference to newly conducted study 307, the incidence of SAEs was lower for natalizumab-treated (5%) than placebo-treated (10%) subjects; exacerbation of CD was the most commonly reported SAE in both treatment groups (3% natalizumab vs. 6% placebo). No deaths occurred during the study.

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 a4-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

The current safety database with respect to longer term treatment is approximately 1100 patients. The applicant has provided a sufficient analysis of the observed events. Aside from the known PML case there were 3 patients with infections typically associated with a compromised immune system. These were *Mycobacterium avium* pneumonia, bronchopulmonary aspergillosis and *Pneumocystic carinii* pneumonia. All 4 patients had evidence of a compromised immune system. The applicant concludes that the potential for serious infections remains a concern and proposes to assess this risk in future studies and with postmarketing pharmacovigilance. During the application procedure for natalizumab for the MS indication the risk for serious infectious complications, in particular PML was discussed extensively. The risk of PML cannot be accurately estimated and the possible co-factors that might contribute to clinical disease are currently unknown. There is no advanced knowledge in this respect and the concerns remain.

- 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 hemoglobin 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

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 labeling. 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.

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 Tysabri. Herpes encephalitis is the most common cause of sporadic viral encephalitis in the USA and typically occurs in immune-competent individuals. There were no cases of encephalitis and there was no safety signal when evaluating CNS herpetic infections in the integrated natalizumab clinical trial safety database. Further to already presented analyses, the applicant reviewed all SAEs reported since the suspension of dosing (28th of Feb 05), up to 30 June 2006, with respect to opportunistic/atypical infections .In total 12 serious opportunistic and atypical infections (excluding PML) were noted from more than

3000 subjects evaluated in the CD and MS clinical trial programs. Of the 12 serious infections, 5 were cases of opportunistic infections, 4 in subjects with CD and one in a subject with MS. The remaining seven cases were serious atypical infections, 6 in subjects with CD and one in a subject with MS. Additional two non-serious cases of CMV hepatitis were noted in subjects with Crohn's disease receiving natalizumab. From the presented data is appears that the risk is highest in the CD population, probably not surprising considering the concomitant therapy used in many patients. It also emphasizes the risk that is inherent to therapy with natalizumab.

- Discussion on clinical safety

In general the incidence of commonly occurring adverse events was balanced between the verum and placebo groups. However, the conclusions can only be preliminary due to the lack of investigation of the relationship of AEs to the study drug. The following common adverse events (preferred terms) were identified in both the placebo-controlled CD and MS studies: headache, fatigue, nasopharyngitis, arthralgia, nausea, and dizziness.

Common adverse events occurring at a 2% higher incidence in natalizumab-treated CD patients over placebo were headache, pharyngolaryngeal pain, influenza-like illness, fatigue, pyrexia, constipation and cough. Likewise, depression, influenza, pharyngitis, and rigors occurred at a 2% higher incidence in the natalizumab-treated MS patients over placebo.

In the pooled analysis of the placebo-controlled CD and MS studies, events more prevalent in the natalizumab-treated subjects are nausea, abdominal pain, vomiting NOS, and pyrexia.

The events most often considered related to study drug were headache, nausea, fatigue, dizziness, influenza-like illness, nasopharyngitis, arthralgia, diarrhoea NOS, vomiting NOS, and pyrexia. With the exception of headache, each of these events was within 1% of the incidence in those who received placebo.

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. This trend is less clear in the infectious AE, in particular in the serious infectious AE which are more scattered although there appears to be peak from the the 7th to 13th infusion. Assessment of the response dossier did not reveal any new safety signal.

2.5 Pharmacovigilance

Detailed description of the Pharmacovigilance system

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

Risk Management Plan

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

The CHMP, having considered the data submitted in the application was of the opinion that the proposed risk minimisation activities were not able to reduce the risks to an acceptable level.

2.6 Overall conclusions, risk/benefit assessment and recommendation

Quality

The quality of this product is considered to be acceptable. 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.

Non-clinical pharmacology and toxicology

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.
Natalizumab is immunogenic in almost all animals within 2-4 weeks depending on the administered dose.

Efficacy

The CHMP considered clinical efficacy not sufficiently proven and the efficacy database not sufficient to unequivocally demonstrate efficacy of natalizumab in Crohn's Disease (CD). The deficiencies pertained to the fact that the primary analysis of the induction study CD301 did not reveal a statistically significant nor clinically relevant effect of natalizumab and that the re-defined efficacy population for the subsequent maintenance study CD303 was not justified since no significant effect of natalizumab was demonstrated in CD301. Subgroup analysis of study CD301, based on a post-hoc calculation of efficacy in a patient population with elevated C-reactive protein (CRP, a marker for ongoing inflammation) was not considered acceptable as proof of efficacy. Using the outcomes from study CD301 and exploratory analyses, the applicant has meanwhile performed a completely new study for induction therapy (CD307). This study confirms the findings of the post-hoc analysis of study CD301, however, the effect size indicates clinically relevant although not outstanding efficacy for the induction of remission.

Lack of prospective data in maintenance treatment in the intended indication

The population studied in CD303, the maintenance study that had already been part of the initial application, is not the population that will be eligible for treatment. The applicant did not perform a maintenance study subsequently to the newly performed induction therapy study CD307. Therefore, there are only results from one single pivotal trial for maintenance therapy of CD, which have been analysed according to the new indication claimed post-hoc. Due to the fact that CD301 (which enrolled patients to be later eligible for enrolment in study CD303) did not enrol patients based on the intended indication claim, the subsequent patient population in the only pivotal trial for maintenance treatment is not representative. This is not considered sufficient as proof of efficacy, as results are not outstandingly efficacious. Applicability of the results to the intended population has to be demonstrated prospectively. The fact remains that data on the true population for which treatment is envisaged are sparse, and a confirmatory trial is considered necessary

Safety

Reduced lymphocyte surveillance as induced by α4-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. Based on these considerations and a considerable efficacy in Relapsing Remitting Multiple Sclerosis (RRMS), the product Tysabri, containing natalizumab, was given a marketing authorisation, but for a highly restricted patient population. However, unlike patients with RRMS, patients with CD are commonly immunosuppressed, also often in case of formal "wash-out" of previous treatments. This makes treatment with natalizumab highly problematic as regards the possible occurrence of severe opportunistic infections including PML.

Regarding opportunistic infections other than PML, there is an elevated risk, for instance pulmonary infections were more common in the CD patients treated with natalizumab, and organisms were isolated that are predominantly seen in immunosuppressed individuals.

In order to reduce the risk for opportunistic infections further, the applicant proposed to restrict the indication to patients who are not on immunosuppressants and are not considered to be immunosuppressed by clinical judgement. But currently it is unknown whether this strategy will be successful in reducing the incidence of opportunistic infections.

- User consultation

A readability test (technical readability/traceability/comprehensibility/applicability) including scoring has been performed on the English version of the Patient Information Leaflet (PIL) and from the results it can be concluded that the relevant information is accessible and understandable for the user.

Risk-benefit assessment

The analysis of the safety data submitted for natalizumab in the treatment of moderately to severely active Crohn's disease for induction and maintenance of sustained response and remission showed the same pattern of adverse events as already revealed in the MS application. There is, however, a need to further evaluate the occurrence of pneumonias. Natalizumab is an entirely new concept in the treatment of CD patients and the safety profile of the product needs further characterization with particular focus on long-term risks of malignancy and infections.

Since the efficacy has not been shown, the rationale of the product has not been justified, prospective confirmative data in the refractory population are not available, and taking into account the other available therapeutic options, the risk-benefit ratio for natalizumab in both the applied and revised (restricted) indication is at present considered negative.

A risk management plan was submitted, including a risk minimisation plan.

The CHMP, having considered the data submitted, was of the opinion that:

- the proposed risk minimisation activities were not able to reduce the risks to an acceptable level

Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered by consensus that the risk-benefit balance of natalizumab in the treatment of severe, active Crohn's disease, in patients who have:

- had an inadequate response to conventional therapy including a TNF-α inhibitor, or who are intolerant to or have medical contraindications to such therapies.

- evidence of active inflammation defined by raised serum levels of C - reactive protein.

Was unfavourable and therefore did not recommend the granting of the marketing authorisation.

The CHMP stated the following grounds for refusal:

- Remission induction therapy in the restricted population (C-reactive protein elevated, anti-TNF-α failure) is not conclusively demonstrated; in subgroup analysis of the different studies the impact of prior anti-TNF failure is not homogenous
- Evidence of efficacy appears modest and is considered insufficient. Furthermore, there is insufficient evidence of maintenance of efficacy.
- For Crohn's disease patients there is considerable risk of development of serious opportunistic infections including Progressive Multifocal Leukoencephalopathy (PML); the risk of long-term maintenance therapy cannot be deduced from presently available data (particularly in these patients where the majority would have received previous immunosuppressive therapy).

- Risk factors for the development of PML and other opportunistic infections have not yet been defined, and the concerns remain; it is unknown whether restriction to patients not considered immuno-suppressed on clinical judgment is sufficient to reduce the risk of opportunistic infections.

3 RE-EXAMINATION OF THE CHMP OPINION OF 19 JULY 2007

Following the CHMP conclusion that the risk/benefit balance of natalizumab in the treatment of patients with severe, active Crohn's disease was unfavourable, the applicant submitted detailed grounds for the re-examination of the grounds for refusal.

The applicant presented a number of arguments regarding the grounds for refusal and also commented upon the proposed risk minimization activities:

According to the applicant, proposed risk minimization activities for Crohn's disease are based on those shown to be successful for MS with additional elements to account for potential differences in the risk between the MS and Crohn's disease populations. The applicant has provided information indicating that more than 11,500 MS patients (May 2007; over 3000 in Europe) initiated therapy with natalizumab since its launch in June 2006 and that the methods used in the EU to minimize risk have resulted in the appropriate patients using natalizumab as monotherapy and a safety profile similar to that observed in clinical trials. As a result, the applicant concludes that the proposed risk minimization plan for Crohn's disease is sufficient to control the risks of natalizumab to an acceptable level in this patient population.

In line with assessor's comments received during re-examination, the following *restricted indication* was proposed:

"Natalizumab Elan Pharma is indicated for the treatment of severe, active Crohn's disease, in patients who have:

- Had an inadequate response to steroids, an immunosuppressant and a TNF-α inhibitor, or who are intolerant to such therapies.

- Evidence of active inflammation, e.g. defined by by C - reactive protein above the upper limit of normal

Natalizumab Elan Pharma can be used alone or in combination with aminosalicylicate products or antibiotics. Brief courses of corticosteroids can be used with natalizumab to treat acute exacerbations of Crohn's disease."

Amongst the list of stated *contraindications*, the following measures were agreed upon:

"Immunocompromised patients with increased risk of opportunistic infections

Concomitant use of immunosuppressive or immunomodulatory medications. Immunosuppressants must be discontinued when embarking on natalizumab and corticosteroids tapered."

Ground #1

#1a Remission induction therapy in the restricted population (C-reactive protein elevated, anti-TNF-α failure) is not conclusively demonstrated;

The applicant emphasises that response in the "restricted population", refractory to other therapies, is of heightened clinical importance due to the limited options available. These alternative options

themselves carry considerable risks, which, in the opinion of the inflammatory bowel disease (IBD) experts consulted by the applicant, pose a potentially greater risk than natalizumab.

Evaluation of natalizumab efficacy was carried out past week 8, as specified in the CHMP's guidelines (Ref. CPMP/EWP/2284/99 Rev. 1) and primary non-responders to infliximab were not excluded from the evaluation unlike assessments performed with other biologics. Results are comparable with those of adalimumab for the treatment of patients who have lost response or were intolerant to infliximab, although evaluations were only carried out to Week 4 in the trial with adalimumab.

The applicant states that it is notoriously hard to bring such a refractory population into remission (a secondary endpoint for the induction studies). However, a statistically significant difference, compared to placebo, was also demonstrated for remission in the "restricted population" at the primary time-point of weeks 8 and 12 (table 20 – shown in the response to issue 2a).

#1b In subgroup analysis of the different studies the impact of prior anti-TNF failure is not homogenous

A favourable effect for both response and remission with natalizumab treatment was seen in both studies, CD301 and CD307. Although it is unclear why there is a difference in remission rates for the CD301 and CD307 placebo groups, such differences across clinical trials are not unique to natalizumab and can reflect differences in inclusion criteria, in the application of such criteria by physicians in different studies, in assessment procedures, or other unidentified factors. Importantly consistency in the direction of treatment effect has been demonstrated in both induction studies.

CHMP position

The CHMP considers study CD301 as a failed study. In that situation any subsequent subgroup analysis has to be interpreted with great caution. It is well-known that immunosupressants have a better efficacy in patients with elevated C-reactive protein (CRP), but the CHMP is not in favour of restricting the indication to such patients as even severely affected Crohn's patients with ongoing inflammation as evidenced by endoscopy, may have non-elevated CRP.

Ground #2

#2a Evidence of efficacy appears modest and is considered insufficient.

The applicant states that following review of the Day 180 Assessment Report the indication statement has been restricted to clarify that natalizumab should only be used for patients who have had an inadequate response to conventional therapies, including a TNF-α inhibitor (SPC). This population, with severe refractory disease, was evaluated following safety concerns, to demonstrate that natalizumab addresses a high unmet need in CD. In this population (anti TNF-α failure, elevated CRP) the evidence shows an important and significant clinical difference. Patients refractory to other therapies have limited options, all of which pose considerable risk. According to the applicant, clinical experts in gastroenterology support this opinion.

Given that efficacy of natalizumab has been demonstrated in the overall ITT population, analysis in biologically plausible sub-populations, with greatest medical need, is statistically sound. In particular, analyses of interactions appear to generally support the idea that the treatment effect observed on the subgroups and the overall patient population is consistent.

Table 19 and table 20 below show the proportion of CD307 subjects in the "restricted population" who achieved response and remission, respectively. Significant and clinically meaningful treatment differences were observed at the primary time point of both Weeks 8 and 12.

Induction data for patients in CD307 who failed prior treatment with a TNF-α inhibitor are compared with the overall (ITT) population data for response and remission in figure 1 and figure 2 respectively. The absolute response and remission rates were lower for both the natalizumab and placebo-treated

patients in the TNF-α Inhibitor Failure Population compared with the ITT Population. This finding is consistent with the hypothesis that such patients are more severe and less responsive to therapy and with findings observed with other biologics evaluating this refractory population. The magnitude of treatment effect however, with natalizumab, was at least as great as that of the ITT Population. It was remarked that patients in the "restricted population" are unlikely to enter spontaneous response or remission, as signified by the low placebo response rates seen in this population.

Table 19: CD307: Proportion of Subjects with a Clinical Response (Subjects with Prior TNF-α Inhibitor Failure)

Visit	Placebo (n= 83) N (%)	Natalizumab (n= 89) N (%)	Odds Ratio (a)	95% CI of Odds Ratio (a)	p-value (a)
Week 4	28 (33.7)	39 (43.8)	1.523	(0.816, 2.840)	0.186
Week 8	18 (21.7)	46 (51.7)	3.885	(1.985, 7.601)	<0.001
Week 12	24 (28.9)	44 (49.4)	2.415	(1.282, 4.550)	0.006
Weeks 4 & 8	13 (15.7)	32 (36.0)	3.022	(1.445, 6.322)	0.003
Weeks 8 & 12	12 (14.5)	34 (38.2)	3.662	(1.733, 7.737)	<0.001
Any Time (b)	38 (45.8)	61 (68.5)	2.605	(1.389, 4.884)	0.003

Note 1: Subjects were classified as treatment failures at any visit after rescue, early withdrawal, or discontinuing use of study drug due to adverse event. If CDAI score was missing for a given time point, the subject was considered a treatment failure for that time point.
2: Response at Weeks 8 & 12 is the primary endpoint.
3: Subjects who left the question 'Has the subject ever taken an anti-TNF agent?' blank on the CRF were excluded.
(a) The adjusted odds ratio, 95% CI, and p-value are from logistic regression adjusting for disease severity (CDAI < 330 vs >= 330) at baseline.
(b) Any time through Week 12.

Table 20: CD307: Proportion of Subjects in Clinical Remission (Subjects with Prior TNF-α Inhibitor Failure)

Visit	Placebo (n= 83) N (%)	Natalizumab (n= 89) N (%)	Odds Ratio (a)	95% CI of Odds Ratio (a)	p-value (a)
Week 4	9 (10.8)	12 (13.5)	1.346	(0.525, 3.452)	0.536
Week 8	7 (8.4)	19 (21.3)	3.065	(1.204, 7.802)	0.019
Week 12	11 (13.3)	20 (22.5)	2.023	(0.885, 4.622)	0.095
Weeks 4 & 8	3 (3.6)	9 (10.1)	3.093	(0.803, 11.916)	0.101
Weeks 8 & 12	4 (4.8)	15 (16.9)	4.474	(1.387, 14.435)	0.012
Any Time (b)	18 (21.7)	27 (30.3)	1.652	(0.814, 3.351)	0.165

Note 1: Subjects were classified as treatment failures at any visit after rescue, early withdrawal, or discontinuing use of study drug due to adverse event. If CDAI score was missing for a given time point, the subject was considered a treatment failure for that time point.
2: Subjects who left the question 'Has the subject ever taken an anti-TNF agent?' blank on the CRF were excluded.
(a) The adjusted odds ratio, 95% CI, and p-value are from logistic regression adjusting for disease severity (CDAI < 330 vs >= 330) at baseline.

(b) Any time through Week 12.

Figure 5: CD307: Response at Both Weeks 8 and 12 in the ITT Population and in Patients who Failed Prior Treatment with a TNF-α Inhibitor

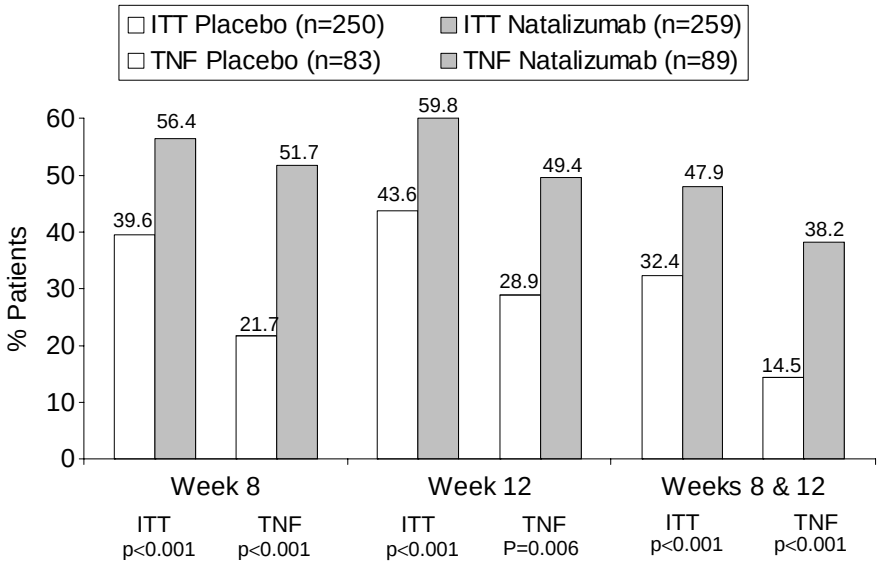
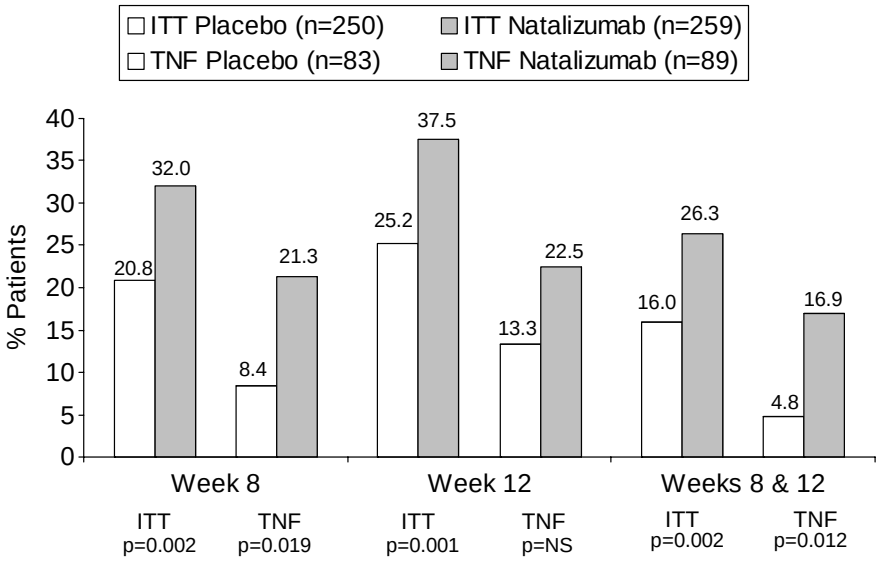


Figure 6 CD307: Remission at Both Weeks 8 and 12 in the ITT Population and in Patients who Failed Prior Treatment with a TNF-α Inhibitor



With respect to one's ability to draw conclusions from a sub-group analysis, it is important to recall that 348 patients were evaluated in the "restricted population" (TNF failure, elevated CRP) for induction (CD301 and CD307). According to the applicant, the data is sufficient to demonstrate both a clinically meaningful and statistically significant benefit in this population.

The applicant concludes that for a generally young patient population who face hospitalization, surgery with possible bowel resection and a potential stoma for their remaining years, it is considered that the evidence of efficacy shows clinically important and very meaningful treatment differences and the applicant disagrees that the findings are modest. As per the indication statements in the SPC for each TNF-α inhibitor, all patients prescribed an anti TNF-α inhibitor are patients with severe disease.

Natalizumab has therefore demonstrated it has an important role to play in the treatment of severe, active disease for patients with limited options.

#2b Furthermore, there is insufficient evidence of maintenance of efficacy.

Study CD303 recruited patients from induction study CD301. All patients were required to be in response at weeks 10 and 12 and have a CDAI score ≤220 at both these visits. The applicant stated that this is a more stringent definition than that used for the primary endpoint of response at week 10 only for CD301 (i.e. a patient in response at week 10 of CD301 may not necessarily be eligible to enter CD303 if s/he did not fulfil the more stringent CD303 entry criteria). The applicant does not agree that findings in CD303 are not justified based on the results of CD301. It was emphasised that even though CD301 did not enrol patients for CD303 based on the claimed indication, analyses of CD303 data using the subgroup of patients who failed TNF-α provided strong evidence of maintenance of response and remission in this TNF-F population. Again, efficacy analysis of the ITT population was statistically significant and it is only due to safety considerations that the subgroup analysis was highlighted.

The applicant states that maintenance of efficacy was demonstrated with natalizumab in the pre-specified ITT population of CD303, with all primary and secondary endpoints being met. In addition, maintenance efficacy was consistently demonstrated in CD303 sub-populations.

Based on the 2001 guideline for CD (Points to consider document CPMP/EWP/2284/99), the applicant did not consider performing a repeat long-term, placebo-controlled maintenance study following the second, 3-month CD307 induction study due to the compelling evidence from the previously completed long-term, placebo-controlled maintenance CD303 study. CD307 patients were therefore enrolled in open-label study CD351 rather than undergoing re-randomisation to placebo in another long-term, placebo-controlled study.

The applicant re-iterates that the 12-month maintenance study CD303 met all primary & secondary endpoints in a pre-defined overall population with robust results. CD303 was adequately powered to demonstrate maintenance of response for a further six months' treatment (i.e., through to Month 9; primary endpoint) and maintenance of remission through to Month 9 (co-primary endpoint). The requirement for a sustained maintenance of response or remission, respectively, at each time point in this natalizumab study was more stringent than other maintenance studies that have required maintenance only at a specified time point.

An analysis of the specific population of patients with elevated CRP at the start of natalizumab therapy in CD301 (i.e., the population that corresponds to the CD307 ITT population) also demonstrated sustained maintenance of response and remission through to Month 15 of CD303 ($p<0.001$ in both cases).

According to the applicant, the requirements of a pivotal maintenance study were fulfilled with CD303 and the maintenance data compare favourably with other biological therapies, which were approved on the basis of one pivotal maintenance study.

The applicant states that evaluating clinically relevant subgroups from a successful pre-specified overall analysis is sound from the perspective of clinical trial methodology. In total 72 patients entered study CD303 who had previously failed therapy with an inhibitor of TNF-α. Significant maintenance of response ($p<0.001$) was shown at the CD303 primary time-point, Month 9, for this population, consistent with the significant findings demonstrated in the ITT population. The subgroup-analysis was not an exploratory attempt to highlight positive data, but was motivated by safety issues emerging after study completion and the desire to restrict treatment to those patients with the highest unmet medical need (i.e., patients who have failed all conventional therapy including a TNF-α inhibitor).

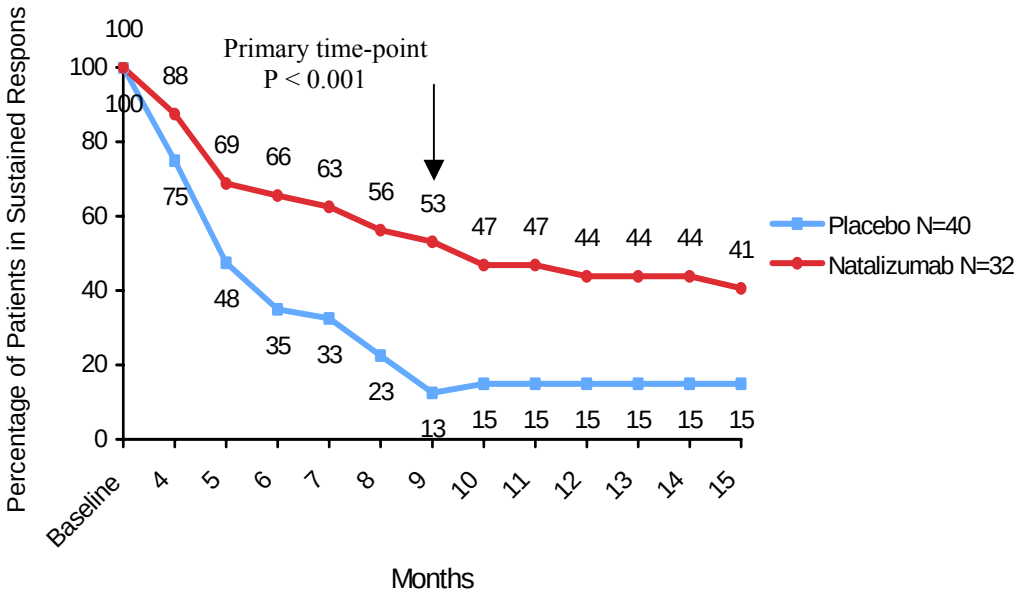
The *primary* outcome for CD303 was the proportion of patients who maintained response at every monthly visit up to and including the CD303 Month 9 visit. This analysis demonstrates that more than twice the proportion of subjects receiving natalizumab, compared with placebo, maintained clinical

response (103/168 [61%] *vs.* 48/170 [28%]; p<0.001). In addition, the proportion of natalizumab-treated subjects who maintained response at all time points through to Month 15 was also more than twice that of placebo subjects (90/168 [54%] *vs.* 34/170 [20%]; p<0.001).

The proportion of natalizumab-treated subjects who maintained clinical remission at every visit through to Month 9 was 44% for natalizumab *vs* 26% for placebo (p=<0.05). More than twice the proportion of natalizumab-treated subjects compared with placebo subjects maintained remission at every visit through to Month 15, 39% *vs* 15%; p<0.001)

In the *anti-TNF failure population*, 53% of patients maintained a sustained response through to Month 9 vs 13% of patients receiving placebo, an absolute difference of 40% (p<0.001), figure 7;

Figure 7: CD303: Proportion of prior anti–TNF α failure population who maintained a response at every assessment through to Month 15



It is worthy of note that concomitant use, or not, of immunosuppressants at baseline had no effect on maintenance efficacy in either the efficacy population or the TNF-α inhibitor failures subgroup.

The applicant concludes that maintenance efficacy has been demonstrated in the primary efficacy population and all subgroups. These findings were consistent, statistically and clinically significant. According to the applicant, evaluating additional numbers of patients for maintenance would be unlikely to alter this conclusion.

CHMP position

The clinical efficacy of natalizumab is based in three studies, two of them as induction therapy (study CD301 and CD307) and one as maintenance therapy (study CD303).

Study CD301 failed to reach the primary endpoint (clinical response) and the contingent primary endpoint (clinical remission) at week 10. The differences between active treatment and placebo were 7.8% for clinical response and 6.5% for clinical remission. In an additional post hoc analysis the applicant identified retrospectively a group of "refractory patients" characterized by an elevated CRP levels (> 2.87 mg/L) and at least one concomitant immunosuppressant, at baseline. In this population Natalizumab was significantly more effective in clinical response (difference=25%) and clinical remission (difference=23%). However, the results in patients with high CRP levels have mainly to be regarded as hypothesis generating only. This new hypothesis was confirmed in study CD307.

Study CD307 demonstrated that natalizumab treatment induces response and remission in a significantly larger proportion of patients than placebo. Efficacy is maintained across the studied population. Sub-group analyses have demonstrated that efficacy is maintained in numerous clinically relevant subsets of patients including patients having previously failed TNF-α inhibitors, patients being intolerant to or having failed immunosuppressants and patients without baseline immunosuppressants However, the number of patients representing the target population is deemed as very low and the follow up limited.

Study CD303 was designed as an add-on study of CD301, to demonstrate that natalizumab was effective to maintain clinical response and remission. Eligible to enter the maintenance study were only patients who responded to treatment (natalizumab or placebo) in study CD301. Primary endpoint (maintained clinical response) and contingent primary endpoint (maintained clinical remission) were reached in a statistically and clinical manner, in each of the visits of the study during 9 months. The differences for maintained clinical remission and response were 33% and 18% respectively for natalizumab treated patients. The overall CD303 population is **not** the population that would be eligible according to the claimed indication; another post-hoc analysis was performed in the target population (a total of 72 patients treated with placebo and active) obtaining similar results.

In conclusion, the CHMP is of the opinion that limited efficacy has been demonstrated with regard to induction of response/remission but that efficacy with regard to maintenance of response/remission has been insufficiently demonstrated even in the proposed limited population of patients failing corticosteroids and immunosupressants and prior TNF-alpha inhibitor therapy.

Ground #3

#3a There is considerable risk of development of serious opportunistic infections including Progressive Multifocal Leukoencephalopathy (PML).

The applicant states that natalizumab is associated with a low risk of opportunistic infections (OI's), including PML. This risk is offset by a need for therapy in patients who have exhausted all other medical therapies prior to consideration of natalizumab. The applicant will put in place, as has been done for MS, a series of measures that are aimed at informing patients of the current risk and establishing an ongoing educational programme to reduce that risk. In addition, natalizumab therapy will be limited to those patients with the greatest need i.e., those who have failed prior treatment with a TNF-α inhibitor, and will be recommended to be continued beyond 3 months only in those demonstrating benefit of therapy.

Based on the available clinical data, to date there have been 3 cases of PML in almost 4000 patients exposed to the medicinal product in clinical studies, and over 3000 that were evaluated as part of the post-suspension Safety Evaluation. The best current estimate of the risk of PML is approximately 1:1000 with 95% confidence intervals ranging from 0.2 to 2.8 per 1000 (1 per 5000 to 1 per 360 patients exposed). The 2 MS cases in which PML occurred had received 28 and 37 consecutive monthly infusions of natalizumab in combination with interferon beta-1a while the single CD case, an individual with long-standing cytopenia following chronic azathioprine use, had received 8 infusions over 18 months. This rate is low, however the seriousness of the event renders even a low incidence of concern. The proposed indication statement would however exclude patients similar to those who previously developed PML while on natalizumab. Since the re-introduction of natalizumab in the USA and initial lunch in Europe, over 11,500 patients have been exposed to natalizumab with no further cases of PML reported through to May 2007.

#3b The risk of long-term maintenance therapy cannot be deduced from presently available data (particularly in these patients where the majority would have received previous immunosuppressive therapy).

The applicant states that there does not appear to be a substantial increased risk of serious infections or OI's, based on whether or not patients had previously received immunosuppressant therapy, including

a TNF-α inhibitor, or were receiving short-term concomitant administration of natalizumab with these products. However, as a further precaution, the applicant has proposed to contraindicate the use of concomitant immunosuppressive medicinal products (which includes TNF-α inhibitors) with natalizumab (SmPC).

Similarly, there does not appear to be a substantial increased risk of serious infections or OI's for patients receiving short-term concomitant administration of natalizumab with corticosteroids. However, the applicant also recommends (SPC) that patients who are receiving steroid therapy at the time of starting natalizumab should commence tapering the steroid dose once a response to natalizumab is achieved. These patients should discontinue the use of steroids within 6 months of starting natalizumab therapy in order to reduce the risk of infections, including OI's. Patients unable to discontinue steroid therapy should discontinue use of natalizumab. Based on the time to response with natalizumab, many patients will be able to commence steroid withdrawal within 4 weeks of their first infusion of natalizumab and data from CD303 indicates that approximately two-thirds of such patients can discontinue steroids within 10 weeks of starting the steroid taper. Thus, many patients may be off steroids within 14 weeks of initiating natalizumab, but a proportion may require longer.

Although the long-term risk of OI's, including PML, cannot be ascertained with certainty currently, steps have been taken to reduce this risk and the ongoing MS risk assessment program will provide information on the risk of monotherapy, in large numbers of patients, well in advance of similar exposure in CD patients.

The recommendation that natalizumab therapy be discontinued in patients who do not achieve a response within three months of the start of therapy, limits the long-term risk only to those with demonstrated benefit. This recommendation, together with the contraindications described above result in a proposed SPC that clearly defines and limits the CD patients who are eligible for treatment with natalizumab.

Ground #4

#4a Risk factors for the development of PML and other opportunistic infections have not yet been defined, and the concerns remain

The applicant states that the main risk factor for OI's, including PML, is immune suppression. Other potential risk factors in those receiving natalizumab, are unknown, but are being investigated in pre-clinical studies as well as in the TOUCH program, the TYGRIS observational study in MS and will also be done in CD-TOUCH in the USA and an observation study in CD patients. In addition to trying to identify further risk factors, the applicant has introduced contraindications to exclude use in patients with evidence of immunocompromise, and in those using immunosuppressive medicinal products concurrently (SPC). Restricting use of natalizumab to that of monotherapy would reduce the utilisation of concomitant immunosuppressive drugs and would likely reduce the overall rate of OI's.

#4b It is unknown whether restriction to patients not considered immuno-suppressed on clinical judgment is sufficient to reduce the risk of opportunistic infections

The applicant states that there is no evidence to support a higher risk of PML in patients with Crohn's disease than those suffering with MS. The CHMP previously evaluated the risk of PML, with natalizumab, when approval was granted in June 2006 for MS. Since that time >11,500 patients have been exposed and no further cases of PML have been identified. Although the duration of exposure is just under 1 year for those on therapy the longest, the lack of further cases in MS when natalizumab is used as monotherapy is encouraging, suggesting that measures implemented to reduce risk, may be effective in achieving this goal. The measures implemented for MS (and planned for CD), including extensive education of practitioners on risk factors, and restrictions in use (SPC) are likely to reduce the risk of OI's in patients receiving natalizumab to an acceptable level, given the benefit in the restricted population.

CHMP position on grounds #3 and #4

The primary safety concern associated with natalizumab are infections, in particular opportunistic infections, including Progressive Multifocal Leukoencephalopathy (PML). For MS, the proposed risk minimization activities have been evaluated by the CHMP and found to be adequate to reduce the risk to an acceptable level compared to the demonstrated efficacy. As Crohn's disease patients, due to their higher rates of previous or current exposure to prednisolone and/or immunosuppressants, generally have a higher risk of being immunocompromised compared to MS and as immunosuppression is believed to be the main risk factor for PML, it is not evident that similar risk minimization activities will be sufficient in Crohn's disease. In order to account for this, the applicant has proposed additional risk minimization activities. These primarily include an attempt to lower the risk of immunosuppression. The applicant proposes to contraindicate treatment in immunocompromised patients and in patients concomitantly using immunosuppressive or immunomodulatory medications. Furthermore, it is required that systemic glucocorticoids should be discontinued within six months. It is accepted that by virtually eliminating immunocompromised patients from the target population, the risk of PML and other opportunistic infections might theoretically be lowered to levels compatible with a positive benefit/risk conclusion.

However, the CHMP expressed residual concerns about the practical value of the risk minimization measures proposed by the applicant

- Most if not all patients with Crohn's disease fulfilling the criteria established by the proposed indication will be immunosuppressed to a greater or lower extent. A contraindication of immunosuppressed patients as a risk minimization measure is felt to be an issue which is difficult to apply in clinical practice and could pose a real challenge to patients and prescribers alike.

- Immunosuppression in these patients is greatly derived from established therapies for Crohn's disease. Recovering of the immune system from a failing therapy course (before a patient can be regarded as candidate for natalizumab therapy) would take some time, during which the disease may flare up again precipitating an urgent need for re-starting a new treatment course.

- Gold criteria are proposed as a tool for defining which patients may be at increased risk. However, no consensus currently exists on the right criteria defining the population (in terms of immune status) which would be candidate for natalizumab therapy. The Gold criteria require further validation before they can be accepted as useful.

- White blood cells (WBC) count is proposed as one of the criteria to be used for defining immunocompromised patients. Even accepting that this could be a baseline marker suggesting immunosuppression, its monitoring value while on natalizumab therapy is questionable since natalizumab therapy on itself affects WBC count.

One additional risk management measure which is proposed is a close neurological monitoring of patients in order to stop natalizumab therapy when the very first suspicion of PML appears. It is however questionable whether stopping natalizumab therapy may have a relevant impact on the clinical evolution of PML.

In conclusion, the CHMP is of the opinion that the proposed measures including Risk Management Plan (RMP) will not be able to limit the safety-concerns to such an extent that the risk of the drug is outweighed by its modest efficacy

Overall conclusion on grounds for re-examination

Efficacy aspects

The CHMP considered the clinical efficacy not sufficiently proven and the efficacy database not sufficient to unequivocally demonstrate efficacy of natalizumab in Crohn's Disease.

The CHMP agreed that the efficacy of natalizumab in the induction of remission of Crohn's disease in the claimed restricted target population has been shown. According to the experts, this is a clinically meaningful effect. However, it remains doubtful if the efficacy data of natalizumab as maintenance therapy in patients with Crohn's disease can be regarded as predictive of a clinically relevant effect in the final proposed target population.

Safety aspects

The applicant proposes a number of risk management measures aimed to reduce the risk of opportunistic infections (especially PML). However, it remains doubtful that the proposed risk minimization measures will effectively reduce that risk.

Benefit/risk assessment

The CHMP acknowledges that patients with severe Crohn's disease failing prior therapy with corticosteroids, immunosupressants as well as TNF-alpha inhibitor therapy is a population in need for new medical therapies. To this end, natalizumab has demonstrated an effect with regard to induction of remission, but not with respect to maintenance of response/remission. Moreover, the CHMP is concerned with the safety of natalizumab in patients with Crohn's disease and do not find that the safety measures including risk management strategies proposed by the applicant will be sufficient to reduce the risk to such an extent that the risk will be outweighed by the limited efficacy. Therefore the benefit/risk ratio, even for the proposed restricted population, is considered negative.

CHMP conclusion on benefit/risk

Having considered the grounds for the re-examination from the applicant, the discussion during the Ad-Hoc Expert Group meeting and the CHMP members' discussion during the oral explanation, the CHMP is of the opinion that the benefit/risk for Natalizumab Elan Pharma in the claimed indication remains negative.

Grounds for refusal

- The applicant has not convincingly demonstrated efficacy with regard to maintenance of remission in the proposed restricted population failing prior therapy with corticosteroids, immunosuppression and TNF-alpha inhibitor therapy.

- The proposed risk management measures proposed by the applicant are considered insufficient to reduce the risk to an acceptable level.