20 July 2023
EMA/359152/2023
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Tyruko

International non-proprietary name: natalizumab

Procedure No. EMEA/H/C/005752/0000

Note

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

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List of abbreviations

ADA	Anti-Drug Antibodies
(TE)(S)AE	(Treatment-emergent)(Serious) Adverse Event
AESIs	Adverse Events of Special Interest
ALT	Alanine Aminotransferase
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
ARR	Annualized Relapse Rate
AST	Aspartate Aminotransferase
$AUC_{0\text{-last}}$	Area under the concentration-time curve from zero to last timepoint
$AUC_{0\text{-inf}}$	Area under the concentration-time curve from zero to infinity
$AUC_{0\text{-12w}}$	Area under the effect time curve from time zero to 12 weeks
$AUEC_{base\_neg}$	Area below the individual baseline value minus the area above the individual baseline in case of time intervals for which the effect time curve exceeded the baseline value
$AUEC_{0\text{-12w}}$	Area under the effect curve from zero to 12 weeks
$AUEC_{4\text{-12w}}$	Area under the effect curve from four to 12 weeks
$AUEC_{0\text{-t}}$	Area under the effect curve from zero to last timepoint
BBB	Blood-brain barrier
BMI	Body mass index
CI	Confidence interval
CL	Clearance
C_{max}	Maximum Concentration
C_{min}	Minimum Concentration
CNS	Central Nervous System
COVID-19	Coronavirus disease 2019
CRO	Contract Research Organization
CS-1	Connecting segment-1
CSR	Clinical Study Report
CSP	Clinical Study Protocol
CTMS	Clinical Trial Management System
CV	Arithmetic Coefficient of variation
gCV	Geometric Coefficient of variation
DSMB	Data and Safety Monitoring Board

ECG	Electrocardiogram
ECLIA	Electrochemiluminescence immunoassay
EDC	Electronic Data Capture
ELISA	Enzyme Linked Immunosorbent Assay
EMA	European Medicines Agency
E_{max}	Maximum effect
E_{min}	Minimum effect
EDSS	Expanded Disability Status Scale
Fab	Fragment antigen-binding
FDA	Food and Drug Administration
FAS	Full Analysis Set
GdE	Gadolinium-Enhancing
IgG	Immunoglobulin G
ICF	Inform Consent Form
IHC	International Council for Harmonisation
IV	Intravenous
JCV	John Cunningham virus
k_{el}	Terminal elimination rate constant
LDL	Low Density Lipoprotein
LLOQ	Lower Limit of Quantification
LS(M)	Least Square (Mean)
MedDRA	Medical Dictionary for Regulatory Activities
MRI	Magnetic Resonance Imaging
MS	Multiple Sclerosis
(s)MAdCAM-1	(soluble) Mucosal addressin cell adhesion molecule-1
NNP	Negative Predictive Value
QC	Quality control
PD	Pharmacodynamics
PK	Pharmacokinetic
PML	Progressive multifocal leukoencephalopathy
PP	Per Protocol
PPV	Positive Predictive Value
PT	Preferred Term

RRMS	Relapsing Remitting MS
(%)RS	(Relative) Receptor saturation
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SAF	Safety Population
SD	Standard Deviation
SE	Standard Error
SDTM	Study Data Tabulation Model
SOC	System Organ Class
SSW	Safety-Switch Population
TFL	Tables, Figures and Listings
$T_{max, E}$	time to E_{max}
t_{max}	Time to C_{max}
t_{min}	Time to C_{min}
$t_{1/2}$	Half-life
VCAM-1	Vascular cell adhesion molecule-1
Vz	Volume of distribution

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Polpharma Biologics S.A. submitted on 24 June 2022 an application for marketing authorisation to the European Medicines Agency (EMA) for Tyruko, through the centralised procedure falling within the Article 3(1) and point 1 of Annex of Regulation (EC) No 726/2004.

During the procedure the applicant changed from Polpharma Biologics SA to Sandoz GmbH.

The applicant applied for the following indication Tyruko is indicated as single disease modifying therapy in adults with highly active relapsing remitting multiple sclerosis (RRMS) for the following patient groups:

- Patients with highly active disease despite a full and adequate course of treatment with at least one disease modifying therapy (DMT) (for exceptions and information about washout periods see sections 4.4 and 5.1)

or

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

1.2. Legal basis, dossier content

The legal basis for this application refers to:

Article 10(4) of Directive 2001/83/EC – relating to applications for a biosimilar medicinal product.

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies).

The chosen reference product is:

Medicinal product which is or has been authorised in accordance with Union provisions in force for not less than 10 years in the EEA:

- Product name, strength, pharmaceutical form: Tysabri, 300 mg, concentrate for solution for infusion
- Marketing authorisation holder: Biogen Netherlands B.V.
- Date of authorisation: 27-06-2006
- Marketing authorisation granted by:
  - Union
- Marketing authorisation number: EU/1/06/346/001

Medicinal product authorised in the Union/Members State where the application is made or European reference medicinal product:

- Product name, strength, pharmaceutical form: Tysabri, 300 mg, concentrate for solution for infusion
- Marketing authorisation holder: Biogen Netherlands B.V.
- Date of authorisation: 27-06-2006
- Marketing authorisation granted by:
  - Union
- Marketing authorisation number: EU/1/06/346/001

Medicinal product which is or has been authorised in accordance with Union provisions in force and to which bioequivalence has been demonstrated by appropriate bioavailability studies:

- Product name, strength, pharmaceutical form: Tysabri, 300 mg, concentrate for solution for infusion
- Marketing authorisation holder: Biogen Netherlands B.V.
- Date of authorisation: 27-06-2006
- Marketing authorisation granted by:
  - Union
- Marketing authorisation number: EU/1/06/346/001

1.3. Information on Paediatric requirements

Not applicable

1.4. Information relating to orphan market exclusivity

1.4.1. Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

1.5. Scientific advice

The applicant received the following Scientific advice on the development relevant for the indication subject to the present application:

Date	**Reference**	**SAWP co-ordinators**
21 April 2017	EMA/CHMP/SAWP/224608/2017	Andrés Trelles, André Elferink

The Scientific advice pertained to the following *quality*, *non-clinical, and clinical* aspects:

- Proposed panel of analytical methods for characterisation and physico-chemical and biological analytical similarity assessment.
- Proposed panel of bioassays for analytical similarity exercises.
- Classification of product quality attributes.
- Potency-related critical quality attributes to establish analytical similarity.
- Approach to analysis of Fab-arm exchange phenomena to demonstrate analytical similarity.
- Use of alternative host cell line for the expression of PB006 as compared to that used by the manufacturer of the reference medicinal product.
- Approach to the establishment and use of the Internal Reference Standard.
- Need to perform animal studies to support the assessment of biosimilarity.
- Adequacy of parallel-group study design to support demonstration of PK/PD similarity between PB006 and the reference product.

- Design of a pivotal PK/PD study in healthy subjects to demonstrate PK and PD bioequivalence in α4-integrin receptor saturation as a marker for the pharmacological effect using PB006, EU-sourced Tysabri, and US-sourced Tysabri: study population, selection of PD marker, dose level, choice of primary and secondary pharmacokinetic, pharmacodynamic, safety, and immunogenicity endpoints, PK and PD statistical analysis, blinded interim assessment, sample size calculation, need for confirmatory efficacy data in patients with multiple sclerosis.

- Design of an immunogenicity and safety study in patients with multiple sclerosis comparing PB006 with EU-sourced Tysabri to rule out any clinical meaningful differences in terms of the immune response.

- ELISA test for qualitative detection of human antibodies to John Cunningham virus.

The applicant received the following Scientific advice on the development relevant for the indication subject to the present application:

Date	**Reference**	**SAWP co-ordinators**
26 January 2023	EMADOC-1700519818-1010854	Ivana Haunerová, Caoimhin Concannon

The Scientific advice pertained to the following *quality* aspects:

- Proposed grouping of type II variations related to analytical method transfer and manufacturing process transfer to additional manufacturing and quality testing site for the active substance; PB006 active substance testing as well as comparability and validation plan to support the transfer; appropriateness of good manufacturing practice (GMP) quality management system to introduce additional identical manufacturing line for PB006 active substance.

1.6. Steps taken for the assessment of the product

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

Rapporteur: Christian Gartner Co-Rapporteur: Simona Badoi

The application was received by the EMA on	24 June 2022
The procedure started on	14 July 2022
The CHMP Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	5 October 2022
The CHMP Co-Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	17 October 2023
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC and CHMP members on	18 October 2022
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	10 November 2022
The applicant submitted the responses to the CHMP consolidated List of Questions on	22 February 2023
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Questions to all CHMP and	05 April 2023

PRAC members on	
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	14 April 2023
The CHMP agreed on a list of outstanding issues in writing and/or in an oral explanation to be sent to the applicant on	26 April 2023
The applicant submitted the responses to the CHMP List of Outstanding Issues on	23 May 2023
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	07 June 2023
The CHMP agreed on a list of outstanding issues in writing and/or in an oral explanation to be sent to the applicant on	22 June 2023
The applicant submitted the responses to the CHMP List of Outstanding Issues on	28 June 2023
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	06 July 2023
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Tyruko on	20 July 2023

2. Scientific discussion

2.1. Problem statement

Not applicable

2.2. About the product

Tyruko (development code PB006) has been developed as a biosimilar to the reference product Tysabri (INN natalizumab), a full-length monoclonal antibody of the IgG4 subclass that targets the α4 integrin component of adhesion molecules found on lymphocytes, monocytes, and eosinophils.

PB006 has been developed to have the same IV dosage form, route of administration, dosing regimen and presentation as the EU reference product Tysabri (hereafter identified as EU-Tysabri). The subcutaneous presentation recently approved for Tysabri is not in the scope of this marketing authorization application for Tyruko.

The intended indication for Tyruko is the same as for the reference product EU-Tysabri, which is indicated for treatment of multiple sclerosis (MS).

Tysabri is indicated as single disease modifying therapy (DMT) in adults with highly active relapsing remitting multiple sclerosis (RRMS) for the following patient groups:

- *Patients with highly active disease despite a full and adequate course of treatment with at least one DMT*

or

- *Patients with rapidly evolving severe RRMS defined by 2 or more disabling relapses in one year, and with 1 or more Gadolinium enhancing (GdE) lesions on brain magnetic resonance imaging (MRI) or a significant increase in T2 lesion load as compared to a previous recent MRI.*

Tyruko is intended to be administered at a fixed dose of 300 mg, infused IV over approximately one hour, every four weeks.

Mode of action

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 also blocks the interaction of α4β7 integrin with the mucosal addressin 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.

In MS, lesions are believed to occur when activated T-lymphocytes cross the blood-brain barrier (BBB). Leukocyte migration across the BBB involves interaction between adhesion molecules on inflammatory cells and endothelial cells of the vessel wall. The interaction between α4β1 and its targets is an important component of pathological inflammation in the brain and disruption of these interactions leads to reduced inflammation. Under normal conditions, VCAM-1 is not expressed in the brain parenchyma. However, in the presence of pro-inflammatory cytokines, VCAM-1 is upregulated on endothelial cells and possibly on glial cells near the sites of inflammation. In the setting of central nervous system (CNS) inflammation in MS, it is the interaction of α4β1 with VCAM-1, CS-1 and osteopontin that mediates the firm adhesion and transmigration of leukocytes into the brain parenchyma and may perpetuate the inflammatory cascade in CNS tissue. Blockade of the molecular interactions of α4β1 with its targets reduces inflammatory activity present in the brain in MS and inhibits further recruitment of immune cells into inflamed tissue, thus reducing the formation or enlargement of MS lesions (Tysabri SmPC, Mar 2022).

2.3. Type of application and aspects on development

The development program comprises 4 clinical studies: 1 pivotal Phase 1 PK/ PD study with PB006, US-Tysabri, and EU-Tysabri in healthy subjects, 1 pivotal Phase 3 study with PB006 versus EU-Tysabri in patients with RRMS, 1 pilot Phase 1 pharmacokinetic (PK)/pharmacodynamic (PD) study with EU-Tysabri in healthy subjects, and 1 supportive Phase 1 safety study with PB006 in healthy subjects.

The 3-arm PK/PD study in healthy subjects (PB006-01-03) was 1 of the 2 pivotal studies in the clinical program demonstrating similarity of PB006 versus US-Tysabri and EU-Tysabri. This PK/PD study aimed to demonstrate PK similarity and included sensitive PD endpoints in order to support the demonstration of similar efficacy in this MAA. In addition, the clinical pharmacology data generated for EU-Tysabri and US-Tysabri in this study established the scientific bridge between both products.

The Phase 3 study in RRMS patients (PB006-03-01) was the second pivotal study in the clinical program. The study aimed to show that there were no clinically meaningful differences with regard to efficacy, immunogenicity and safety between PB006 and EU-Tysabri in the target population.

Scientific advice was obtained from the European Medicines Agency (EMA) in 2017, early in the development of PB006. In this meeting, the design of the proposed clinical studies with PB006 was discussed, and main design aspects were aligned with the agency, including:

- PK and PD endpoints in the pivotal PK/PD study PB006-01-03 (with α4-integrin % relative receptor saturation [%RS] as primary PD endpoint)
- Efficacy endpoints in the Phase 3 study PB006-03-01 (with magnetic resonance imaging (MRI) activity as primary endpoint and ARR as secondary endpoint)

The global development program of PB006 was subsequently also discussed with the United States Food and Drug Administration (FDA) in BPD Type 2 meetings in 2017, 2019 and 2020.

In the Scientific Advice meeting in 2017, EMA has confirmed that an evaluation of similar clinical efficacy based on MRI assessment is required. In contrast, the US FDA will evaluate similarity of efficacy of PB006 with the reference product by means of PD endpoints. The EMA considers PD data as supportive for evaluation of similarity of efficacy.

Subsequent to the meeting with the EMA, various design aspects of the clinical studies (e.g., including the choice of PD endpoints and their analysis in the pivotal PK/PD study) were discussed with the FDA and adjustments to the study designs were made to meet FDA requirements. In particular, the FDA requested to include sensitive PD endpoints in the pivotal PK/PD study, which would serve as basis for FDA's assessment of similar efficacy between PB006 and the reference product. In addition, a pilot study was requested to enable the selection of sensitive PD endpoints.

The most relevant adjustments that were implemented into the PB006 clinical program after the initial discussion of the program with the EMA in 2017 are outlined in the following.

Study Tysabri Pilot-01-01:

- A pilot study was conducted, to collect PK/PD data with 3 different doses of EU-Tysabri in order to establish an appropriately sensitive PK/PD study setting, including the dose selection and primary PD endpoints, for the pivotal PK/PD study with PB006.

Study PB006-01-03:

- The primary PD endpoint α4-integrin RS was complemented with baseline-adjusted CD19+ B-cell counts as a co-primary PD endpoint.
- For the primary analysis of α4-integrin RS, $AUEC_{0\text{-}12\ weeks}$ was chosen. As additional analysis, a descriptive analysis of $AUEC_{4\text{-}12\ weeks}$ was conducted.
- For the analysis of co-primary PD endpoints, a pooling approach for both comparators (EU- and US-Tysabri) was chosen, due to the high variability of the CD19+ endpoint. The pooling approach for the primary analysis (i.e., only one comparison biosimilar candidate vs reference) increases the number of subjects in the pooled reference group.
- To power the study for a CD19+ PD assessment, the sample size was increased from N=255 (sample size discussed with EMA in 2017) to N=453 subjects (N=151 per treatment group).
- CD34+, VCAM and MAdCAM were selected as secondary PD endpoints.

Study PB006-03-01:

A single transition of a subset of RRMS patients (N=30) from treatment with EU-Tysabri after 24 weeks to PB006 was implemented on the request of the FDA. Safety and immunogenicity data collected after the transition were used to investigate whether the transition from the reference product to the biosimilar drug may be associated with any hypersensitivity or increased immunogenicity.

The key elements and the design of the clinical studies are considered to be in line with the recommendations received from the EMA in 2017. Additional efficacy endpoints included thereafter and an increase in sample size further facilitate the assessment of similarity inefficacy between PB006 and the reference product.

Scientific advice on non-clinical issues

In the scientific advice procedure EMA/CHMP/SAWP/224608/2017 the applicant asked for agreement that no animal studies are required to support the assessment of biosimilarity between PB006 and Tysabri. The CHMP accepted this strategy as far as analytical and functional comparability of PB006 and EU-Tysabri can be demonstrated in vitro, which is in line with current guidance (guideline on Similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues (EMEA/CHMP/BMWP/42832/2005 Rev1), and the guideline on Similar biological medicinal products containing monoclonal antibodies: non-clinical and clinical issues (EMA/CHMP/BMWP/403543/2010)).

National scientific advice was also provided in 2016. The minipig animal model was accepted as a relevant species for Natalizumab, however, a pre-clinical study in lieu of a human PK study for formulation bridging was not accepted. It was agreed that no *in vivo* toxicity studies need to be performed.

The applicant performed a comparative subchronic repeat-dose toxicity study in cynomolgus monkeys, including PK analysis, with PB006 and EU-Tysabri

Coronavirus disease 2019 (COVID-19) related considerations

The pivotal PK/PD study PB006-01-03 and the pivotal confirmatory Phase 3 study PB006-03-01 were conducted during the COVID-19 pandemic. In the PK/PD study, recruitment was temporarily held from March to August 2020 (when around one-third of the subjects had been enrolled) and safety measures were implemented to protect subjects from a COVID-19 infection after dosing (e.g., reducing maximum subject age to 54 years, including polymerase chain reaction [PCR] tests for severe acute respiratory syndrome coronavirus type 2 [SARS CoV-2], increasing in-house period from 3 to 8 days and including home-quarantine up to Day 14).

The Phase 3 study in RRMS was (after Data Safety Monitoring Board [DSMB] endorsement) still enrolling and treating patients as of March 2020, when COVID-19 was declared a pandemic by the World Health Organization; the last patient was randomized in May 2020.

For study PB006-03-01, all COVID-19 AEs (according to Medical Dictionary for Regulatory Activities [MedDRA] coding) were identified and summarized. In order to assess the impact of COVID-19 on safety and efficacy of this study, the following analyses were performed:

- Study discontinuations and protocol deviations due to COVID-19 were summarized.
- Demography data for confirmed COVID-19 patients were summarized.
- Protocol deviations due to COVID-19 were summarized by country.
- A sensitivity analysis (for primary endpoint) was to be performed on the pre- COVID per protocol (PP) population.

These additional analyses are in line with the guidance from the EMA on the management of clinical trials during the COVID-19 pandemic (Guidance on the management of clinical trials during the COVID-19 pandemic, 2022).

2.4. Quality aspects

2.4.1. Introduction

Natalizumab, the active substance contained in Tyruko, also referred of as PB006, is a recombinant humanised anti-α4-integrin antibody produced in a Chinese Hamster Ovary (CHO) cell line by recombinant DNA technology.

Tyruko was developed as biosimilar to the reference medicinal product (RMP) Tysabri. The finished product is presented as sterile, colourless, and clear to slightly opalescent concentrate for solution for infusion, containing 300 mg of natalizumab as active substance (each vial contains 15 mL of concentrate). Other ingredients are: sodium chloride, histidine, histidine hydrochloride, monohydrate, polysorbate 80 and water for injections.

It is presented as a 300 mg/15 mL concentrate for solution for infusion in single-use Type I glass vial.

The product is available in type I borosilicate glass vials with bromobutyl stoppers and aluminium seals with a flip-top cap.

The subcutaneous route of administration, which was approved for Tysabri, was not in the scope of Tyruko development.

2.4.2. Active substance

2.4.2.1. General information

PB006 is a humanised monoclonal antibody of the IgG4κ subclass consisting of two heavy (HC) and two light chains (LC), connected by four inter-chain disulfide bonds. Antibodies of the IgG4 subclass are characterised by a shorter hinge region in comparison to antibodies of the IgG1 subclass, leading to a reduced flexibility of the hinge region. As expected for an IgG4 antibody, natalizumab demonstrates reduced binding to Fcγ receptors and lack of ability to fix complement *in vitro*. The molecular weight of the intact deglycosylated natalizumab molecule, as measured by mass spectrometry, is 146 kDa. Each heavy chain has one N-linked glycosylation site at Asn300.

As other complex glycoproteins, PB006 displays a natural amount of microheterogeneity in terms of a different degree of glycosylation and post-translational modifications of amino acids. The C-terminal lysine residues of the heavy chains (Lys450) are mostly cleaved by cellular proteases during cell culture growth. As other antibodies of the IgG4 class, natalizumab contains 12 intra-chain disulfide bonds. The two heavy chains are connected in the hinge region by two inter-chain disulfide bonds and the light chain is linked to the heavy chain by an inter-chain disulfide bond. Further details on product variants due to common sources of post-translational and processing modifications of PB006 were presented by the applicant.

2.4.2.2. Manufacture, characterisation and process controls

Manufacturing process

The active substance is manufactured at Polpharma Biologics S.A., Ul. Trzy Lipy 3, Gdańsk, Pomorskie 80-172, Poland. All sites involved in the manufacture and storage of the cell banks and manufacture, quality control testing of the active substance operate in accordance with EU GMP.

PB006 active substance is produced based on a CHO cell line. To manufacture of PB006 active substance, a fed-batch process with preceding expansion stages, followed by primary recovery and a series of purification steps, typical for monoclonal antibodies, including several chromatography steps, virus inactivation, virus-filtration and ultra-/diafiltration (UDF) steps was established. Used media, critical process parameters (CPPs), critical in-process controls (IPCs), hold times and column lifetimes were presented. No reprocessing is foreseen for intermediates and PB006 active substance. The manufacturing process was sufficiently described.

Control of materials

The control of raw materials section was split into an upstream process (USP) and a downstream (DSP) part. All raw materials used in USP and DSP are of non-animal and non-human origin, while cell culture media are chemically defined and free of animal-derived raw materials. All ingredients are certified as BSE/TSE-free. A complete list of all raw materials used for manufacture of PB006 was provided. Non-compendial raw materials are tested against in-house specifications, which were disclosed and found acceptable. The qualitative and quantitative composition of cell culture media and feeds was not provided, and justified by being intellectually protected. The composition of all buffers and media and their physico-chemical properties was disclosed. Chromatography resins were listed, including respective suppliers. Provided information on compendial and non-compendial raw materials and respective control strategy used to manufacture PB006 is acceptable, except cell culture media and supplements used in the USP, and purchased from an external supplier. Information about the composition of these media was provided, and thus the compliance of respective control strategies is confirmed.

In relation to cell line development, the applied cloning strategy was explained. Cell line development was sufficiently described and is acceptable.

The procedure for the manufacture of the MCB and WCB was described. Cell line characterisation data and IPCs were presented. Genetic stability was assessed, and the nucleotide sequence of the transgene was confirmed in the MCB, working cell bank (WCB) and the post-production cell bank (PPCB). Gene copy numbers were comparable in the MCB, WCB, PPCB and the end-of -production cell bank (EOPCB), and flanking LC and HC nucleotide sequences were confirmed. A limit of *in vitro* cell age (LIVCA) study was performed at manufacturing scale to ensure the genetic stability of the recombinant production cell line until the end of production (EOP) with extended number of cell passages, including the demonstration of adventitious agents safety (such as viruses and mycoplasma). Taken together, characterisation of cell banks was performed by state-of-the-art technologies and found acceptable.

Control of critical steps and intermediates

The applicant performed a quality attribute criticality assessment. A first criticality assessment evaluation was performed at the beginning of the development to identify and classify the criticality of the product quality attributes (QAs) to patient safety and drug efficacy. The assessment was later updated to reflect additional knowledge as well as health authority feedback/requirements.

The applicant gave an overview of process controls applied for the active substance manufacturing process. Process parameters and IPCs were split into categories based on their criticality. A list of process parameters was provided for the USP and the DSP, and included each step of the manufacturing process. IPCs were also listed for the USP and the DSP. Corresponding method applied to assess respective IPC was indicated, as well as a short justification of criticality and the acceptable range. Process control and IPC lists are complete, and acceptance ranges and limits are tight enough and justified by experimental data. Hold times were validated at commercial scale during PPQ runs. Additional analytical procedures for controlling critical steps were briefly described. Taken together, safety and efficacy related quality attributes were controlled at critical process steps. Presented control strategy is complete and aligned with guidance documents, thus appropriate for this class of biologics. The applicant performed a

comprehensive criticality assessment evaluation of PB006 quality attributes based on impact and criticality of respective parameters. A detailed list of CQA's was presented. The overall risk of each quality attribute was adjusted, if e.g. multiple orthogonal methods were applied to assess the same quality attribute. The evaluation procedure was explained in enough details, scientifically justified and thus is acceptable for this molecule.

Process validation

Process validation consisted of the assessment of USP and DSP process performance qualification (PPQ) runs at commercial scale, dispensing homogeneity of active substance, in-process material hold-time, process-related and product-related impurity clearance, and supportive studies including resin re-use and storage studies, extractable- and leachable assessment, transport validation and continued process verification.

All CPP and key process parameter (KPP) remained within proposed ranges for USP validation activities. In addition, all IPCs of the USP process, as well as all IPCs of media, feed and supplement solutions testing met the pre-defined limits. Deviations that occurred during the USP process were assessed to have no adverse impact on process performance or on product quality attributes.

The results obtained during the manufacture and analysis of the PPQ batches demonstrate the consistency of the downstream process. This provides confirmation and demonstrates that the DSP process can consistently provide product of defined quality and in specifications and that the process can be considered validated.

A dispensing homogeneity study was performed in order to demonstrate the capability of the equipment and the process to perform homogeneous dispensing of the compounded active substance over the entire dispensing process. These parameters were shown to be efficient in order to obtain homogenous in-process material. Evaluation of the results of the samples taken during dispensing demonstrated that the PB006 dispensing process has no impact on product quality and consistently delivers a homogenous active substance meeting with the specifications.

Impurity clearance was assessed at respective clearance steps during process validation. Again, all PPQ batches were analysed and confirmed that the downstream processing is able to efficiently and reproducibly reduce levels of product-related impurities below specified acceptance limits.

Resin re-usage studies were performed for chromatography resins and demonstrated no adverse effects on process performance and product quality. The absence of protein carryover was also confirmed at manufacturing scale by performing a mock run (resin carry-over).

A leachable and extractable assessment was performed for polymeric materials used during manufacturing, processing and storage of PB006 active substance in order to identify potential risks caused by material in direct or indirect contact with media, buffers, in-process materials and the active substance. No organic compound or element could be released at toxicologically relevant concentrations from either process consumable. The applicant's approach is supported.

Transportation validation was performed for the temperature controlled shipment of active substance using qualified shipment containers. A transport simulation (mechanical stress, reduced air pressure) was carried out in an ISTA lab, using worst case simulations. All requirements as defined in the transport validation protocol for PB006 were met, and the applicant's conclusion that the transportation process is considered validated is supported. After successful process qualification process performance and product quality are monitored as part of the continued process verification (CPV) to ensure that the manufacturing process is maintained under control throughout the commercial phase. It is a planned lifecycle management program to ensure that the manufacturing process remains in a state of control.

This is achieved through the systematic collection, analysis, and trending of product related and process related data.

Manufacturing process development

The overview of PB006 active substance manufacturing process history is presented below.

After successful USP and DSP development, consolidation runs were performed and process characterisation (PC) studies defined process parameter criticality and its ranges. The process was upscaled to the commercial scale for production of pre-clinical and clinical material, after its robustness was confirmed in consolidation runs.

In the early development program of PB006, a change in cell banks (MCB and WCB) was implemented; a comprehensive comparability exercise at active substance level confirmed the comparability of both cell banks, and that the finished product manufactured from the initial cell bank is representative for the commercial product.

Early process development activities were initiated, and optimisation of the process was conducted and the final verification of the optimised process was performed. The final setting was confirmed by assessing several quality attributes using representative USP starting material. The final process was locked after the manufacture of consolidation runs.

Process characterisation included a pre-PC risk assessment to assess the process- and product related risks which might impact on process performance and/or product quality, in order to define process parameters for further investigation during PC studies. Finally, the development of a control strategy including justifications for IPC classifications was achieved. The representativeness of the initial WCB for the USP PC characterisation studies has been also confirmed. Resin reuse studies were performed using a representative down-scaled model.

The development of the manufacturing process and the process characterisation were well and comprehensively described. Development activities were performed according to guidance documents, including comparability exercises when critical changes were implemented into the manufacturing process. The provenience and the use of each development batch is clearly indicated. The control strategy is considered state-of-the-art, and was based on an in depth understanding of each process step, supported by intense and well-coordinated development activities. This part of the dossier is found acceptable.

Characterisation

Elucidation of structure

PB006 active substance was thoroughly characterised on the physicochemical/biophysical level and at the level of *in vitro* functional activity using a broad panel of analytical procedures to investigate all relevant quality attributes. The applied method panel included orthogonal analytical methods for in-depth analysis of higher order structures, size and charge variants and the assessment of functional properties of natalizumab.

Taken together, proposed methodological portfolio consists of a broad spectrum of orthogonal and state of the art methods, which enable an in-depth assessment of the active compound. All applied methods were validated, qualified or at least classified as suitable for the intended use. On top of the in-depth assessment of the active compound, charge-variants and hydrophobic-variants were isolated, and assessed for their specific physico-chemical properties. Taken together, PB006 active substance was sufficiently characterised and respective part of the dossier is considered acceptable.

Impurities

Clearance of the most important product-related impurities was sufficiently addressed in the PB006 active substance process validation chapter. It is agreed that no further evaluation of product related impurities is required.

Respective studies demonstrated that the downstream process is able to consistently reduce levels of process-related impurities to meet the limits of the PB006 active substance specification.

Qualitative and quantitative studies were conducted to analyse the extractable profiles and is acceptable for the most important consumables used to manufacture PB006 active substance. Overall, the conclusion that it is highly unlikely that an identified or unidentified extractable or leachable chemical from the process equipment may affect the biological safety of PB006 is agreed.

An extractables study performed on the container closure system for PB006 finished product is assessed in the finished product section.

2.4.2.3. Specification

Specifications

The active substance release and shelf life specifications include control of identity, purity and impurities, potency and other general tests.

The proposed battery of assays to control PB006 CQAs is composed of orthogonal and state-of-the-art methods.

Overall, the active substance release and shelf life specifications are considered acceptable.

Analytical procedures

Analytical procedures used to control PB006 active substance were described.

Validation of non-compendial methods was carried out according to ICH Q2(R1) guideline. All predefined acceptance criteria were met, and thus proposed methods are considered valid within their respective ranges. Compendial methods were qualified for their intended use, but respective qualification protocols were not submitted. Thus the applicant justified the performance in sample matrix of compendial methods used to assess bioburden and bacterial endotoxins.

Batch analysis

The applicant presented batch analysis data from manufacturing scale batches. They confirm that the established manufacturing process is able to deliver PB006 active substance with a consistent and predefined quality profile, and the proposed control strategy besides minor adaptations of specified acceptance limits is acceptable.

Reference Standard

The development reference standards were prepared for characterisation, qualification and routine testing until preparation of the Interim Reference Standard. The Interim Reference Standard was further qualified as Primary Reference Standard (PRS) prior to the first Working Reference Standard (WRS), which are the two reference standards currently in use. After establishing the PRS, any subsequent WRS will be calibrated against it (two-tiered approach). Based on the re-test results, storage conditions have been confirmed. WRSs will be used for the future routine testing of PB006 active substance and finished product batches and will be characterised against the PRS.

Development, primary and working reference standards have been adequately bridged via potency assays. The reference standard control strategy is properly described.

Container closure system

The container closure system for PB006 active substance is composed of a PET bottle and a high-density polyethylene (HDPE) screw cap. An appropriate description of the container closure has been provided.

In summary, the proposed container closure system is justified for storing PB006 active substance regarding stability, integrity and compatibility of the medicinal product. All materials of the container closure system are described in the Ph. Eur. and their qualitative composition is determined by the supplier. Absence of interaction studies has been properly justified and a representative supplier product certificate has been provided.

2.4.2.4. Stability

The applicant initially claimed a 36-month shelf life (5 ± 3ºC).

The stability of PB006 active substance was performed at long term storage (5 ± 3ºC), accelerated (25 ± 2°C / 60 ± 5% R.H.) and stressed (40 ± 2°C / 75 ± 5% R.H.) conditions. Accelerated and stress stability studies were performed to confirm the PB006 active substance stability-indicating profile, and provided assurance that changes in the purity and potency are detected. Stability studies of PB006 active substance were designed in accordance with guidance documents ICH Q5C and ICH Q1A(R2), considering testing frequency, storage conditions, representativeness of batches, container closure system and the applied methodological portfolio.

The shelf-life of PB006 active substance was determined at long term conditions based on the totality of available real-time stability data. All test parameters remained within the pre-defined shelf-life specifications for all available pull-points. Stability studies will be pursued, and data provided according to the post-approval stability commitment.

Data and graphical representations from accelerated and stress stability studies were presented.

Taken together, design of stability studies, choice of stability batches and proposed shelf-life specification is appropriate.

Overall, taking into account the totality of the data submitted by the applicant, the proposed shelf-life for the active substance is acceptable.

The applicant committed to finalise ongoing stability studies to confirm proposed shelf-life of PB006 active substance at long-term storage conditions, and to place on stability at least one commercial batch each calendar year, as long as production occurs during the respective calendar year. Stability-indicating parameters will be studied, while the assessment of quality attributes which have shown to be unaffected in presented stability programs will be omitted. In case of any confirmed OOS result or unexpected stability issue, the applicant will inform the agency and propose appropriate corrective actions. The proposed post-approval stability commitment is acceptable.

2.4.3. Finished Medicinal Product

2.4.3.1. Description of the product and pharmaceutical development

PB006 finished product is a sterile, colourless, and clear to slightly opalescent concentrate for solution for infusion, presented in Type I borosilicate glass vials not siliconised, with bromobutyl stoppers and

aluminium seals with a flip-off cap. No overage is required. The nominal fill volume of PB006 finished product is 15 mL per vial. Each vial contains an appropriate excess volume (overfill) that allows withdrawal of the labelled amount of the finished product from the vial.

The finished product is formulated with sodium chloride, histidine, histidine hydrochloride monohydrate, polysorbate 80 and water for injections. All excipients are widely used in the manufacturing of parenteral pharmaceutical preparations and, comply with the Ph. Eur. Certificate of analyses for all four excipients are provided. The finished product does not contain any novel excipients or any excipients of human or animal origin.

The procedure followed to determine the overfill volume is described and results have been provided.

Characterisation studies covering physicochemical and biological properties of natalizumab were performed using PB006 active substance as well as PB006 finished product (for selected quality attributes). An overview of the QTPP of PB006 is presented. In order to define the acceptable ranges for the quality attributes (QA) of the proposed biosimilar, the range and variability of the reference product was evaluated using multiple batches of Tysabri. CQAs were identified by the risk assessment performed in accordance with ICH Q9. The criticality of each quality attribute is defined by combining the impact and uncertainty assessments according to the concepts discussed in the dossier.

The PB006 finished product manufacturing process employs standard methods for production of parenteral products that cannot be terminally sterilised. PB006 is a concentrate for solution for infusion which is manufactured by sterile filtration and subsequent aseptic filling. PB006 active substance and PB006 finished product have the same qualitative composition and no compounding is performed in the PB006 finished product manufacturing process. The same process was used for manufacturing of finished product during clinical trials.

Changes in the manufacturing process have been properly described. PB006 finished product used for non-clinical studies was manufactured at the initial manufacturing site. Afterwards, the clinical manufacturing process was established at the commercial manufacturing site. Differences introduced into the PB006 finished product manufacturing process are related to the change of the manufacturing site.

Comparability between pre-clinical and clinical manufacturing processes has been confirmed by available batch release data and stability data. No differences in the degradation rates and profiles were observed between the pre-clinical and clinical batches.

Based on the change of the manufacturing site and the additional experience gained during PB006 finished product manufacturing, adjustments for some of the manufacturing steps were introduced to optimise the process for the commercial manufacturing. Based on the results gathered during the development studies and the increased product knowledge, a failure mode effect analysis (FMEA) risk assessment of the PB006 finished product manufacturing process was conducted prior to PPQ campaign to define a product control strategy and process action limits. Selection of the PB006 finished product primary packaging for clinical trials was based on the outcome of the reverse engineering evaluation of the RMP Tysabri. Extractable studies were not performed on the glass vials, which are non-polymeric and widely used for pharmaceutical drugs, and are considered to be of low risk and not requiring evaluation for extractable. The discussion on the toxicological evaluation and the final outcome have been provided.

The applicant explained that a change of the vial became necessary, due to a decision of the manufacturer to discontinue production of the vial chosen for clinical batches of PB006. An alternative manufacturer was selected, and implemented prior to finished product process validation for future commercial production. Comparability to the previously used vial was assessed. Studies performed confirmed the suitability of the vials used in commercial production. Microbiological quality of the

container closure system selected was confirmed. The applicant performed in-use compatibility studies to ensure physicochemical compatibility of PB006 finished product with the clinical administration materials at various stages of product development A detailed justification for the low risk classification and the omission of shear force stress degradation studies have been provided. The in-use compatibility of PB006 finished product with clinical administration items was successfully proved.

2.4.3.2. Manufacture of the product and process controls

Manufacturing process

Manufacturing of the finished product, including primary and secondary packaging and quality control and release testing facilities are listed in the dossier. All sites are compliant with EU GMP.

PB006 finished product and active substance have the same qualitative and quantitative composition.

PB006 finished product is manufactured according to a standard manufacturing process which includes pooling of active substance, bioburden reduction filtration, sterile filtration, filling and stoppering, crimping, visual inspection, storage and final packaging. A flow chart of the manufacturing process which include all process steps, CPPs and critical IPCs is presented. The steps are properly described. The batch number system is described. Established hold times have been validated and described.

Process controls

The overall control strategy presented includes definition of process parameters and IPCs. Process parameters have been classified in CPPs, KPPs and non-KPP. Any excursion outside of the established acceptable ranges during the GMP production is investigated and the possible impact on quality will be assessed according to the site internal procedures. Definition of IPCs has been done according to ICH guidelines. For the control of output parameters whose variability has the potential to affect a CQA in the final product (critical IPC), an acceptance criterion or an action limit is defined, and optionally an additional alert limit. Definition of process parameters and IPCs has been properly described. The control strategy presented is appropriate to ensure adequate product quality and manufacturing process consistency.

Process validation

To ensure that the process is consistent and robust, several activities have been conducted throughout the process design and process qualification (PQ) stages. A pre-PPQ risk assessment has been performed ahead of process validation according to FMEA. Validation of the manufacturing process has been done with three consecutive batches manufactured according to the PPQ protocol, from active substance batches fully representative. Test procedures applied to process validation samples and results from analytical method validation are discussed. Validation of PB006 finished product manufacturing process is discussed. The validation strategy is properly described and considered acceptable.

The membrane filters used for bioburden reduction and subsequent sterilising filtration of active substance are sterilised, and respective qualification reports were provided. Filters have been validatedFilter validation has been performed according to EMA Guideline on "the sterilisation of the medicinal product, active substance, excipient and primary container" (EMA/CHMP/CVMP/QWP/850374/2015). Validation of the aseptic filling process in the sterile area of the finished product manufacturing facilities was initially performed with three consecutive successful media fills and is periodically re-validated by media fills.

The suitability in terms of safety of the polymeric process contact materials that are used during the finished product manufacturing process has been evaluated. Mandatory E&L studies for the primary packaging and the final filter of the finished product were performed. The summary reports for the PB006

active substance and finished product elemental impurity risk assessment and for the elemental impurity clearance study have been provided. Vials are washed, depyrogenated and sterilised. The validation process for the depyrogenation step and vial washing step are reported. Rubber stoppers are pre-washed and sterilised. Validation of rubber sterilisation is reported.

Process performance and product quality will be monitored as part of the continued process verification (CPV) to ensure that the manufacturing process is maintained under control throughout the commercial phase.

2.4.3.3. Product specification

Specifications

The finished product release and shelf-life specifications include test methods for physical state, coloration, clarity, subvisible particles, visible particles, osmolality, extractable volume, pH, excipient concentration, identity, purity, sterility, bacterial endotoxins, content and biological activity (potency).

The quality attributes selected for release and stability of PB006 finished product are considered adequate and in line with the guidelines ICH Q6B, EMA/CHMP/BWP/532517/2008 and EMEA/CHMP/BWP/157653/2007. Related analytical tests are acceptable. For compendial methods the respective references to Ph. Eur. and USP are included.

Acceptance criteria for PB006 finished product release and stability testing, as well as limits for PB006 in-process testing, were set based on the available data from PB006 finished product batches, and PB006 finished product stability studies.

Overall, the finished product release and shelf life specifications are considered acceptable.

Analytical procedures

For methods identical to the active substance testing, the applicant refers to the corresponding active substance sections.

For compendial methods, the applicant refers to the corresponding Ph. Eur. Monographs. Non-compendial tests have been validated according to ICH Q2(R1) and a validation report for the instrumental method has been provided. Verification reports for the compendial microbiological methods are presented.

Characterisation of impurities

A risk analysis in accordance with ICH Q3D guideline was performed to evaluate the potential presence of elemental impurities in PB006, covering raw materials, excipients, manufacturing equipment and utilities as possible sources of elemental impurities. Mentioned strategy and the applicant’s conclusion that no additional control measures are required is acceptable.

A nitrosamine risk assessment was performed and considered raw materials, single use materials and manufacturing process conditions used to manufacture PB006 active substance. Respective summary report was provided, and the conclusion that there is no risk of presence of nitrosamine impurities in the PB006 active substance is supported.

Batch analysis

Results of all batch analyses are presented. The history of release and shelf-life specifications and procedures and changes for analyses of commercial processes are presented. Batch to batch consistency has been confirmed among all batches tested

Container closure system

The container closure system is a clear Type 1 non-siliconised glass vial, closed with a 20 mm bromobutyl rubber stopper coated with fluoropolymer, and crimped with 20 mm flip-top aluminium seal caps). Technical drawings of stopper and vial, with critical dimensions, are provided. The secondary packaging system is described. Specifications for vial and stopper have been provided. Compliance to Ph. Eur./pharmacopoeia is stated. Compliance status of the cap is not applicable since it is not in contact with the product, which is acceptable. Suitability of the container closure system for storage has been confirmed. Risk assessment has been conducted on all materials in contact with product. Extractable/leachable studies have been performed only on the rubber stopper.

2.4.3.4. Stability of the product

The applicant proposed a shelf-life of 36 months at 2 to 8°C for the undiluted product and an in-use storage up to 24 hours at 2 to 8°C for the diluted product.

The shelf-life claim is based on available data from stability studies performed in accordance with to the guidelines ICH Q5C and Q1A. Stability was tested at long-term condition (5 ± 3ºC for 48 months), accelerated condition (5 ± 2°C / 60 ± 5% R.H. for 6 months) and stress condition (40 ± 2°C / 75 ± 5% R.H. for 6 months).

The applicant included batches manufactured according to the commercial process, batches of different age and batches derived from different active substance batches. Samples were stored in an upright (intended storage condition only) and inverted position, protected from light.

Results show that all batches remained within the shelf-life specifications after storage at the intended condition of 5 ± 3ºC. Significant changes are observed when samples are stored at 40 ±2°C / 75±5 %RH: Since no significant differences in stability behaviour were observed between the batches tested, the applicant claimed a shelf-life of 36 months, based on a minimum of 3 batches with the longest real time stability data.

Photostability was assessed in accordance to the guideline ICH Q1B. Studies confirmed that PB006 finished product is light sensitive and should be protected from light in the secondary packaging cardboard box. Exposure to stress conditions like oxidation, freeze-thaw, low pH and mechanical stress has been tested within the biosimilarity analytical assessment.

Overall, the acceptable shelf-life for the finished product in unopened vial is 3 years when stored at 2°C to 8°C protected from light.

From a microbiological point of view, after dilution with sodium chloride 9 mg/mL (0.9%) solution for injection, immediate use is recommended. If not used immediately, the diluted solution must be stored at 2°C to 8°C and infused within 24 hours of dilution. In-use storage times and conditions prior to use are the responsibility of the user.

2.4.3.5. Biosimilarity

The applicant has conducted an extensive comparative analytical assessment of PB006 and Tysabri, addressing more than 40 quality attributes to demonstrate that PB006 is identical to the EU approved RMP in amino acid sequence and similar in physicochemical and functional tests, using a broad panel of sensitive and orthogonal analytical methods.

Analytical similarity of PB006 was assessed in a comprehensive analytical similarity exercise using EU-sourced Tysabri RMP. The clinical study (PB006-01-03) was conducted with US-Tysabri and thus, the

applicant performed a three-way analytical similarity assessment between PB006, EU-Tysabri, and US-Tysabri. The biosimilarity exercise includes the comparison of physicochemical, biophysical and *in vitro* functional properties, forced degradation studies and stability studies under long-term, accelerated and stress conditions. Tables and figures summarising the individual results and data distribution for each parameter, chromatograms, spectra, dose-response curves etc. have been included. The presentation of data was well structured and of high quality.

An in-depth analysis of the RMP and the biosimilar as well as prior knowledge was the base for establishment of the QTPP. Each quality attribute identified was classified according to the risk to potentially have an impact on activity/efficacy, pharmacokinetics/pharmacodynamics (PK/PD), immunogenicity and safety as well as the uncertainty of the impact. Similarity was primarily concluded from min-max ranges obtained from EU-Tysabri, pre-defined quality ranges (QRs) were considered as secondary criterion. QR is defined as QR = (μR − KσR, μR + KσR) where μR is the sample mean, σR is the sample standard deviation based on reference product lots, and the multiplier K (in principle, the higher the risk category, the lower the multiplier). Overall, the critical quality attributes assessment was well described and in the main the outcome of the risk assignment appears reasonable.

The analytical similarity study includes PB006 (natalizumab) data from finished product batches manufactured at full commercial scale. Biosimilarity was evaluated against an appropriate number of batches of the reference medicinal product, EU-approved Tysabri. A reduced but appropriate number of batches has been used for primary structure analysis by LC-MS as well as for ADCC, CDC, ADCP, binding to FcγR and C1q methods. US-sourced Tysabri products have been used in the clinical study (PB006-01-03). Therefore, US-Tysabri batches have additionally been included in the analytical similarity analyses. A bridging report of EU-approved and US-licensed Tysabri has been provided by the applicant.

Ages of the PB006 and Tysabri batches at the time of analytical testing vary within the expiry date. The batches seem to reflect a range of expiration dates and product ages. Justification for the statistical approaches were provided, and these are considered acceptable as supportive evidence of biosimilarity. As presented by the applicant, all the assays used in the biosimilarity study are demonstrated to be suitable for their intended purpose and are either qualified, validated, fit for purpose or are compendial methods. The chosen methods are considered standard methods that are state-of-the-art and are suitable to characterise and compare relevant structural and functional features of the natalizumab finished product in comparison to the RMP. Analytical methods cover primary and higher order structure, potency/binding and Fab arm exchange kinetics as well as purity and product related variants. For each parameter under investigation, the methodology and performance of the analyses were well described, batches used and experimental data derived were well presented including raw data or chromatograms/spectra where applicable.

Similarity between PB006 and Tysabri

For many quality attributes, PB006 was demonstrated to be analytically highly similar to EU-Tysabri. Observed differences are adequately addressed by the applicant and are not expected to impact clinical performance of the product. In addition, analytical comparability of US-Tysabri to EU-Tysabri has been sufficiently demonstrated as presented in a separate bridging report.

Primary and higher order structure

Primary structure of PB006 was characterised for amino acid sequence by peptide mapping with 100% confirmation of the sequence. Intact deglycosylated protein, heavy chain deglycosylated and light chain masses of PB006 and RMP were demonstrated to be similar to that of theoretical masses. The same locations of posttranslational modifications (N-terminal pyroglutamic acid, oxidation, deamidation, N-glycosylation) have been detected in peptide mapping MS analyses of PB006 and Tysabri samples.

Higher order structure was characterised for secondary and tertiary structure. The spectra of higher order structures are similar between PB006 and the RMP and, minor differences in the overall secondary structure are not considered impactful as the region impacted does not show strong straightforward correlation to the secondary structure of the protein.

Fab-arm exchange kinetics

Fab-arm exchange kinetics, a process typical for IgG4 molecules has been characterized using FRET in reducing and physiological (serum) mimicking conditions as suggested within an EMA-SA. The exchange of half-molecules of PB006 was within min-max ranges set by the EU-RMP under both conditions and are thus regarded as similar.

Functional characterisation

Natalizumab is binding to the α4-subunit of α4β1 and α4β7 integrins expressed on the surface of all leukocytes except neutrophils inhibiting the α4-mediated adhesion of leukocytes to their counter-receptor(s) vascular cell adhesion molecule-1 (VCAM-1), and mucosal adressin cell adhesion molecule-1 (MAdCAM-1). The main mechanism of action (MoA) was evaluated by an ELISA method and SPR. Analysing binding to α4β1 and α4β7 by ELISA, relative potency values of PB006 batches were comparable to EU-Tysabri and US batches. Orthogonal SPR analyses showed similar relative potencies and measured binding affinities constants between compared PB006, EU-Tysabri and US-Tysabri batches. In turn, inhibition of interaction between VCAM-1 and α4 integrin evaluating the MoA, was demonstrated by three potency methods, relevant cell-based assays included, and the results were comparable. Highly comparable results have been provided for the inhibition of interaction between MAdCAM-1 and α4 integrin. As natalizumab does not exert Fc-effector functions, the absence of such functions was demonstrated for all three products.

Limited Fcγ receptor binding could be demonstrated for PB006, EU-RMP and US-Tysabri targeting various Fcγ receptor isoforms. In comparison to a positive control C1q activity by natalizumab could not be shown. Binding of natalizumab to FcRn was shown to have comparable relative potencies and binding affinity constants.

Although individual outliers have been detected, it is agreed that biosimilarity regarding biological functionality was demonstrated.

Molecular heterogeneity

Molecular heterogeneity was characterised for size, charge heterogeneity and N-glycosylation. Results indicated similar structural, size, and charge heterogeneity between PB006 and the RMP Tysabri.

Size heterogeneity was assessed by multiple orthogonal, state-of-art methods and comparable profiles and chromatograms/electropherograms were observed indicating similarity. The size variants and their levels are similar. A larger non-glycosylated heavy chain (NG-HC) peak was observed in EU/US-Tysabri samples in comparison to PB006. However, this is a desirable effect and indicates consistent high degree glycosylation using CHO host cell (vs murine cells used for Tysabri). Antibody fragments in PB006 are low and similar to EU/US-Tysabri.

Free thiols and correct disulphide bridging were analysed with higher free amounts of SH groups detected for PB006 in comparison to EU and US-Tysabri. The same trend is detected by analyses of non-reduced peptides. The applicant argues that differences detected are small and related to the semi-quantitative method variability. As no effect on relative potency and higher order structure could be detected, the conclusion is followed that there is only low risk to be clinically meaningful. Further, neither thioether bonds nor incorrectly bound disulphide bonds were detected.

Charge heterogeneity was verified. Overall PB006 and Tysabri were found to be similar in terms of charge heterogeneity with the exception of a basic peak at approximately 30 min elution time detected in Tysabri batches. Consequently, higher main charge variants for PB006 and lower basic variants have been reported in comparison to EU-Tysabri. The applicant investigated the basic fragment of the signal peptide present in Tysabri samples in detail. Acidic variant levels were found to be highly similar in PB006 batches as compared to EU-approved Tysabri. Deamidation occurs at very low levels and no major sites of deamidation have been found within CDR and non-CDR regions of natalizumab. No concern is raised for slight differences shown between PB006 and the RMP.

Levels of isoaspartic acid and glycation were shown to be comparable. N-terminal sequence heterogeneity shows almost 100% conversion of N-terminal glutamine to pyroglutamate for all analysed natalizumab samples. An incompletely processed signal peptide was detected in EU/US-Tysabri which seems to be cell line specific. Absence in PB006 is considered beneficial. No differences in C-terminal lysine formation were determined between PB006 and the RMP. C-terminal leucine (L445) amidation however is reported for PB006 at very low levels whereas this posttranslational modification is not present in Tysabri (murine host cell). Amidation is not considered unnatural, thus no clinical impact is expected.

Low oxidation levels of methionine residues analysed by multiple orthogonal methods observed in PB006 and EU/US-Tysabri were reported. Major oxidation sites are HC M255 and HC M431 both located at the constant region of the antibody. M255 showed higher oxidation levels in PB006 when compared to EU-Tysabri. However, as shown in the functional assessment section, potency was found to be highly similar and FcRn binding was not affected by Met oxidation in the constant domain. Slightly higher oxidation levels for PB006 were obtained by other applied analytical methods, which correlates with the presented site-specific results. The hydrophobic variants analyses showed higher results for PB006 in comparison to the EU/US-Tysabri samples, correlating with results from the above discussed C-terminal leucine amidation. It was concluded by the applicant that the hydrophobic variants did not impact protein folding (data from higher order structures assays) and had no influence on protein potency. Corresponding data have been provided.

N-glycosylation was characterized for site occupancy by panel of orthogonal methods. N-glycosylation site occupancy was identified at EEQFN(300)STYR peptide with slightly higher results in comparison to EU/US-Tysabri samples. Similar results were provided by other orthogonal analyses. Comparable glycan profiles with minor differences in N-glycans present were shown. While the following N-glycan groups could be detected (main, high mannose, afucosylated, sialylated and galactosylated glycans), the immunogenic α(1,3)-galactosylation could not be identified in PB006 natalizumab samples analysed. Differences reported include higher values for galactosylated, afucosylated and high mannose N-glycans as well as lower values for main glycan and sialylated glycan species for PB006 samples in comparison to EU/US-Tysabri. However, all reported values are within min-max and/or QR ranges of EU-Tysabri.

Finished product attributes

Protein concentration was calculated using the experimentally determined extinction coefficient. The applicant provided experimentally determined absorbance coefficients for PB006 and EU-RMP. Measured UV 280 concentration values were used for all PB006 batches and for the majority of EU-approved Tysabri batches during the biosimilarity studies. The limited use of nominal concentration values for Tysabri does not affect the similarity claim. A justification has been provided. Clinical doses in the Phase 1 PK/PD study were calculated based on the truly measured antibody concentration, instead of the labelled concentration.

Overall, at the quality level similarity between PB006 and EU-sourced Tysabri could be demonstrated for most quality attributes in a comprehensive analytical similarity exercise. Comparability between EU- and US-sourced Tysabri, which was used as comparator in the clinical trial, could be demonstrated in the

analytical similarity exercise. The uncertainties concerning differences observed in some parameters of PB006 in comparison to the RMP and issues regarding missing information are considered resolved.

The following table summarises the outcome of the analytical similarity exercise:

Table 1: Summary of analytical biosimilarity assessment

Category	Product Attributes	Evaluation method	Key findings[1]
Identity and structure	Primary Structure	Peptide mapping-UV	Identical
		LC-MS	Identical
	Molecular mass	LC-MS	Comparable
	Higher order structure	FTIR	Comparable
		CD	Comparable
		Fluorescence spectroscopy	Comparable
	Fab arm exchange kinetics	FRET in PBST-DTT conditions	Comparable
		FRET in physiologically relevant conditions	Comparable
Potency and binding	Binding to α4β1-integrin	ELISA	Comparable
		SPR	Comparable
	Binding to α4β7-integrin	ELISA	Comparable
		SPR	Comparable
	Inhibition of interaction between VCAM-1-and α4 integrin	ELISA	Comparable
		Cell based assay	Comparable
		Flow cytometry	Comparable
	Inhibition of interaction between MAdCAM-1-and α4 integrin	ELISA	Comparable
	ADCC	Cell based assay	Comparable (no significant activity)
	CDC	Cell based assay	Comparable (no significant activity)
	ADCP	Cell based assay	Comparable (no significant activity)
	Binding to Fcγ receptors	SPR	Comparable (low binding)
	Binding to C1q	ELISA	Comparable (no significant binding)
	Binding to FcRn	SPR	Comparable
Product related variants	Dimers	SEC	Comparable
		SV-AUC	Comparable
	HMWI	SEC	Comparable
		SV-AUC	Comparable
	Total aggregates	SEC	Comparable
		SV-AUC	Comparable
	Monomer peak (Purity)	SEC	Comparable
		SV-AUC	Comparable
		CE-SDS non-reduced	Comparable
	Antibody fragments (LMWI)	CE-SDS non-reduced	Comparable
		CE-SDS reduced	Comparable
	Half antibodies	CE-SDS non-reduced	Comparable

Category	Product Attributes	Evaluation method	Key findings[1]
	Free thiols	Ellman's assay	Higher values for PB006, differences justified
Product related variants	Open disulfide bonds	LC-MS	Higher values for PB006, differences justified
	Acidic variants	CEX	Comparable
		cIEF	Comparable
	Basic variants	CEX	Lower values for PB006, differences justified
		cIEF	
	Main charge variant	CEX	Higher values for PB006, differences justified
		cIEF	
	Deamidation in the non-CDR	LC-MS	Comparable
	Deamidation in the CDR	LC-MS	Comparable
	C-terminal amidation	LC-MS	Higher values for PB006, differences justified
	Isoaspartic acid	Isoquant	Comparable
	Glycation	LC-MS	Comparable
	C-terminal lysine	LC-MS	Comparable
		CEX-CpB	Comparable
	N-terminal pyro-glutamate	LC-MS	Comparable
	Oxidation in the non-CDR	LC-MS (absolute method)	Higher values for PB006, differences justified
		RP-UPLC (Fc region oxidation)	
	Oxidation in the non-CDR/CDR region	HIC	Comparable
	Hydrophobic variant		Higher values for PB006, differences justified
	Oxidation in the CDR	LC-MS	Comparable
	N-glycosylation site occupancy	CE-SDS reduced	Higher values for PB006, differences justified
		LC-MS	
	Galactosylated N-glycans	HILIC	Higher values for PB006, differences justified
	Sialylated N-glycans	HILIC	Lower values for PB006, differences justified
	Afucosylated N-glycans	HILIC	Comparable
	High mannose N-glycans	HILIC	Comparable
	Main glycan	HILIC	Comparable
	Galactose-α-1,3-galactosylation	xCGE-LIF	Not detected in PB006
Strength and composition	Natalizumab concentration	UV-Vis Spectroscopy	Comparable
	Extractable volume	Gravimetric	≥ nominal volume (15.0 mL)
Particles	Visible particles	Visual inspection	Fulfills regulatory requirements
	Subvisible particles	LO	Fulfills regulatory requirements
		MFI	Comparable

[1] Comparable means all batches are within min/max ranges and/or Quality Ranges

Stability

The applicant presented a detailed dataset from long-term (5 ± 3°C up to 6 months), accelerated (25 ± 2°C/60 ± 5% R.H. up to 6 months) and stress (40 ± 2°C/75 ± 5% R.H. up to 6 months) stability studies as well as forced degradation studies (multiple stress conditions), in order to establish degradation profiles and to provide a direct stability comparison of PB006 and Tysabri.

No significant differences were observed under long-term and accelerated storage conditions up to and including the 6-month timepoint. Minor differences have been observed for stressed samples between PB006 and Tysabri, however no clinically meaningful impact is expected.

Bridging data of EU-approved and US-licensed Tysabri

An analytical bridging report has been provided between EU-approved Tysabri and US-licensed Tysabri for supporting the conclusions drawn from the PB006 clinical studies. The report consists of data from establishment of QTPP ranges. Although minor differences were detected, it is agreed that the analytical bridge is established with regards to identity and structure, potency and binding, product-related variants, strength and composition as well as degradation pathways and degradation kinetics.

Overall, taking into account the totality of the data provided, biosimilarity of Tyruko with Tysabri is considered demonstrated from a quality point of view.

2.4.3.6. Adventitious agents

Viral clearance studies were performed according to guidance documents. It is agreed that the PB006 active substance purification process provides sufficient capacity for virus clearance.

Process characterisation and validation studies were performed using scale down models, which were established for each step of the PB006 active substance manufacturing process. Enough details on each model were given, and the representativeness of each single manufacturing step was studied based on representative material. Results confirmed that down-scaled models for all manufacturing steps were suitable and representative of the manufacturing scale behaviour for the measured attributes, and therefore suitable for use in process characterisation and validation studies. Virus validation studies were performed with the same down scaled models as described in Section 3.2.R Scale Down Model Qualification Active substance.

Viral safety in relation to starting materials as well as virus validation were sufficiently addressed and depicted in the dossier, and the applicant's strategy is considered acceptable.

The documentation provided by the applicant confirms that active substance and finished product pose negligible risk for transmission of TSE and BSE and in relation to mycoplasma.

In summary, the adventitious agents safety evaluation is considered acceptable.

2.4.4. Discussion on chemical, pharmaceutical and biological aspects

Active substance

The applicant has developed PB006 as biosimilar to EU approved Tysabri (RMP), and submitted a well written quality part of the dossier. In brief, the QTPP of the biosimilar was designed based on the RMP, which formed the basis for all further development steps. Critical quality attributes were defined based on a risk assessment in combination with a comprehensive assessment of the active compound, based on a portfolio of validated or at least qualified orthogonal, and state-of-the-art methods. The manufacturing process and respective control strategy were developed based on the QTPP and identified CQAs, and validated. Active substance specification in combination with the manufacturing process control strategy covers all relevant quality attributes of PB006, and thus the manufacturing process

supplies the active substance at consistent and acceptable quality. Batches used for preclinical and clinical development were representative for the commercial manufacturing process, and stability data support the claimed active substance shelf-life of up to 24 months at long term storage conditions (5 ± 3ºC).

Finished product

Tyruko is manufactured according to a standard manufacturing process. No further changes to the formulation are introduced within the manufacturing process of PB006 finished product. All process steps, CPPs and critical IPCs are discussed.

Pharmaceutical development, manufacture and control of the finished product have been properly described. The results presented indicate consistency and uniformity of important product quality characteristics. The control strategy is sufficient to guarantee consistent/ satisfactory quality/performance of the product.

The proposed shelf life of 36 months when stored at the intended storage condition (2-8 °C, protected from light) is supported by appropriate data.

Biosimilarity

Overall, at the quality level biosimilarity between PB006 and EU-sourced Tysabri, which was used as clinical comparator, could be demonstrated for most quality attributes in a comprehensive analytical similarity exercise. In the PK/PD study EU-and US-sourced Tysabri were applied, and biosimilarity between both products could be demonstrated in the analytical similarity exercise.

Overall, taking into account the totality of the data provided, biosimilarity of Tyruko with Tysabri is considered demonstrated from a quality point of view.

2.4.5. Conclusions on the chemical, pharmaceutical and biological aspects

The overall quality of Tyruko is considered acceptable when used in accordance with the conditions defined in the SmPC. The different aspects of the chemical, pharmaceutical and biological documentation comply with existing guidelines.

In conclusion, based on the review of the data provided, the marketing authorisation application for Tyruko is considered approvable from the quality point of view.

2.4.6. Recommendation(s) for future quality development

None.

2.5. Non-clinical aspects

2.5.1. Introduction

Analytical and functional similarity studies of PB006 to both US- and EU-Tysabri are described and discussed in the Quality Assessment Report. No additional non-clinical PD studies, neither *in vitro* nor *in vivo*, were performed for this MAA. However, a comparative four-week repeated-dose toxicity study including a supportive toxicokinetic evaluation was conducted to compare the systemic PK and toxicological profile between PB006 and EU-Tysabri in Cynomolgus monkeys following IV infusion.

2.5.2. Pharmacology

No *in vivo* PD animal studies investigating analytical, physiochemical and functional similarity between PB006 and its referenced medicinal product (RMP) Tysabri (sourced from EU) were conducted in addition to the analytical biosimilarity assessment (see quality assessment).

2.5.3. Pharmacokinetics

Neither stand-alone comparative PK studies nor separate absorption, distribution, metabolism and/or excretion studies were performed with PB006 and EU-Tysabri. A comparative four-week repeat-dose toxicity study including supportive toxicokinetic evaluation was conducted to compare the systemic PK profile between PB006 and EU-Tysabri in Cynomolgus monkeys (3 animals/sex/treatment group) following IV infusion. Natalizumab (PB006 or Tysabri) was given every other day at dose levels of 3 or 30mg/kg body weight. Blood samples for toxicokinetic analysis were obtained predose and several hours post dose after the end of infusion on test days 1 and 30. To measure the concentrations of free Natalizumab (PB006 or Tysabri) in the cynomolgus monkey serum samples, a quantitative antibody sandwich enzyme linked immunosorbent assay (ELISA) was validated. All projected validation parameters and acceptance criteria, as calibration of a standard curve, accuracy, precision, freeze/thaw stability, long term stability and determination of a LLOQ (Lower Limit of Quantification), were met. In addition, an ELISA assay for determination of PB006 and EU-Tysabri in application formulation samples and an ELISA method for determination of anti-drug antibodies (ADAs) against Natalizumab (PB006 or EU-Tysabri) in cynomolgus monkey serum were validated. All validation results fulfilled the necessary requirements.

Exposure ratios based on C_{max} (maximum concentration) and $AUC_{0\text{-last}}$ (area under the concentration-time curve from time 0 to the time of the last measured concentration) were observed to be highly similar for all treatment groups on day 1, whereas on test day 30 differences were noted between the C_{max} and AUC values of PB006 and Tysabri in the high dose groups (males: 2831.3 µg/mL [SD: 604.6] of PB006 and 1708.6 µg/mL [SD: 651.9] of Tysabri; females: 1764.3 µg/mL [SD: 736.1] of PB006 or 858.7 µg/mL [SD: 868.2] of Tysabri). According to the applicant, this observation was considered to be due to ADA formation. To mention, in the high dose groups, only one animal was ADA positive on study day 30, specifically a female monkey of the Tysabri treatment group, which had already been ADA positive pre-dose on day 1. Therefore, this explanation might be applicable for females, whereas it cannot explain the differences observed between both Natalizumabs in male animals. Almost all animals in the low dose group of PB006 and Tysabri were found to be ADA positive on test day 30 and showed low serum levels of the drug substance, although female animals treated with Tysabri had significant lower ADA levels (one of them had no ADAs at all), with corresponding slightly higher Natalizumab levels in serum samples, compared to PB006 treated female monkeys. Hence, ADA formation seems to have neutralizing character mirrored by decreased levels of Natalizumab, especially in PB006 treated female animals. The lack of detection of ADAs that presumably also appeared in the high dose groups of PB006 and Tysabri, could be attributed to either immunotolerance due to high dose treatment or to the masking of these antibodies that reacted and were saturated with the test or reference item, respectively, and therefore, not quantifiable. Nevertheless, the low group size (3 animals/sex/group) in conjunction with individual data variability is limiting the sensitivity of the test system and therefore does not allow to reliably distinguish between identified differences of PB006 and Tysabri treated animals.

2.5.4. Toxicology

2.5.4.1. Single dose toxicity

No dedicated single-dose toxicological studies were conducted with PB006.

2.5.4.2. Repeat dose toxicity

The applicant conducted a 4-week repeat-dose toxicity study in cynomolgus monkeys to evaluate and compare potential toxicological findings and the toxicokinetic profile of PB006 with its referenced medicinal product Tysabri, sourced from EU. The 4 week-duration of this study is consistent with the posology of PB006 which is to be administered by IV infusion once every 4 weeks. Therapy may be continued up to 2 years (after that therapy should be considered only following a reassessment of the potential for benefit and risk). However, up to 6 months repeated-dose toxicity studies have been performed with Tysabri and as analytical and functional similarity of PB006 to EU-Tysabri was demonstrated in *in vitro* studies, longer duration toxicity studies of PB006 were not conducted and are not considered relevant for this biosimilar development, in which reference is made to EMA's previous findings on safety and efficacy of Tysabri. The Cynomolgus monkey is considered an adequate model for the toxicity study of PB006, as Cynomolgus monkeys were consistently used in the original nonclinical studies performed for registration of the reference product Tysabri.

Animals were assigned to 5 treatment groups (control group, low and high dose groups of PB006 and Tysabri; with 3 animals/sex/group) receiving IV infusions of Natalizumab (0, 3 or 30mg/kg b.w.) every other day. No findings and noteworthy differences were observed with PB006 or Tysabri treated animals regarding local tolerance, mortality, clinical signs (systemic tolerance), body weight and body weight gain, food and drinking water consumption, electrocardiogram (ECG), circulatory functions, clinical biochemistry, urinalysis, ophthalmological and auditory examinations, macroscopic systemic post mortem findings, organ weights and histopathology. Observed differences between control groups and PB006 and RMP treated animals may likely be due to the limited sample size (3 animals/sex/group) and variability within individual animals.

However, some changes in haematological parameters were reported by the applicant. Both high dose groups (30mg/kg of PB006 or Tysabri) showed an increase in the absolute number of the total and differential leucocyte count and in the number of reticulocytes, which are considered common and reproducible effects of natalizumab in Cynomolgus monkeys. Furthermore, again concerning the high dose groups, an increase in the absolute number of all lymphocyte subtypes compared to the control was noted. Overall, with some exceptions (e.g. sup./cyt. T-cells and act. sup./cyt. T-cells for PB006, 30mg/kg; act. sup./cyt. T-cells and double negative T-cells for Tysabri, 30 mg/kg), these observations were slightly more obvious in male animals. Again, differences between the treatment groups might be relativized by disparities of values from animals within the same treatment groups and limited sample size. Male and female animals treated with 3 or 30 mg/kg Natalizumab (PB006 or Tysabri) revealed to have elevated myeloid : erythroid ratios. Low and high dosage groups of PB006 treated animals seemed to be slightly more affected compared to animals treated with the RMP.

ADA formation was investigated in the course of the 4-week repeat-dose toxicity study in cynomolgus monkeys. Almost all animals in the low dose groups of PB006 and Tysabri were found to be ADA positive on test day 30 with corresponding low levels of Natalizumab in serum samples. Female animals treated with 3mg/kg Tysabri had significantly lower ADA levels (one out of three revealed to have none at all), compared to 3mg/kg PB006 treated female monkeys, which correlates with slightly higher Natalizumab levels in serum samples in the RMP treated group. In contrast to the low dose groups, no ADAs were

reported for the high dose groups of PB006 and Tysabri, with the exception of one female animal of the EU-Tysabri group that already showed to have ADAs prior to its first Natalizumab infusion.

The applicant determined the No-Observed-Adverse-Effect-Level to be above 30 mg/kg PB006 or EU-Tysabri (IV, every second day for 4 weeks), with the justification that all findings were considered to be anticipated PD effects of natalizumab being a monoclonal antibody binding to α4 integrin.

2.5.4.3. Genotoxicity

No dedicated genotoxicity studies were conducted with PB006.

2.5.4.4. Carcinogenicity

No dedicated carcinogenicity studies were conducted with PB006.

2.5.4.5. Reproductive and developmental toxicity

No dedicated developmental and reproductive studies were conducted with PB006.

2.5.4.6. Toxicokinetic data

A comparative four-week repeat-dose toxicity study including a supportive toxicokinetic evaluation was conducted to compare the systemic PK profile between PB006 and EU-Tysabri in cynomolgus monkeys (3 animals/sex/treatment group) following IV infusion. For further details please refer to section 2.5.3. Pharmacokinetics.

2.5.4.7. Local Tolerance

No local tolerance studies were performed with PB006 given that all the excipients used in the final commercial formulation are commonly used in currently approved biologics with the same exposure levels and same intended route of administration. Moreover, even if stand-alone local tolerance studies of PB006 have not been performed, in the 4 weeks repeated dose toxicity study in cynomolgus monkey, no test item-related signs of local intolerance have been shown.

2.5.4.8. Other toxicity studies

No dedicated "Other toxicity studies" were conducted with PB006.

2.5.5. Ecotoxicity/environmental risk assessment

In line with the Guideline on the environmental risk assessment (EMEA/CHMP/SWP/4447/00 corr 2) in case of products containing proteins, the applicant provided a justification that the use of Tyruko is unlikely to result in a significant risk to the environment.

This is acceptable.

2.5.6. Discussion on non-clinical aspects

Pharmacodynamics

No *in vivo* PD animal studies were conducted in addition to the analytical *in vitro* biosimilarity assessment. This is accepted and in agreement with the EMA Guideline on similar biological medicinal products (CHMP/437/04 Rev 1; 2014) and the EMA Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues (EMEA/CHMP/BMWP/42832/2005 Rev 1).

For review of the biosimilar comparability exercise, please refer to the discussion and conclusion section of the respective quality assessment.

Pharmacokinetics

Overall, methods of analysis were adequately validated and the applicant's approach can be regarded as appropriate. The results of the validations confirmed that the methods employed were suitable for the detection of Natalizumab (PB006 and Tysabri) in application formulations and cynomolgus monkey serum samples, and for the detection of ADAs against PB006 or EU-Tysabri in cynomolgus monkey serum.

The similarity between the originator and the biosimilar product was assessed in the *in vitro* quality biocomparability testing. In contrast to *in vitro* methods, *in vivo* studies in animals are not necessarily considered informative for the PK similarity / comparability exercise. Due to the high variability, these models are often too insensitive. This conclusion concerns both PK comparisons and comparisons on safety. Based on these considerations, these comparative studies in animal models have been discouraged in a previous Scientific Advice (EMA/CHMP/SAWP/224608/2017).

Additionally, ADA data from animals are not predictive for the clinical situation, and comparative data on ADA are of uncertain relevance. Potential differences in immunogenicity between PB006 and Tysabri should be evaluated in the clinical setup. Therefore, the conducted *in vivo* study can at most be considered supportive, but not as evidence of true clinical similarity.

As indicated by the applicant, the comparative 4-week repeat-dose toxicity study in cynomolgus monkeys with PB006 and EU-Tysabri, including toxicokinetic evaluations, was conducted to satisfy requirements laid out by other health authorities at that time to support entry into global clinical development. The study design, as well as provided data (e.g. results of ADA and Natalizumab concentration evaluations) are considered adequate and sufficient for this purpose. Toxicokinetic data evaluation revealed that there were no noteworthy differences between PB006 and Tysabri with respect to their toxicokinetic profiles in monkeys. As demonstrated in the Tysabri EPAR, natalizumab shows a pk typical profile of monoclonal antibodies, with dose-dependent but not dose-proportional increases in C_{max} and AUC values and increases in elimination half-lives with increasing dose. This profile is probably the result of saturation of the major antibody clearance pathway (Fc-mediated phagocytosis).

In this sense, it is acknowledged that the *in vivo* study was conducted due to expectations from regulatory bodies competent for non-European regions. Though not endorsed, these *in vivo* testing studies are accepted.

Toxicology

Generally, studies regarding toxicology, including developmental and reproductive toxicity studies, are not required for non-clinical testing of biosimilars according to the EMEA/CHMP/BMWP/42832/2005 Rev1 guideline. Neither are studies regarding safety pharmacology, carcinogenicity and local tolerance. Scientific advice was provided by the EMA (EMA/CHMP/SAWP/224608/2017), supporting the proposed strategy of the applicant to not conduct *in vivo* animal studies to assess biosimilarity between PB006 and its RMP Tysabri. Nevertheless, a comparative 4-week repeat-dose toxicity study in cynomolgus monkeys was conducted to satisfy requirements laid out by other health authorities to support entry into global clinical development.

Overall, the study design is regarded as appropriate in terms of species selection, used dosages, frequency and route of administration. Observed findings, such as increased haematological parameters (lymphocytes, reticulocytes; myeloid: erythroid ratio), were seen in all animals treated with either PB006 or Tysabri. Altered trafficking of lymphocytes, seen as an increase in white blood cells, was already reported in the EPAR of Tysabri (EPAR Tysabri, latest updated version 8th of June 2022) and was described to be reversible without any adverse toxicological changes. In this comparative repeat-dose toxicology study with PB006 and EU- Tysabri, no recovery groups were included. Although this would have been of interest, the lack of a recovery period is accepted, because reported findings of Natalizumab are already known for Tysabri and related to its pharmacological activity. A main issue remains the limited number of animals per treatment group (3/sex/group), which does not allow to reliable distinguish between differences in findings of PB006 and Tysabri treated animals and individual data variability. Based on the results of the study and taking into consideration the intrinsic variability of the model, the limited sample size and the absence of adverse histopathological findings in any of the animals, there was no noteworthy difference between the animals treated with PB006 and the animals treated with Tysabri from a toxicity and toxicokinetic point of view. All findings were considered to be anticipated PD effects of natalizumab as a monoclonal antibody binding to α4 integrin, i.e inhibition of lymphocyte migration from blood vessels into the surrounding tissues. Blocking of the α4 integrin also allows migration of the immature red blood cells from the bone marrow and consequently, the increase in progenitor cell circulation. No new unexpected toxicities were identified for PB006. The marginal differences observed are considered to be within the biological variability. The toxicological similarity assessment raised no concerns. Therefore, data obtained from this repeat-dose toxicology study can be regarded as supportive information in addition to the *in vitro* similarity/comparability exercise, discussed in the quality part of the dossier.

Regarding the ADA assessment of this study, the applicant provided a discussion for possible reasons for ADA detection prior to first infusion on day 1 for female animal number 30 of the 30 mg/kg b.w. Tysabri treatment group. It was confirmed that according to the protocol only treatment naïve animals were used in the repeat-dose toxicology study in cynomolgus monkeys and that due to the consistent discrepancy at both pre- dose and on day 30, contamination of the serum samples is unlikely. The existence of pre-existing drug-reactive antibodies as well as other non-antibody interferences in the ELISA assay (e.g. matrix effects leading to the binding of the biotinylated antibody) was discussed, whereat a non-ADA mechanism seems to be more likely (as no ADAs developed in other animals in the high dose group due to interference with high Tysabri levels). Furthermore, serum natalizumab levels of this female animal were similar to the serum natalizumab concentrations in other animals of this dose group and ADA detection at pre-dose in female number 30 had no influence on data interpretation in this study.

Environmental Risk Assessment

The active substance is a natural substance, the use of which will not alter the concentration or distribution of the substance in the environment. Therefore Tyruko is not expected to pose a risk to the environment.

2.5.7. Conclusion on the non-clinical aspects

The non-clinical dossier can be considered acceptable.

2.6. Clinical aspects

2.6.1. Introduction

GCP aspects

The Clinical trials were performed in accordance with GCP as claimed by the applicant.

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

The development program comprises 4 clinical studies: 1 pivotal Phase 1 PK/ PD study with PB006, US-Tysabri, and EU-Tysabri in healthy subjects, 1 pivotal Phase 3 study with PB006 versus EU-Tysabri in patients with RRMS, 1 pilot Phase 1 PK/PD study with EU-Tysabri in healthy subjects and 1 supportive Phase 1 safety study with PB006 in healthy subjects.

- **Tabular overview of clinical studies**

Table 2: Clinical development program of PB006

Study Identifier	Location of Study Report	Objective of Study	Study design and Type of Control	Test Products; Dosage Regimen; Route of Administration	Number (N) of Subjects (treated)	Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report
[Tysabri Pilot-01-01]	Module 5.3.3.1	Safety/immuno-genicity, PK, PD	Single-center, randomized, 3-arm parallel group study	EU-Tysabri Single-dose, at 1, 3 or 6 mg/kg IV infusion	36 subjects N=12 per dose group	Healthy subjects	1 day	Completed; Full
[PB006-01-02]	Module 5.3.3.1	Safety/immuno-genicity	Single-center, single-arm, open-label study	PB006 Single-dose, 300 mg IV infusion	10 subjects	Healthy subjects	1 day	Completed; Full
[PB006-01-03]	Module 5.3.4.1	PK, PD, safety/immuno-genicity	Multi-center, randomized, double-blind, 3-arm parallel group study	PB006, US-Tysabri or EU-Tysabri Single-dose, 3 mg/kg IV infusion	450 subjects PB006: N=149 US-Tysabri: N=150 EU-Tysabri: N=151	Healthy subjects	1 day	Completed; Full
[PB006-03-01]	Module 5.3.5.1	Efficacy, safety/immuno-genicity, PK	Multi-center, randomized, double-blind, 2-arm parallel group study	PB006 or EU-Tysabri 300 mg, every 4 weeks, for a total of 48 weeks (12 infusions) IV infusion	264 subjects PB006: N=131 EU-Tysabri: N=133*	Patients with RRMS	48 weeks	Completed; Full

EU=European Union, IV=intravenous, N=number of subjects, PD=pharmacodynamic, PK=pharmacokinetic, RRMS=relapsing-remitting multiple sclerosis, US=United States.
* A subset of 30 patients enrolled in the EU-Tysabri group was switched after 24 weeks from treatment with EU-Tysabri to treatment with PB006 for the remaining period. Thus, a total of 161 patients were treated with PB006.

2.6.2. Clinical pharmacology

2.6.2.1. Pharmacokinetics

The **PB006-01-03**. was a Phase 1 multicenter, double-blind, randomized, single-dose, 3-arm, parallel-group PK/PD study in healthy subjects, to investigate the similarity between PB006 versus US-Tysabri and versus EU-Tysabri.

Study **PB006-03-01** was a phase 3 confirmatory efficacy and safety study in RRMS patients and is presented in detail under efficacy section. Apart from efficacy evaluation based on MRI endpoints, relapse rates and disability status, this study also assessed natalizumab trough concentrations and immunogenicity.

Analytical methods

For quantification of MAdCAM-1 and VCAM-1 levels in human serum, the applicant has applied an automated ELISA system based on the Simple Plex ELLA multiplex platform together with combined MAdCAM-1 and VCAM-1 analysis kits purchased from R&D Systems. In brief, diluted samples and running buffer are pipetted into the inlet of a cartridge. Samples are directed by microfluidic channels into different compartments, where they interact with analyte specific capture and labelled detection antibodies pairs. After fully automated, sequential incubation and washing steps fluorescence is measured through glass nano reactor tubes. Each cartridge lot of the assay is already provided in a pre-validated format, including pre-validated ranges from 0.003 ng/ml to 13 ng/ml and from 0.6 ng/ml to 83 ng/ml for MAdCAM-1 and VCAM-1, respectively. Factory calibration curves are generated on a lot-to-lot basis, including assessment of accuracy and precision over the acceptance range. The manufacturer also confirms robustness of the assay for the duration of the cartridge shelf life. No additional calibration samples nor calibration curves were generated. Partial re-validation of the assay for each cartridge included the assessment of precision for QC (quality control) samples, the evaluation of a prozone (hook effect), selectivity (haemolyzed, lipemic samples), drug tolerance (confirmed up to 328 µg/ml), freeze/thaw stability, bench-top stability for up to 24 hours, freeze/thaw stability and frozen sample storage stability. Activities were performed at ICON Bioanalytical Laboratories, NL and at PRA Health Sciences, NL. Samples were applied only once but measured in triplicates, and standard settings in the software were used for data evaluation, acceptance and rejection (>10.0% coefficient variation of the RFU) criteria. QC samples (low, medium and high concentration) were prepared by using reagents (controls and assay diluents) from the kit. Thus some aspects of the method validation such as determination of precision and prozone effect were performed in assay buffer. Blank human serum was used as endogenous QC sample, and to monitor the longitudinal assay performance. All pre-defined validation parameters were met. Frozen storage stability at -70°C was demonstrated for at least 65 weeks for MAdCAM-1. For VCAM-1 endogenous QC samples were measured over a period of 65 weeks, confirming stability of study samples. Taken together, provided validation program covered most but not all aspects: Assay intermediate precision and repeatability were not indicated, and it is unclear if a matrix representative for the clinical samples was used by the manufacturer of the kit for the assessment of accuracy and range. The applicant has applied assay buffer for re-validation of precision and for the assessment of a potential prozone effect. The applicant provided further details on validation activities and justified the omission of the human serum matrix for the assessment of the precision and prozone effect. Proposed automated ELISA multiplex MAdCAM-1 and VCAM-1 assay seems suitable for the intended application.

The applicant has developed and validated for assessment of PK two sandwich ELISA to quantify "unexchanged" and total Natalizumab in human serum samples. The term "unexchanged" refers to bivalent antibodies which have not undergone Fab-arm exchange. Thus this assay is detecting "functional" Natalizumab, which fulfils the purpose of a PK assay, and total amounts of Natalizumab by two different ELISA. In brief, Natalizumab is captured in the functional Natalizumab setting by an immobilised anti-Natalizumab Fab (Fragment antigen-binding) and detected via an HRP-labelled anti-Natalizumab-MAb conjugate. For the total Natalizumab setting, a human monoclonal Natalizumab specific IgG1 antibody is used as coating agent, while detection occurs via a HRP-labelled murine anti-human IgG4 antibody. Standard curves were fitted in both cases using a 4 parameter logistic model, and used for the evaluation of concentrations of unknown samples. Both methods seem scientifically justified. They were fully validated for their precision, selectivity (in normal and in patient serum), minimal required dilution, range (from 60-400 ng/ml in both cases), integrity of dilution and robustness by assessing the impact of haemolytic and lipemic serum, as well as freeze-thaw, bench top, and long-term stability for samples and stock solutions). Specific recognition of "unexchanged" Natalizumab in the first ELISA setting was sufficiently demonstrated. The validation matrix was a pool of a suitable number of patient serum samples, and thus representative. All reagents, their provenience and the most

important methodological details were reported. Validation reports and respective amendments were provided. All predefined acceptance criteria of study plans were met which qualifies both PK assays for their intended use.

Administration of Natalizumab is associated with increased numbers of CD19+ B cells and increased numbers of immature CD34+ progenitor cells released from the bone marrow. The applicant has developed a battery of FACS based assays for immuno-phenotyping of whole blood cells, in order to assess bio-similarity of PB006, Tysabri EU and Tysabri US. Samples were analyzed with a flow cytometer (FACSCanto II) equipped for measuring FITC, PE, PerCP-Cy5.5, PE-Cy7, APC and APC-Cy7. BD FACSCanto Clinical Software v3.1 was used for data acquisition. For data processing, De Novo FCSExpress 6 Flow Clinical Edition was used.

Quantification of CD19+ B-cells in human whole blood was part of this comparability study. CD19 may be involved in activation and proliferation of B lymphocytes and is expressed at all stages of maturation, but gets lost on plasma cells. In brief, whole blood samples are treated with anti-coagulant K3EDTA and fixation reagent Cyto-Chex BCT. Staining is performed with BD Multitest™ 6-color TBNK Reagent, while only B-cells were reported in this study. A validation run is performed each day including the use of setup beads with stable fluorescence intensity through time to ensure stable performance of the flow cytometer. Prior to the staining of cells, a white blood cell (WBC) count was carried out to ensure that the total WBC count was between below $33.0x10^6$ WBC/mL. Two separate validation reports for the same analytical procedure were provided for the NL and the US site. A cross-validation of bioanalytical laboratories was performed. The assay was validated for its repeatability, intermediate precision, and reproducibility between Assen (NL) and Lenexa (US) Bioanalytical Laboratories. Inter-operator reproducibility and inter-analyst data analysis reproducibility were evaluated. Blank matrix consisted of whole blood donations from three individuals collected at 5 time-points. Inter-assay reproducibility was analysed in three runs on three separate days. Stability of whole blood and stained cells as well as carry-over were also assessed. Tysabri EU, Tysabri US and PB006 had the same outcome with regards to intra-assay reproducibility, whole blood stability and stained cell stability. Therefore it was concluded that the assay is measuring all 3 compounds in a comparable way. The method met the requirements of the validation plan and was considered suitable for determination of CD19+ B cells in human whole blood samples.

The same quantification protocol for CD34+ cells in human whole blood was applied in studies PLP19696-19696X-O (NL) and PLP19696-19696XP (US). Two separate bioanalytical reports were provided. The single tube BD™ Stem Cell Enumeration kit was used enabling simultaneous enumeration of viable dual positive CD45+/CD34+ hematopoietic stem cell populations. The absolute number of positive cells in the sample was determined by comparing cellular events to a known number of fluorescent beads released by BD Trucount reagent. Concentration, number and percentage of CD34+ cells of the CD45+ cell population were reported. WBC counts prior to each measurement ensured that samples were in the linear range of the assay. Validation parameters included intra-assay reproducibility, inter-assay reproducibility, interoperator reproducibility, inter-analyst-data-analysis reproducibility, stability of whole blood, stability of stained cells, carry-over and cross-validation of NL and US bioanalytical laboratories. PB006, EU-Tysabri and US-Tysabri were comparable within the assay, and the method was found to be suitable for the intended application.

Saturation of α4-integrin on leukocytes surfaces is part of Natalizumab's mechanism of action. α4-integrin %RS in human whole blood is assessed by the same flow cytometry assay, which was validated in studies PLP19696-19696X-L (NL) and PLP19696-19696X-N (US), for analysis of samples collected at EU and US sites, respectively. Two separate bioanalytical reports were provided. In brief, Leukocytes are quantified via CD45, and α4-integrin bound Natalizumab by addition of mouse anti-human IgG4 Fc-PE. Maximum %RS was assessed in a second aliquot of each sample where unlabelled PB006 was added. The method was validated for its intra-assay reproducibility, inter-assay reproducibility, interoperator

reproducibility, inter-analyst-data-analysis reproducibility, stability of whole blood, stability of stained cells, carry-over and cross-validation of bioanalytical laboratories and found to be suitable for analysis of clinical samples. Again PB006, EU-Tysabri and US-Tysabri were comparable within the assay, and the method was found to be suitable for the intended application.

Bioequivalence (Study PB006-01-03)

Study PB006-01-03 was a multicenter PK/PD study in healthy subjects, to support the demonstration of similarity between PB006 versus EU-Tysabri and versus US-Tysabri.

Methods

Study design

This was a randomized, double-blind study with 3 parallel arms in 453 healthy male and female subjects. Subjects received a single dose of 3 mg/kg PB006, EU-approved Tysabri, or US-licensed Tysabri as an IV infusion over a 60-minute period. Dosing was followed by PK and PD sampling for 85 days and a final follow-up visit 6 months (24 weeks) after dosing to assess new neurological symptoms that could be suggestive for progressive multifocal leukoencephalopathy (PML). Safety was monitored throughout the study by repeated clinical and laboratory evaluations. Samples were collected for immunogenicity assessments for 85 days.

The study was performed at six clinical sites, 4 of which were in the US, 1 in the Netherlands, and 1 in Poland.

Study participants

Healthy male and female subjects aged 18 to 65 years (18 to 54 years after implementation of protocol amendment 3), with a body mass index (BMI) of 18.5 to 30.0 kg/m^2 (body weight 50 – 92 kg), who were anti-John Cunningham virus (JCV) antibody negative and tested negative for SARS-CoV-2 before dosing were eligible for this study.

Treatments

Subjects were randomized in a 1:1:1 ratio to PB006, US-Tysabri or EU-Tysabri and received single IV infusions of 3 mg/kg natalizumab.

The study drug was administered on day 1 with the subject in the upright position. Subjects fasted overnight for at least 10 hours following a light supper on the evening before. Breakfast was consumed after the end of the IV infusion. During fasting, no fluids were allowed except water; water was not allowed from 2 hours prior to dosing until the end of the IV infusion. A fasting period of at least 4 hours was required before obtaining clinical laboratory samples at all time points.

Objectives

The primary objective of the study was to demonstrate PK and PD similarity of PB006 to both US-licensed Tysabri and EU-approved Tysabri.

Outcomes/endpoints

PK endpoints

The primary PK endpoint was $AUC_{0\text{-}inf}$ of total natalizumab, which was compared between PB006, EU-Tysabri, and US-Tysabri in a 3-way comparison. This comparison was part of a hierarchical testing procedure. As a prerequisite for the pooling of the reference, PK similarity needed to be established (PK bridge between EU-Tysabri and US-Tysabri) and a pre-defined pooling criterion had to be fulfilled. For the 3 pairwise comparisons of $AUC_{0\text{-}inf}$ the 90% CI (confidence interval) for the ratio of the test and reference products should be contained within the acceptance interval of 80.00% to 125.00%.

As secondary PK endpoints, $AUC_{0\text{-}t}$, C_{max}, and t_{max} (time to C_{max}) of total natalizumab were selected to support the PK comparability of PB006 to EU-Tysabri and US-Tysabri. Further secondary PK endpoints were $AUC_{0\text{-}inf}$, $AUC_{0\text{-}t}$, C_{max}, and t_{max} of unexchanged natalizumab. Other PK parameters were analyzed descriptively.

PD endpoints

For PD, two co-primary endpoints were selected: $AUEC_{0\text{-}12w}$ (area under the effect curve from zero to 12 weeks) of baseline-adjusted CD19+, and $AUEC_{0\text{-}12w}$ of α4-integrin %RS. As an additional analysis, $AUEC_{4\text{-}12w}$ (area under the effect curve from 4 to 12 weeks) for α4-integrin %RS was specified. For these parameters the 95% CI for the ratio of the test and reference products should be contained within the acceptance interval of 80.00% to 125.00%.

Secondary PD endpoints included E_{max} (maximum effect) and t_{max} of baseline-adjusted CD19+, $AUEC_{base\_neg}$, (area below the individual baseline value minus the area above the individual baseline in case of time intervals for which the effect time curve exceeded the baseline value), E_{min} (minimum effect), t_{min} (time to minimum concentration) of sVCAM and sMAdCAM and $AUEC_{0\text{-}t}$ (area under the effect curve from zero to last timepoint), E_{max}, t_{max} of CD34+.

Prior to each PK sampling point, each subject was to remain at least 5 minutes in a supine position. Blood samples of 6 mL each were taken for analysis of total and unexchanged natalizumab in plasma samples. Blood samples of approximately 11 mL were collected for measurement of relative a-integrin receptor saturation (a-integrin %RS) and CD19+ and CD34+ cells. Blood samples of approximately 6 mL were collected for measurement of sVCAM and sMAdCAM.

Sample size

The sample size calculation was based on the following assumptions:

- Test/Reference ratio:
  - PK: 0.95–1/0.95 for $AUC_{0\text{-}inf}$,
  - PD: 0.95–1/0.95 for $AUC_{0\text{-}12w}$ for baseline-adjusted CD19+ and $AUC_{0\text{-}12w}$ for α4-integrin %RS
- Significance level: 5% (Two one-sided tests corresponding to 90% CIs for the Test/Reference ratio)
- Similarity margin: 0.8000–1.2500
- Randomization ratio: 1:1:1

The assumptions for the coefficients of variation for the arithmetic means (CV) and for the geometric means (gCV) were based on the data of the pilot study Tysabri Pilot-01-01.

The demonstration of PD similarity was planned to be conducted while combining the data of the US and EU-reference products for the statistical analysis of the primary PD endpoint baseline-adjusted CD19+ $AUEC_{0\text{-}12w}$ and α4-integrin %RS $AUEC_{0\text{-}12w}$ subsequent to having established the PK bridge between the 2 reference products and meeting the predefined pooling criterion.

For the 3-way comparisons of PK a between-subject gCV of 36% was assumed for the first step of the hierarchical testing strategy. For the PD hypothesis, tested in the second step of the hierarchical model, a between-subject gCV of 56% was assumed. For the sample size consideration, the PD endpoint α4-integrin %RS was neglected, since the variability was considered lower compared to CD19+ $AUEC_{0\text{-}12w}$.

In earlier protocol versions 82 evaluable subjects per arm were seen required to ensure an overall power of at least 80% (93% power for each of the 3 pairwise comparisons resulting in 0.93x0.93x0.93=0.804) including the comparison of US-licensed Tysabri vs EU-approved Tysabri establishing the PK bridge.

According to the latest protocol version, the analysis of baseline-adjusted CD19+ $AUEC_{0\text{-}12w}$ pooling US-licensed Tysabri and EU-approved Tysabri subsequent to having established the PK and thus the scientific bridge resulted in a 1:2 randomization ratio.

Total N=411 evaluable subjects (137 evaluable subjects per arm) was derived by updated sample size calculations, using a 90% CI for the geometric mean ratio, which resulted in a power of 93%. Using a 95% CI, which was seen required to meet global regulatory expectations, for the geometric mean ratio resulted in a power of 88%. The power above 80% seemed to be appropriate to account for the additional PD endpoint α4-integrin %RS.

After accounting for 10% drop-outs, the total number of subjects to be randomized was calculated to be 453 subjects (151 per treatment group). The 10% drop-out rate included subjects that terminated prematurely (discontinued from the study or withdraw consent), as well as subjects with protocol deviations that warranted their exclusion from the analysis of the primary endpoint.

Randomisation

After obtaining informed consent, subjects were to be screened according to the inclusion and exclusion criteria. Subjects who met all eligibility criteria were supposed to receive a subject number upon inclusion in the study. Subjects were planned to be randomly assigned in a [1:1:1] ratio to receive study intervention. Stratification was to be performed according to body weight class in order to ensure balance across the study arms (50kg to 65kg, >65kg to 80kg, >80kg to 92kg). No gender stratification was deemed necessary, as gender was not found to have an effect on the PK properties of natalizumab. The study was planned to be performed at 6 sites by competitive recruitment. The clinical study protocol (CSP) defined 3 weight groups in order to ensure balance across the study arms; however, all eligible subjects were to be admitted in the study. For that reason, 18 randomization lists were created, 1 per site and weight group combination. Subjects were to receive the subject number just prior to dosing and according to the randomization code generated. The subject number was to ensure identification throughout the study. Replacement subjects were to be administered the same treatment as the subject replaced. The final randomization list was created, reviewed, and approved by 2 designated biostatisticians who were not members of the study team. After the final randomisation list was approved, it was transferred to the responsible pharmacy and kept in a restricted area.

Blinding

Subjects were planned to be randomly assigned in a [1:1:1] ratio to receive study intervention. Investigators were planned to remained blinded to each subject's assigned study intervention throughout the course of the study. In order to maintain this blind, an otherwise uninvolved third party (e.g, site pharmacy staff) was planned to be responsible for the dilution and dispensing of all study intervention and delivery to site.

A sealed envelope that contained the study intervention assignment for each subject was planned to be provided to the Investigator. The sealed envelope was to be retained by the Investigator (or representative) in a secured area. In case of an emergency, the Investigator had the sole responsibility for determining if unblinding of a subject's treatment assignment was warranted.

If the Investigator decided that unblinding was warranted, the Investigator had to make every effort to contact the Sponsor prior to unblinding a subject's treatment assignment, unless this could delay emergency treatment of the subject. If a subject's treatment assignment was unblinded, the Sponsor was to be notified within 24 hours after breaking the blind.

Statistical methods

<u>Analysis Populations</u>

Safety Population

All subjects assigned to study intervention and who were dosed with study intervention were to be included in this population. Subjects were to be analysed according to the study drug received.

Pharmacokinetic Population

All subjects randomized to study intervention and who were dosed with study intervention and have not experienced any major protocol deviations that might impact PK results and provide sufficient samples to allow calculation of the PK endpoint $AUC_{0\text{-}inf}$ were to be included in this population.

Pharmacodynamic/Target Receptor Engagement Population

All subjects randomized to study intervention and who were dosed with study intervention and did not experience any major protocol deviations that might impact the PD results and provided sufficient samples to allow calculation of the primary PD endpoints $AUEC_{0\text{-}12w}$ of baseline-adjusted CD19+ and/or α4-integrin %RS were to be included in this population.

There were several analysis sets defined in addition to the sets originally defined in the SAP: CD19+ Set, RS/RO Set – primary analysis for $AUEC_{0\text{-}12w}$, RS/RO Set – sensitivity analysis for $AUEC_{0\text{-}12w}$, RS/RO Set – main analysis for $AUEC_{4\text{-}12w}$, RS/RO Set – sensitivity analysis for AUEC4-12w, and PD Other Set – analysis set for VCAM, MadCAM, CD34.

General aspects of statistical analyses

Baseline (demographic) data was planned to be summarized by treatment group, PK, PD, and safety results by treatment group and time point.

Statistical analysis methods for primary endpoints

All PK analyses were to be performed on the PK Set.

For the analysis of the primary PK parameter $AUC_{0\text{-}inf}$ of total natalizumab a 3-way comparison was to be performed containing the comparison of PB006 to both US-licensed Tysabri and EU-approved Tysabri as well as the PK bridge between US-licensed Tysabri and EU-approved Tysabri. The 3-way comparison was the first step of the 2-step hierarchical testing procedure. For each comparison an analysis of variance (ANOVA) was to be performed on the ln-transformed primary PK parameter $AUC_{0\text{-}inf}$. The ANOVA was to include calculation of least-squares means (LSM) for the treatments. The ratios of LSM were to be calculated using the exponentiation of the LSM differences from the analyses on the corresponding ln-transformed PK-parameters. The 90% CIs were to be calculated and back-transformed to the original scale. PK similarity was to be concluded if the respective CIs for $AUC_{0\text{-}inf}$ were completely included in the similarity margin of 0.8000 to 1.2500.

All PD analyses were to be performed on the relevant PD population.

The comparison for PD similarity regarding the primary PD endpoint $AUEC_{0\text{-}12w}$ of baseline-adjusted CD19+ and $AUEC_{0\text{-}12w}$ of α4-integrin %RS was considered the second step of the 2-step hierarchical testing procedure. The data of US-licensed Tysabri and EU-approved Tysabri were planned to be combined/pooled for the comparison with PB006 if:

- the analytical and PK bridge has demonstrated similarity between US-licensed Tysabri and EU-approved Tysabri and
- the subsequently described predefined pooling criterion for the respective PD parameter (see further below) was fulfilled.

For the analysis of the primary PD endpoint $AUEC_{0\text{-}12w}$ of baseline-adjusted CD19+, an ANCOVA was to be performed on the ln-transformed PD parameter baseline-adjusted $AUEC_{0\text{-}12w}$ of baseline-adjusted CD19+. The ANCOVA model was to include the ln-transformed individual baseline value of CD19+ as

a covariate. The ANCOVA was to include calculation of LSM for the treatments. The ratios of LSM were to be calculated using the exponentiation of the LSM difference from the analyses on the ln-transformed PD parameter. The 90% and 95% CIs were to be back-transformed to the original scale.

Predefined pooling criterion:
The ANCOVA comparing only US-licensed Tysabri and EU-approved Tysabri was to be calculated as described above for CD19+. If the 95% CI back-transformed baseline-adjusted $AUEC_{0\text{-}12w}$ of baseline-adjusted CD19+ did not completely fall outside of [95.00%, 105.00%], the reference data was planned to be pooled for the primary analysis. If the data could not be pooled, the unpooled analysis was planned to be presented.

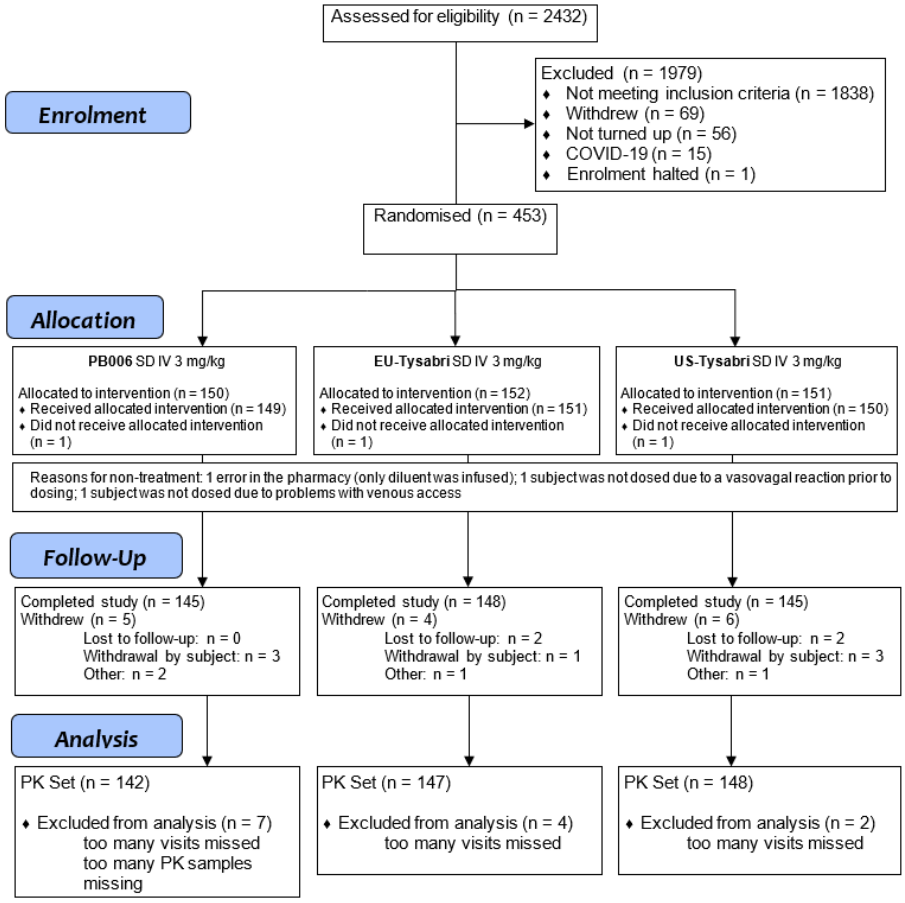
After the analytical and the PK bridge were established and the predefined pooling criterion for CD19+ was fulfilled, an ANCOVA model as described above was to be calculated comparing PB0006 and the pooled reference (US-licensed Tysabri and EU-approved Tysabri).
Similarity was to be concluded if the 90% CI (for FDA) and 95% CI (for EMA) for baseline-adjusted $AUEC_{0\text{-}12w}$ of baseline-adjusted CD19+ was completely included in the similarity margin of 0.8000 to 1.2500.

The analysis of the primary PD endpoint $AUEC_{0\text{-}12w}$ of α4-integrin %RS was analogously performed to the analysis for $AUEC_{0\text{-}12w}$ of baseline-adjusted CD19+.

Results

Participant flow

Figure 1: Participant flow



Recruitment

For successful recruitment, two sequential screening visits were performed. At screening 1 (between day -56 and day -2), subjects had to be tested negative for JCV. Screening 2 was then performed between day -28 and day -1 (admission). Follow-up was performed at the ambulatory visit on day 85 (end of study), and at an ambulatory visit on day 169 ±7 days (6 months).

The first screening took place on 30 Oct 2019, the last follow-up was performed on 10 Mar 2021.

Conduct of the study

The study was performed according to the CSP dated 30 Sep 2019, and 6 CSP amendments were issued during clinical execution of the study, comprising 4 general CSP amendments and 2 CSP amendments applicable to 1 country only (Poland). These changes to the protocol included extensive modifications to both primary endpoints as well as sample size, and other variables of the study. A summary of changes to the study protocol is presented in table below.

Table 3: Summary of Changes to Protocol PB006-01-03

Document	Date of Issue	Summary of Changes
Original CSP	30 Sep 2019	Not applicable
CSP Version 2.0/ Amendment 1	05 Dec 2019	Overall Rationale for the CSP amendment: To incorporate clarifications and corrections deemed necessary from the previous version of the CSP. Changes introduced were: • Objectives, endpoints, and populations required for each agency (FDA and EMA) were separately defined • Clarification for Inclusion Criterion 3 that weight was checked at Screening 2 and Day -1, but BMI was only calculated at Screening 2 • Clarification that mean values should be used for the triplicate measurements in Exclusion Criterion 13 • Corrections of inconsistencies and minor clarifications
Note to file	09 Dec 2019	Maximum weight of study subjects reduced to 92 kg to ensure that a maximum of 1 vial is used per dose. Information included in CSP Version 3.0. No subjects were randomized before this Note to file was issued.
CSP Version 3.0/ Amendment 2	12 Dec 2019	Changes introduced were: • Maximum weight of study subjects reduced from 95 kg to 92 kg to ensure that a maximum of 1 vial is used per dose • Highest stratification group amended to >80 kg to 92 kg • Clarification that a JCV test can be performed again if a subject who was otherwise screening positive had his first negative JCV test longer than 56 days prior to dosing
CSP Version 4.0/ Amendment 3	20 Jun 2020	Overall rationale for CSP Amendment 3: Implementing measures to be taken to protect subjects after dosing from a COVID-19 infection. Changes introduced were: • Reduce the maximum inclusion age from 65 years to 54 years before dosing • Inclusion of PCR SARS-CoV-2 tests, update of the ambulatory activities, increase of the in-house period from 3 to 8 days and inclusion of home quarantine up to Day 14 • Update of the Benefit/Risk Assessment, the risk assessment for increase of serious infections was classified as both high impact and low possibility • Exclusion of subjects with a history or evidence of SARS-CoV-2 infection in the last month prior Screening 1 or having been in confirmed contact with SARS-CoV-2 positive subjects in the last 2 weeks before dosing • Corrections of inconsistencies and minor clarifications
Note to file	02 Jul 2020	According to CSP Version 4.0 (Table 4, Table 5 and Section 8.2.2) all vital signs assessments, except temperature, should be done in triplicate for the listed timepoints (Screening 2 and Day -1).

Document	Date of Issue	Summary of Changes
		This Note to file explained that the oxygen saturation was also not required in triplicate for the timepoints listed in the CSP Version 4.0. This was updated in CSP Version 4.1.
CSP Version 4.1/ Amendment 4	14 Aug 2020	Changes to the CSP requested by the IEC in Poland only. Changes introduced were: • Clarifications on controlled and home quarantine. • Clarifications on oxygen saturation procedures.
CSP Version 5.0/ Amendment 5	28 Aug 2020	The revision of endpoints was agreed with the FDA in a Type 2 meeting, which included to add CD19+ AUEC as scientific most relevant PD parameter. This parameter is now defined as primary endpoint and the sample size of the study was adjusted accordingly. Changes introduced were: • Increase of sample size to 453 subjects, 151 subject per arm • Correction of primary PK endpoint $AUC_{0\text{-}inf}$ and related objective • Demotion of CD19+ E_{max} to a secondary endpoint • Use of pooled EU-approved Tysabri and US-licensed Tysabri data as a comparison with PB006 in terms of $AUEC_{0\text{-}12w}$ of baseline-adjusted CD19+ • Removal of $t_{1/2}$, CL, and K_e as secondary PK parameters • To align endpoints among regulatory regions and following the scientific advice discussion, the primary PD endpoint on α4-integrin %RS was adjusted to $AUEC_{0\text{-}12w}$ and the partial receptor saturation $AUEC_{4\text{-}12w}$ was removed* • Modification of the definition of the analysis sets • Update of PK Analysis Methods • Update of PD and Target Receptor Engagement Analysis Methods • Typographical and editorial changes
CSP Version 5.1	28 Aug 2020	Changes the same as the changes implemented for CSP Version 5.0 but based on CSP Version 4.1 (Poland only)
Note to file	01 Oct 2020	Information to the site in Poland that 1 of the batches of study drug had a slightly lower concentration of study drug. The site was asked to try to limit the subject weight to 90.4 kg though weights up to 92.0 kg could be feasible.
Note to file	29 Apr 2021	CSP deviations on site level were described

%RS=relative receptor saturation; ADA=antidrug antibody; AUEC=area under the effect curve; BMI=body mass index; CSP=clinical study protocol; CD19+=Cluster of Differentiation 19 activation; COVID-19=coronavirus disease-19; EMA=European Medicines Agency; FDA=Food and Drug Administration; IEC=independent ethics committee; JCV=John Cunningham virus; PCR=polymerase chain reaction; PD=pharmacodynamic(s); PK=pharmacokinetic(s); SAP=statistical analysis plan; SARS-CoV-2=severe acute respiratory syndrome coronavirus-2 * Despite being removed as endpoint from the protocol, $AUEC_{4\text{-}12w}$ was calculated and presented in line with the SAP

Protocol deviations

Throughout the study 675 protocol deviations were reported for 246 subjects from all 6 sites. The frequency of the most important protocol deviations are presented below.

Important protocol deviations related to COVID-19 were reported for 5 subjects, 3 in the Netherlands, all related to compliance with quarantine requirements, and 2 in the US, both related to use of medication without consulting the Investigator.

Table 4: Summary of Important Protocol Deviations

	3 mg/kg sd IV PB006 (N=149)	3 mg/kg sd IV Tysabri EU (N=151)	3 mg/kg sd IV Tysabri US (N=150)	Overall (N=450)
Deviation Category	**n (%)**	**n (%)**	**n (%)**	**n (%)**
Assessment Safety	-	-	1 (0.7)	1 (0.2)
Exclusion Criteria	-	-	2 (1.3)	2 (0.4)
Inclusion Criteria	-	1 (0.7)	1 (0.7)	2 (0.4)
Informed Consent	11 (7.4)	12 (7.9)	12 (8.0)	35 (7.8)
Other	-	1 (0.7)	-	1 (0.2)
Overdose/Misuse	2 (1.3)	1 (0.7)	1 (0.7)	4 (0.9)
Prohibited Co-Medication	3 (2.0)	2 (1.3)	3 (2.0)	8 (1.8)
Study Drug	-	2 (1.3)	-	2 (0.4)
Visit Window	-	1 (0.7)	-	1 (0.2)
Protocol Deviations Related to COVID-19	2 (1.3)	1 (0.7)	2 (1.3)	5 (1.1)
US	1 (0.7)	-	1 (0.7)	2 (0.4)
the Netherlands	1 (0.7)	1 (0.7)	1 (0.7)	3 (0.7)

IV=intravenous; n=number of subjects in this category; N=number of subjects receiving study drug; Tysabri EU=EU-approved Tysabri; Tysabri US=US-licensed Tysabri

Baseline data

A total of 450 subjects between 18 and 61 years of age and with a BMI between 18.6 and 30.3 kg/m^2 were dosed with the study drug in the study.

Demographic and baseline characteristics were similar across groups. The majority of subjects were white. The ratio of males and females was balanced in all 3 groups. Mean age was 31 years, mean weight was approximately 72 kg and mean height was 172-173 cm across groups. Approximately half of the subjects in each group were in the weight class >65 kg to ≤80 kg.

Table 5: Demographic and baseline characteristics in study PB006-01-03 (PK population)

	PB006 N=142	EU-Tysabri N=147	US-Tysabri N=148
Age (years)			
Mean (SD)	31 (11)	31 (11)	31 (11)
Sex, n (%)			
Male	78 (54.9)	68 (46.3)	71 (48.0)
Female	64 (45.1)	79 (53.7)	77 (52.0)
Race, n (%)			
White	118 (83.1)	122 (83.0)	132 (89.2)
Black or African-American	16 (11.3)	15 (10.2)	10 (6.8)
Asian	3 (2.1)	5 (3.4)	3 (2.0)
White + Black or African-American	2 (1.4)	1 (0.7)	0
White + Asian	1 (0.7)	0	0
White + American Indian or Alaska Native	0	2 (1.4)	0
American Indian or Alaska Native	2 (1.4)	1 (0.7)	2 (1.4)
Native Hawaiian or Other Pacific Islander	0	1 (0.7)	0
Unknown	0	0	1 (0.7)
Weight (kg)			
Mean (SD)	72.5 (10.4)	71.4 (10.4)	72.4 (10.4)
Height (cm)			
Mean (SD)	173 (9)	172 (10)	172 (9)
Body mass index (kg/m^2)			
Mean (SD)	24.3 (2.7)	24.1 (2.9)	24.4 (2.7)
Weight class, n (%)			
≤ 65 kg	34 (23.9)	37 (25.2)	38 (25.7)
> 65 kg to ≤ 80 kg	73 (51.4)	77 (52.4)	76 (51.4)
> 80 kg to ≤ 92 kg	35 (24.6)	33 (22.4)	34 (23.0)

N=Number of subjects in treatment group, n=number of subjects in category, PK=pharmacokinetic, SD=standard deviation.

The difference between the subjects enrolled before and after the study stop due to COVID-19 was limited and primarily due to the small number of subjects randomized before the study stop (n=16) when compared to the number of subjects randomized after the study restart (n=434).

In the PK Set, 437 subjects between 18 and 61 years of age and with a BMI between 18.6 and 30.3 kg/m^2 were included. There was no relevant difference between the demographics in the Safety Set and in the PK Set.

In the PD Set (Other), 438 subjects between 18 and 61 years of age and a BMI between 18.6 and 30.3 kg/m^2 were included. In the PD Set (CD19+), 437 subjects between 18 and 61 years of age and a BMI between 18.6 and 30.3 kg/m^2 were included. In the PD Set (Primary α4-integrin %RS), 389 subjects between 18 and 61 years of age and a BMI between 18.7 and 30.3 kg/m^2 were included. There were no relevant differences between the demographics in the Safety Set and those in the PD Set (Other), the PD Set (CD19+), or the PD Set (Primary a-integrin %RS).

Administration of the study drug was performed by the Investigator or authorized designee as an IV infusion over 60 minutes. There was no indication of noncompliance based on observations during study drug administration. Compliance was further confirmed by bioanalytical assessment of natalizumab in serum samples.

Numbers analysed

The PK Set consists of 437 subjects; 13 subjects were not included in the PK Set because they dropped out before the serum natalizumab levels approached LLOQ or they missed 2 or more consecutive visits before the serum natalizumab levels approached LLOQ.

The PD Set (CD19+) consists of 437 subjects; 13 subjects were not included in the PD Set (CD19+) because they dropped out before the CD19+ levels returned to baseline or they missed 3 or more consecutive visits before the CD19+ levels returned to baseline.

The PD Set (Primary α4-integrin %RS) consists of 389 subjects; 61 subjects were not included in the PD Set (Primary α4-integrin %RS) because they dropped out before the α4-integrin %RS levels returned to baseline, samples were analyzed outside the validation stability window, or they missed 3 or more consecutive visits before the α4-integrin %RS levels returned to baseline.

The PD Set (Sensitivity α4-integrin %RS) consists of 432 subjects; 18 subjects were not included in the PD Set (Secondary α4-integrin %RS) because they dropped out before the α4-integrin %RS levels returned to baseline or they missed 3 or more consecutive visits before the α4-integrin %RS levels returned to baseline.

Table 6: Subject disposition and analysis sets in study PB006-01-03 (all subjects)

	PB006 N=150 n (%)	EU-Tysabri N=152 n (%)	US-Tysabri N=151 n (%)
Randomized	150 (100)	152 (100)	151 (100
Safety population*	149 (99)	151 (99)	150 (99)
PK population	142 (95)	147 (97)	148 (98)
PD population (other)	145 (97)	148 (97)	145 (96)
PD population (CD19+)	142 (95)	147 (97)	148 (98)
PD population (Primary α4-integrin %RS)	126 (84)	137 (90)	126 (83)
PD population (Sensitivity α4-integrin %RS)	141 (94)	146 (96)	145 (96)
Dosed	149 (99)	152 (100)	150 (99)
Completed	145 (97)	148 (97)	145 (96)
Withdrew	5 (3)	4 (3)	6 (4)
Reason for withdrawal:			
Lost to follow-up	0	2 (1)	2 (1)
Withdrawal by subject	3 (2)	1 (1)	3 (2)
Other	2 (1)	1 (1)	1 (1)

PD=pharmacodynamic, PK=pharmacokinetic, SAF=safety population.*Three subjects did not receive study drug after randomization.

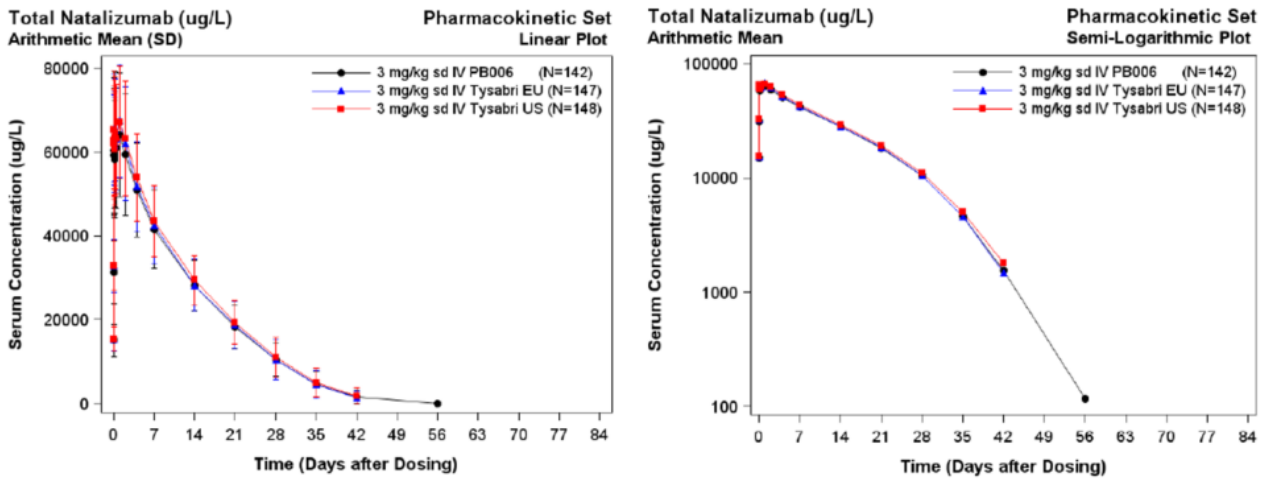
Outcomes and estimation

A. Pharmacokinetic Results

A1. Total natalizumab

Concentration-time profiles of total natalizumab were similar in the 3 treatment groups over the entire sampling period. A rapid increase in total natalizumab was observed, with concentrations reaching nearly maximum values already at the end of the 1-hour infusion period. Concentrations remained high (at values of around 60000 µg/L) until Day 3 and subsequently declined. From Day 57 onwards, no natalizumab concentrations above the LLOQ were observed in any of the groups.

Figure 2: Arithmetic mean (SD) plasma concentration-time profiles of total natalizumab (PK population) in study PB006-01-03, linear plot (left) and semi-log plot (right)



The primary PK parameter was $AUC_{0\text{-}inf}$ of total natalizumab. For this parameter, a 3-way comparison was performed containing the comparison of PB006 to both US-Tysabri and EU-Tysabri, as well as the PK bridge between US-Tysabri and EU-Tysabri. The 3-way comparison was part of a hierarchical testing procedure. The hierarchical testing procedure foresees that subjects in the treatment arms receiving US-Tysabri and EU-Tysabri, respectively, can be pooled for the primary PD analysis. As prerequisite for the

pooling of the reference, PK similarity needs to be established and the pre-defined pooling criterion has to be fulfilled. Results from the statistical analysis are presented below.

Table 7: Statistical analysis of the primary PK parameter $AUC_{0\text{-}inf}$ of total natalizumab (PK population) in study PB006-01-03

Parameter	Treatment	n	Geometric LS Means	Pairwise comparison		
				Pair	Ratio	90% CI
$AUC_{0\text{-}inf}$ (mg.h/L)	PB006	141	22118	PB006 vs EU-Tysabri	0.9864	0.9410, 1.0340
	EU-Tysabri	147	22424	PB006 vs US-Tysabri	0.9491	0.9054, 0.9948
	US-Tysabri	148	23306	EU-Tysabri vs US-Tysabri	0.9622	0.9184, 1.0080

$AUC_{0\text{-}inf}$=area under the concentration time curve from time zero to infinity, CI=confidence interval, LS=least square, n=number of subjects with data available, PK=pharmacokinetic, vs=versus. The analysis was performed on natural log (ln) transformed parameters using an analysis of variance model with treatment as a fixed effect. Similarity could be concluded if the 90% CI fell completely in the margin of 0.80 to 1.25.

PK similarity was demonstrated for all comparisons (PB006 vs EU-Tysabri, PB006 vs US-Tysabri, EU-Tysabri vs US-Tysabri). For all pairwise comparisons, the 90% CIs were completely included in the similarity margin of 0.80 to 1.25. Thus, the primary PK endpoint was met.

C_{max} and $AUC_{0\text{-}t}$ of total natalizumab were secondary PK parameters and supportively evaluated in the same manner as for the primary analysis using $AUC_{0\text{-}inf}$. Results from this statistical analysis are presented below.

Table 8: Statistical analysis of the secondary PK parameters C_{max} and $AUC_{0\text{-}t}$ of total natalizumab (PK population) in study PB006-01-03

Parameter	Treatment	n	Geometric LS Means	Pairwise comparison		
				Pair	Ratio	90% CI
C_{max} (mg/L)	PB006	142	68.6	PB006 vs EU-Tysabri	0.9565	0.9215, 0.9929
	EU-Tysabri	147	71.7	PB006 vs US-Tysabri	0.9621	0.9269, 0.9987
	US-Tysabri	148	71.3	EU-Tysabri vs US-Tysabri	1.0059	0.9694, 1.0438
$AUC_{0\text{-}t}$ (mg.h/L)	PB006	142	22014	PB006 vs EU-Tysabri	0.9840	0.9387, 1.0316
	EU-Tysabri	147	22371	PB006 vs US-Tysabri	0.9468	0.9032, 0.9925
	US-Tysabri	148	23251	EU-Tysabri vs US-Tysabri	0.9622	0.9183, 1.0081

$AUC_{0\text{-}t}$=area under the concentration time curve from time zero to last measurable concentration, CI=confidence interval, Cmax=maximum concentration, LS=least square, n=number of subjects with data available, PK=pharmacokinetic, vs=versus. The analysis was performed on natural log (ln) transformed parameters using an analysis of variance model with treatment as a fixed effect. Similarity could be concluded if the 90% CI fells completely in 0.80 to 1.25.

PK similarity was further supported by results from secondary endpoints. For both parameters (C_{max} and $AUC_{0\text{-}t}$) and for all comparisons (PB006 vs EU-Tysabri, PB006 vs US- Tysabri, EU-Tysabri vs US-Tysabri), the 90% CIs were completely included in 0.80 to 1.25.

The PK parameters t_{max}, $t_{1/2}$ (half-life), CL (clearance), k_{el} (terminal elimination rate constant) for total natalizumab were analyzed descriptively. A summary of the PK parameters for total natalizumab is presented below.

Table 9: Summary of PK parameters for total natalizumab (PK population) in study PB006-01-03

	PB006 N=142	**EU-Tysabri N=147**	**US-Tysabri N=148**
AUC_{0-inf} (mg.h/L)	n=141	n=147	n=148
Geometric mean (geometric CV%)	22118 (25.2)	22424 (25.1)	23306 (23.5)
Arithmetic mean (SD)	22766 (5240)	23083 (5411)	23894 (5058)
C_{max} (mg/mL)	n=142	n=147	n=148
Geometric mean (geometric CV%)	68.6 (22.4)	71.7 (17.5)	71.3 (18.1)
Arithmetic mean (SD)	70.1 (14.2)	72.8 (13.0)	72.3 (12.2)
t_{max} (h)	n=142	n=147	n=148
Median (min – max)	12.00 (1.00-96.05)	12.00 (1.00-96.00)	9.04 (1.00-101.07)
AUC_{0-t} (mg.h/L)	n=142	n=147	n=148
Geometric mean (geometric CV%)	22014 (25.4)	22371 (25.2)	23251 (23.5)
Arithmetic mean (SD)	22667 (5255)	23030 (5403)	23839 (5056)
AUC_{extra} (%)	n=141	n=147	n=148
Arithmetic mean (SD)	0.2 (0.3)	0.2 (0.4)	0.2 (0.4)
$t_{1/2}$ (h)	n=141	n=147	n=148
Geometric mean (geometric CV%)	90.0 (40.3)	87.6 (37.4)	87.5 (32.6)
Arithmetic mean (SD)	106 (165)	96.2 (68.2)	92.6 (37.3)
CL (mL/h)	n=141	n=147	n=148
Geometric mean (geometric CV%)	9.72 (24.9)	9.48 (26.1)	9.22 (24.1)
Arithmetic mean (SD)	10.0 (2.64)	9.82 (2.78)	9.49 (2.51)
Vz (mL)	n=141	n=147	n=148
Geometric mean (geometric CV%)	1262 (42.6)	1198 (36.5)	1164 (33.2)
Arithmetic mean (SD)	1469 (1943)	1291 (662)	1228 (427)
k_{el} (1/h)	n=141	n=147	n=148
Geometric mean (geometric CV%)	0.00770 (40.4)	0.00792 (37.4)	0.00792 (32.6)
Arithmetic mean (SD)	0.00810 (0.00213)	0.00833 (0.00235)	0.00829 (0.00240)

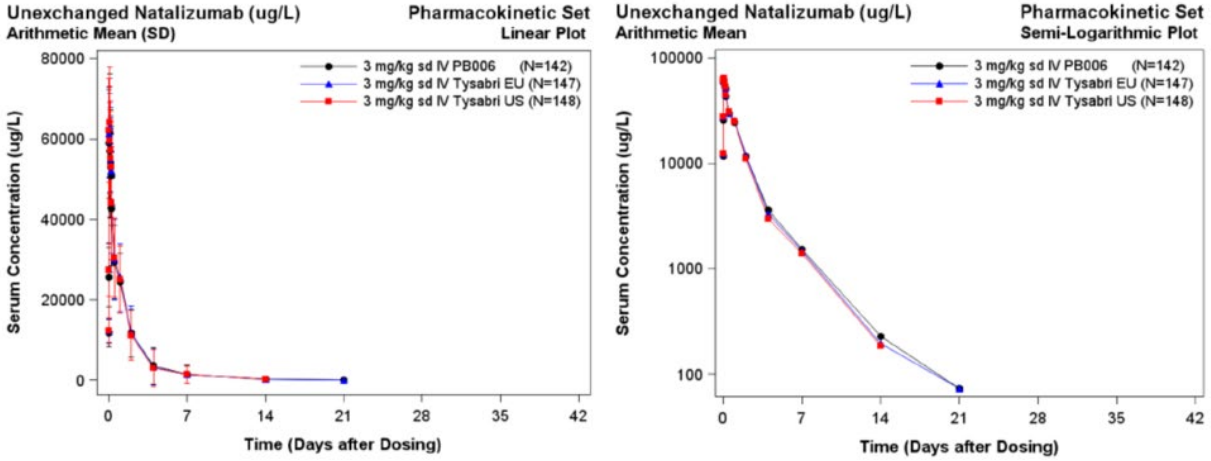
AUC_{0-inf}=area under the concentration time curve from time zero to infinity, AUC_{0-t}=area under the concentration time curve from time zero to last measurable concentration, AUC_{extra}=percentage of estimated part for the calculation of AUC_{0-inf}, calculated as ($AUC0_{-inf}$ - AUC_{0-t})/ AUC_{0-inf})*100%, CL=clearance, C_{max}=maximum concentration, CV=coefficient of variation, k_{el}=terminal elimination rate constant, N=number of subjects, n=number of subjects with data available, PK=pharmacokinetic, SD=standard deviation, t½=half-life, t_{max}=time to C_{max}, V_z=volume of distribution.

PK parameters were similar for all 3 treatment groups. Geometric mean C_{max} ranged from 68.6 to 71.7 mg/L across groups, with CV being between 17.5% and 22.4%. Geometric mean AUC_{0-inf} ranged from 22118 to 23306 mg.h/L, with CV being between 23.5% and 25.2%. Median t_{max} was 12 hours for PB006 and EU-Tysabri and 9 hours for US- Tysabri. Mean $t_{1/2}$ ranged between 92.6 and 106 hours across groups. An AUC_{extra} of 0.2% indicates that the duration of PK observation period has been sufficient.

A2. Unexchanged natalizumab

Concentration-time profiles of unexchanged natalizumab were similar in the 3 treatment groups over the entire sampling period. A rapid increase in unexchanged natalizumab was observed, with concentrations reaching near maximum values already at the end of the 1 hour infusion period. From Day 29 onwards, no natalizumab concentrations above the LLOQ were observed in the PB006 and EU-Tysabri groups, while for US-Tysabri no natalizumab concentrations above the LLOQ were observed from Day 22 onwards.

Figure 3: Arithmetic mean (SD) plasma concentration-time profiles of unexchanged natalizumab (PK population) in study PB006-01-03, linear plot (left) and semi-log plot (right)



The PK parameters C_{max}, $AUC_{0\text{-}inf}$ and $AUC_{0\text{-}t}$ of unexchanged natalizumab were secondary endpoints and evaluated as supportive evidence (the reported CIs were of exploratory nature, i.e., they were not assessed by pre-specified similarity margins). Results from the statistical analysis are presented below.

Table 10: Statistical analysis of PK parameter C_{max}, $AUC_{0\text{-}inf}$, and $AUC_{0\text{-}t}$ of unexchanged natalizumab (PK population) in study PB006-01-03

Parameter	Treatment	n	Geometric LS Means	Pairwise comparison		
				Pair	Ratio	90% CI
$AUC_{0\text{-}inf}$ (mg.h/L)	PB006	141	1713	PB006 vs EU-Tysabri	0.9987	0.9231, 1.0804
	EU-Tysabri	146	1715	PB006 vs US-Tysabri	1.0208	0.9438, 1.1041
	US-Tysabri	148	1678	EU-Tysabri vs US-Tysabri	1.0222	0.9457, 1.1048
C_{max} (mg/L)	PB006	142	61.4	PB006 vs EU-Tysabri	0.9381	0.8874, 0.9916
	EU-Tysabri	147	65.5	PB006 vs US-Tysabri	0.9440	0.8931, 0.9978
	US-Tysabri	148	65.0	EU-Tysabri vs US-Tysabri	1.0063	0.9525, 1.0631
$AUC_{0\text{-}t}$ (mg.h/L)	PB006	142	1692	PB006 vs EU-Tysabri	1.0037	0.9267, 1.0873
	EU-Tysabri	147	1686	PB006 vs US-Tysabri	1.0164	0.9385, 1.1008
	US-Tysabri	148	1665	EU-Tysabri vs US-Tysabri	1.0126	0.9356, 1.0960

$AUC_{0\text{-}inf}$=area under the concentration time curve from time zero to infinity, $AUC_{0\text{-}t}$=area under the concentration time curve from time zero to last measurable concentration, CI=confidence interval, C_{max}=maximum concentration, LS=least square, n=number of subjects with data available, PK=pharmacokinetic, vs=versus. The analysis was performed on natural log (ln) transformed parameters using an analysis of variance model with treatment as a fixed effect. Similarity could be concluded if the 90% CI fell completely in 0.80 to 1.25.

For all 3 parameters similar results were seen for PB006, EU-Tysabri and US-Tysabri.

As supportive evidence, the PK parameters of unexchanged natalizumab were evaluated in the same manner as described for total natalizumab. A summary of the PK parameters for unexchanged natalizumab is presented below.

Table 11: Summary of PK parameters for unexchanged natalizumab (PK population) in study PB006-01-03

	PB006 N=142	**EU-Tysabri N=147**	**US-Tysabri N=148**
AUC_{0-inf} (mg.h/L)	n=141	n=146	n=148
Geometric mean (geometric CV%)	1713 (42.6)	1715 (42.9)	1678 (41.0)
Arithmetic mean (SD)	1873 (899)	1884 (952)	1833 (935)
C_{max} (mg/mL)	n=142	n=147	n=148
Geometric mean (geometric CV%)	61.4 (37.1)	65.5 (17.4)	65.0 (30.4)
Arithmetic mean (SD)	64.1 (13.7)	66.4 (11.4)	67.0 (13.1)
t_{max} (h)	n=142	n=147	n=148
Median (min – max)	1.50 (1.00-48.00)	1.50 (1.00-6.08)	1.50 (1.00-48.00)
AUC_{0-t} (mg.h/L)	n=142	n=147	n=148
Geometric mean (geometric CV%)	1692 (43.1)	1686 (44.8)	1665 (41.1)
Arithmetic mean (SD)	1853 (898)	1861 (953)	1820 (935)
AUC_{extra} (%)	n=141	n=146	n=148
Arithmetic mean (SD)	0.7 (0.6)	0.7 (0.5)	0.8 (0.7)
$t_{1/2}$ (h)	n=141	n=146	n=148
Geometric mean (geometric CV%)	73.0 (70.5)	68.9 (61.8)	71.9 (75.1)
Arithmetic mean (SD)	90.1 (75.9)	80.3 (47.7)	91.7 (83.7)
CL (mL/h)	n=141	n=146	n=148
Geometric mean (geometric CV%)	125 (45.3)	124 (43.3)	128 (40.1)
Arithmetic mean (SD)	137 (56.0)	134 (49.5)	137 (49.5)
Vz (mL)	n=141	n=146	n=148
Geometric mean (geometric CV%)	13198 (79.5)	12316 (69.9)	13278 (80.1)
Arithmetic mean (SD)	17009 (15037)	14854 (9723)	17352 (16608)
k_{el} (1/h)	n=141	n=146	n=148
Geometric mean (geometric CV%)	0.00950 (70.5)	0.0101 (61.8)	0.00964 (75.1)
Arithmetic mean (SD)	0.0117 (0.00887)	0.0119 (0.00800)	0.0120 (0.00875)

AUC_{0-inf}=area under the concentration time curve from time zero to infinity, AUC_{0-t}=area under the concentration time curve from time zero to last measurable concentration, AUC_{extra}=percentage of estimated part for the calculation of AUC_{0-inf}, calculated as ($AUC0_{-inf}$ - AUC_{0-t})/ AUC_{0-inf})*100%, CL=clearance, C_{max}=maximum concentration, CV=coefficient of variation, k_{el}=terminal elimination rate constant, N=number of subjects, n=number of subjects with data available, PK=pharmacokinetic, SD=standard deviation, t½=half-life, t_{max}=time to C_{max}, V_z=volume of distribution.

PK parameters for unexchanged natalizumab were similar for all 3 treatment groups.

A3. %Fab-arm exchange

Natalizumab is a full-length antibody of the IgG4 subclass. It consists of 2 heavy and 2 light chains connected by 4 inter-chain disulfide bonds. Assessment of natalizumab in serum is complicated by the ability of human IgG4 antibodies to undergo Fab-arm exchange *in vivo*. Such exchange generates IgG4 molecules of mixed specificity comprising a natalizumab heavy-light chain pair coupled to an endogenous IgG4 heavy-light chain pair of unknown specificity (Shapiro et al. 2011).

Since exchanged and unexchanged species cannot be quantified independently using a single ELISA, a quantitation strategy was developed employing 2 ELISAs: one measuring total natalizumab including both unexchanged (i.e., intact) and exchanged molecules, and the second measuring only unexchanged (intact) natalizumab. The presence and amount of exchanged natalizumab in serum was calculated by the difference in values obtained in the 2 assays. This approach was previously developed for quantification of Tysabri and is summarized in the article by Shapiro et al. in 2011.

Mean values for %Fab-arm exchange increased to >95% by Day 8 for PB006 (95.4%), EU- Tysabri (95.9%), and US-Tysabri (96.2%). Maximum values reached 100% in individual subjects at Day 8 for the 3 treatments.

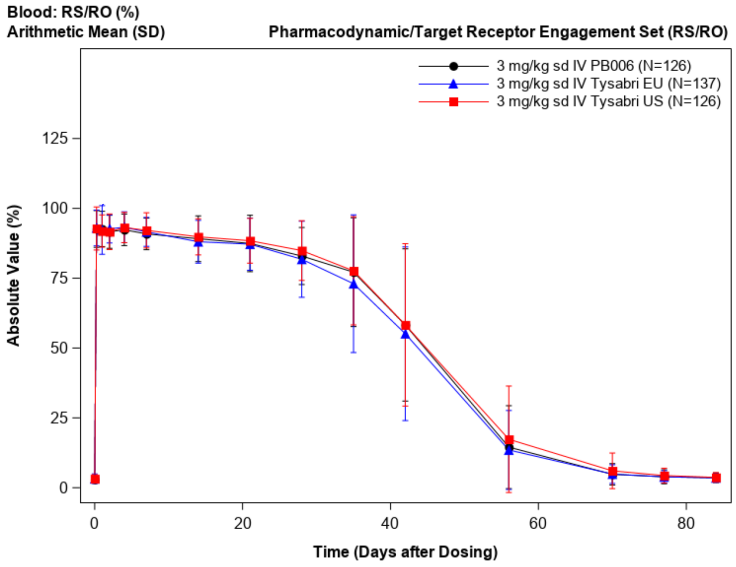
Overall, Fab-arm exchange was rapid and complete, with similar results for all 3 treatment groups.

B. Pharmacodynamic Results

B1. α4-integrin %RS

The α4-integrin %RS profiles over time were similar in all treatment groups. Mean α4-integrin %RS increased to a level close to the maximum value (>90%) at the first postdose time point (6 hours) for all treatments.

Figure 4: Arithmetic Mean of α4-integrin %RS Whole Blood-Time Profile (PD Set [Primary α4-integrin %RS])



%RS=relative receptor saturation; IV=intravenous; N=number of subjects; RO=receptor occupancy; sd=single dose

After administration of 3 mg/kg PB006, a mean maximum response of 92.6% was reached after 24 hours. A mean α4-integrin %RS level >80% was maintained until Day 29. After Day 29 the α4-integrin %RS value showed a decline to a value similar to the baseline value (<5% α4-integrin %RS) at Day 71.

After administration of 3 mg/kg EU-approved Tysabri, a mean maximum response of 93.2% was reached on Day 5 (96 hours). A mean α4-integrin %RS level >80% was maintained until Day 29. After Day 29 the α4-integrin %RS value showed a decline to a value similar to the baseline value at Day 71.

After administration of 3 mg/kg US-licensed Tysabri, a mean maximum response of 93.1% was reached on Day 5 (96 hours). A mean α4-integrin %RS level >80% was maintained until Day 29. After Day 29 the α4-integrin %RS value showed a decline to a value similar to the baseline value at Day 78.

One of the 2 co-primary PD endpoints was $AUEC_{0\text{-}12w}$ for α4-integrin %RS. Similarity was concluded if the 90% CI and 95% CI for baseline-adjusted $AUEC_{0\text{-}12w}$ of α4-integrin %RS was completely included in the similarity margin of 0.80 to 1.25.

The results from the primary PD analysis of α4-integrin %RS are presented in below table.

Table 12: Statistical analysis of the primary PD endpoint AUEC0-12w of α4-integrin %RS (PD [Primary α4-integrin %RS] population) in study PB006-01-03

Parameter	Treatment	n	Geometric LS Means	Pairwise comparison		
				Pair	Ratio	95% CI
$AUEC_{0\text{-}12w}$ α4-integrin %RS (%*h)	PB006	126	99003	PB006 vs pooled Tysabri	0.9933	0.9523, 1.0362
	Pooled Tysabri	263	99668			

$AUEC_{0\text{-}12w}$=area under the effect time curve from time zero to 12 weeks, CI=confidence interval, LS=least square, n=number of subjects with data available, PD=pharmacodynamic, RS=receptor saturation.

When comparing $AUEC_{0\text{-}12w}$ of α4-integrin %RS between PB006 (test) and pooled Tysabri (reference) using an ANCOVA (analysis of covariance) model, the point estimates were close to 1 and the 95% CI for the geometric LS means of $AUEC_{0\text{-}12w}$ was narrow, between 0.9523 and 1.0362. The 95% CI was completely included in the similarity margin of 0.80 to 1.25. Thus, the primary PD endpoint α4-integrin %RS was met.

Results for the individual comparisons (using 95% CIs), i.e., PB006 vs EU-Tysabri, PB006 vs US-Tysabri and EU-Tysabri vs US-Tysabri, are presented below.

Table 13: Statistical analysis of the PD endpoint AUEC0-12w of α4-integrin %RS, individual comparisons (PD [Primary α4-integrin %RS] population) in study PB006-01-03

Parameter	Treatment	n	Geometric LS Means	Pairwise comparison		
				Pair	Ratio	95% CI
$AUEC_{0\text{-}12w}$ α4-integrin %RS (%*h)	PB006	126	99003	PB006 vs EU-Tysabri	1.0142	0.9667, 1.0641
	EU-Tysabri	137	97616	PB006 vs US-Tysabri	0.9711	0.9247, 1.0198
	US-Tysabri	126	101949	EU-Tysabri vs US-Tysabri	0.9575	0.9126, 1.0046

$AUEC_{0\text{-}12w}$=area under the effect time curve from time zero to 12 weeks, CI=confidence interval, LS=least square, n=number of subjects with data available, PD=pharmacodynamic, RS=receptor saturation.

For all comparisons, the point estimates were close to 1 and the 95% CIs were completely included in the similarity margin of 0.80 to 1.25.

Additional analysis based on $AUEC_{4\text{-}12w}$

Mean $AUEC_{4\text{-}12w}$ was an additional analysis to the primary analysis of α4-integrin %RS to meet EMA requirements. Mean $AUEC_{4\text{-}12w}$ was similar for the 3 treatments at approximately 40% of the mean $AUEC_{0\text{-}12w}$ (compare Table 14 Table 15).

Sensitivity Analysis for Samples Analyzed Outside the Validation Stability Window

To assess the effect of the samples analyzed outside the validation stability window for α4-integrin %RS, the PD parameters $AUEC_{0\text{-}12w}$ and $AUEC_{4\text{-}12w}$ for α4-integrin %RS were also calculated including all samples analyzed outside the validation stability window.

Inclusion of samples analyzed outside the validation stability window for α4-integrin %RS (sensitivity analysis) did result in changes in the values for $AUEC_{0\text{-}12w}$ and $AUEC_{4\text{-}12w}$. The value for $AUEC_{0\text{-}12w}$ calculated for PB006 and EU-approved Tysabri increased 1% and 4% and for US-licensed Tysabri decreased 2%. The value for $AUEC_{4\text{-}12w}$ calculated for PB006 and EU-approved Tysabri increased 1% and

10% and for US-licensed Tysabri decreased less than 1% (Table 16). These changes were not expected to influence the outcome of the study.

Table 14: Summary Statistics of Primary PD Parameters for α4-integrin %RS (PD Set [Primary α4-integrin %RS])

Parameter (unit)	Statistic	Treatment 3 mg/kg IV PB006 N=126	3 mg/kg IV Tysabri EU N=137	3 mg/kg IV Tysabri US N=126
$AUEC_{0\text{-}12w}$ (%*h)	Mean (SD)	100,685 (17,724)	99,704 (19,447)	103,687 (18,656)
	CV (%)	17.6	19.5	18.0
	Min-Max	43,341-146,484	54,217-135,299	59,516-144,957
	Geom mean (gCV%)	99,003 (19.1)	97,616 (21.6)	101,949 (18.9)

%RS=relative receptor saturation; AUEC=area under the effect curve; CV=coefficient of variation; gCV=coefficient of variation (geometric mean); Geom=geometric; IV=intravenous; Max=maximum; Min=minimum; N=number of subjects; PD=pharmacodynamic(s); Tysabri EU=EU-approved Tysabri; Tysabri US=US-licensed Tysabri

Table 15: Supportive Analysis of PD Parameters for α4-integrin %RS (Safety Set)

Parameter (unit)	Statistic	Treatment 3 mg/kg IV PB006	3 mg/kg IV Tysabri EU	3 mg/kg IV Tysabri US
$AUEC_{4\text{-}12w}$ (%*h)	n	136	141	136
	Mean (SD)	41,336 (14,939)	40,498 (16,708)	43,054 (17,250)
	CV (%)	36.1	41.3	40.1
	Min-Max	1718-84,384	5058-72,943	3294-82,297
	Geom mean (gCV%)	37,712 (53.1)	35,704 (62.3)	38,655 (56.0)

%RS=relative receptor saturation; AUEC=area under the effect curve; CV=coefficient of variation; gCV=coefficient of variation (geometric mean); Geom=geometric; IV=intravenous; Max=maximum; Min=minimum; n=number of subjects with data available; PD=pharmacodynamic(s); Tysabri EU=EU-approved Tysabri; Tysabri US=US-licensed Tysabri
Note: This analysis is based on the safety set and including subjects with enough values to calculate an AUEC4-12w.

Table 16: Sensitivity Analysis of PD Parameters for α4-integrin %RS (PD Set [Sensitivity α4-integrin %RS])

Parameter (unit)	Statistic	Treatment 3 mg/kg IV PB006	3 mg/kg IV Tysabri EU	3 mg/kg IV Tysabri US
$AUEC_{0\text{-}12w}$ (%*h)	n	141	146	145
	Mean (SD)	102,102 (19,779)	103,474 (39,258)	102,123 (19,252)
	CV (%)	19.4	37.9	18.9
	Min-Max	44,447-218,019	54,217-482,038	54,381-144,957
	Geom mean (gCV%)	100,255 (19.5)	99,479 (26.3)	100,220 (20.1)
$AUEC_{4\text{-}12w}$ (%*h)	n	144	146	145
	Mean (SD)	42,821 (17,789)	44,238 (37,838)	42,824 (17,121)
	CV (%)	41.5	85.5	40.0
	Min-Max	2825-161,038	5058-418,783	3771-82,297
	Geom mean (gCV%)	38,966 (51.0)	36,924 (67.4)	38,468 (55.7)

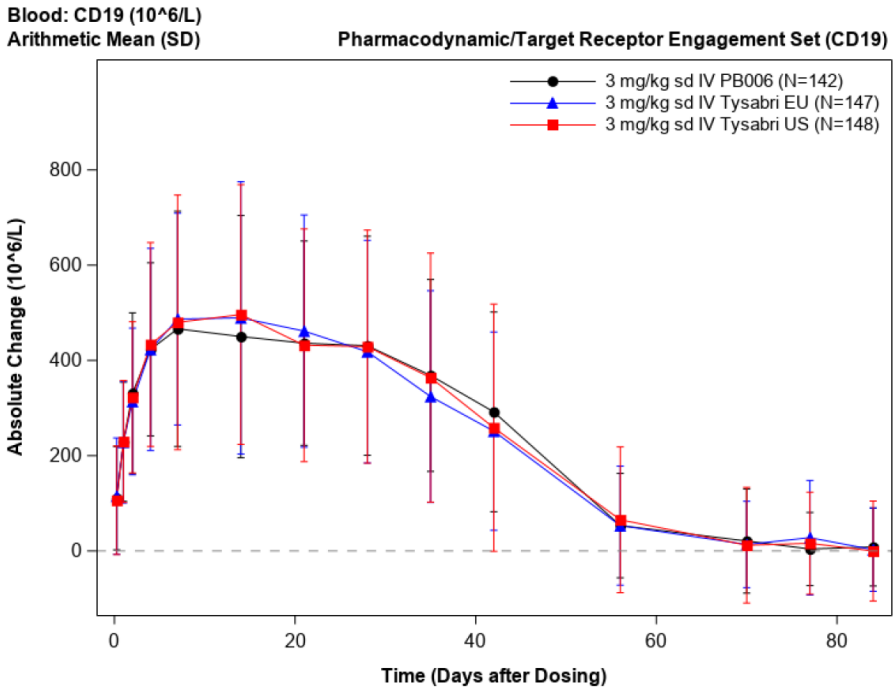
%RS=relative receptor saturation; AUEC=area under the effect curve; CV=coefficient of variation; gCV=coefficient of variation (geometric mean); Geom=geometric; IV=intravenous; Max=maximum; Min=minimum; n=number of subjects with data available; PD=pharmacodynamic(s); Tysabri EU=EU approved Tysabri; Tysabri US=US-licensed Tysabri. Note: Sensitivity analysis - samples outside of the controlled stability window were included. Note: The analysis of $AUEC_{4\text{-}12w}$ is based on the safety set and including subjects with enough values to calculate an $AUEC_{4\text{-}12w}$.

B2. CD19+

The CD19+ profiles over time were similar in all treatment groups. Mean CD19+ levels increased rapidly after administration of all treatments. At the first postdose time point (6 hours) mean CD19+ levels increased for all treatments.

A time profile of mean CD19+ levels is presented below.

Figure 5: Arithmetic Mean Change from Baseline CD19+ (10^6/L) Level - Time Profile (PD Set [CD19+])



IV=intravenous, N=number of subjects, sd=single dose, SD=standard deviation.

For subjects receiving 3 mg/kg PB006, the mean absolute CD19+ level at baseline was 251 10^6/L. After 6 hours the mean CFB was 111 10^6/L. Baseline-adjusted CD19+ levels increased further to reach a maximum mean on Day 8 (466 10^6/L). From Day 8 to Day 29 baseline-adjusted CD19+ levels were stable (CFB >400 10^6/L) and started to decline from Day 36 onwards to return to baseline on Day 78 (CFB 4 10^6/L).

For subjects receiving 3 mg/kg EU-approved Tysabri, the mean absolute CD19+ level at baseline was 255 10^6/L. After 6 hours the mean CFB was 115 10^6/L. A maximum mean CFB was reached on Day 15 (489 10^6/L). From Day 15 to Day 29, baseline-adjusted CD19+ levels were stable (CFB >400 10^6/L) and started to decline from Day 36 onwards to return to baseline on Day 71 (CFB 14 10^6/L).

For subjects receiving 3 mg/kg US-licensed Tysabri, the mean absolute CD19+ level at baseline was 266 10^6/L. After 6 hours the mean CFB was 106 10^6/L. A maximum mean CFB was reached on Day 15 (496 10^6/L). From Day 15 to Day 29, baseline-adjusted CD19+ levels were stable (CFB >400 10^6/L) and started to decline from Day 36 onwards to return to baseline on Day 71 (CFB 12 10^6/L).

$AUEC_{0\text{-}12w}$ for baseline-adjusted CD19+ was the co-primary PD parameter. Similarity was concluded if the 90% CI and 95% CI for baseline-adjusted $AUEC_{0\text{-}12w}$ of baseline-adjusted CD19+ was completely included in the similarity margin of 0.80 to 1.25.

The results from the primary PD analysis of CD19+, based on 95% CIs, are presented below.

Table 17: Statistical analysis of the primary PD endpoint $AUEC_{0-12w}$ of baseline- adjusted CD19+ (PD [CD19+] population) in study PB006-01-03

Parameter	Treatment	n	Geometric LS Means	Pairwise comparison		
				Pair	Ratio	95% CI
$AUEC_{0-12w}$ baseline-adjusted CD19+ (10^6/L*h)	PB006	142	423080	PB006 vs pooled Tysabri	1.0159	0.8955, 1.1525
	Pooled Tysabri	295	416453			

$AUEC_{0-12w}$=area under the effect time curve from time zero to 12 weeks, CI=confidence interval, LS=least square, n=number of subjects with data available, PD=pharmacodynamic

When comparing $AUEC_{0-12w}$ of baseline-adjusted CD19+ between PB006 (test) and pooled Tysabri (reference) using an ANCOVA model, for all comparisons, the point estimates were close to 1 and the 95% CI for the geometric LS means of $AUEC_{0-12w}$ was narrow, between 0.8955 and 1.1525. The 95% CI was completely included in the similarity margin of 0.80 to 1.25. Thus, the primary PD endpoint CD19+ was met.

Results for the individual comparisons (using 95% CIs), i.e., PB006 vs EU-Tysabri, PB006 vs US-Tysabri and EU-Tysabri vs US-Tysabri, are presented in below table.

Table 18: Statistical analysis of the PD endpoint $AUEC_{0-12w}$ of baseline-adjusted CD19+, individual comparisons, (PD [CD19+] population) in study PB006-01-03

Parameter	Treatment	n	Geometric LS Means	Pairwise comparison		
				Pair	Ratio	95% CI
$AUEC_{0-12w}$ baseline-adjusted CD19+ (10^6/L*h)	PB006	142	423080	PB006 vs EU-Tysabri	1.0163	0.8787, 1.1754
	EU-Tysabri	147	416312	PB006 vs US-Tysabri	1.0156	0.8783, 1.1744
	US-Tysabri	148	416593	EU-Tysabri vs US-Tysabri	0.9993	0.8653, 1.1541

$AUEC_{0-12w}$=area under the effect time curve from time zero to 12 weeks, CI=confidence interval, LS=least square, n=number of subjects with data available, PD=pharmacodynamic

For all comparisons, the point estimates were close to 1 and the 95% CIs were completely included in the similarity margin of 0.80 to 1.25.

For the secondary PD endpoint baseline-adjusted E_{max} of CD19+ geometric mean ratios and corresponding 90% CI and 95% CI were calculated, following the same methodology as described for the primary PD endpoints.

For the secondary PD endpoints baseline-adjusted E_{max} and t_{max} of CD19+ a descriptive evaluation was performed, and results based on 95% CIs are provided in Table 20.

Results from the statistical analysis of baseline-adjusted E_{max} of CD19+ are presented below.

Table 19: Statistical analysis of the secondary PD endpoint baseline-adjusted E_{max} of CD19+ (PD [CD19+] population) in study PB006-01-03

Parameter	Treatment	n	Geometric LS Means	Pairwise comparison		
				Pair	Ratio	95% CI
E_{max} baseline-adjusted of CD19+ (10^6/L)	PB006	142	566	PB006 vs EU-Tysabri	0.9961	0.9081, 1.0926
	EU-Tysabri	147	568	PB006 vs US-Tysabri	1.0317	0.9407, 1.1316
	US-Tysabri	148	548	EU-Tysabri vs US-Tysabri	1.0358	0.9452, 1.1350

$AUEC_{0-12w}$=area under the effect time curve from time zero to 12 weeks, CI=confidence interval, LS=least square, n=number of subjects with data available, PD=pharmacodynamic

Table 20: Summary of PD parameters for CD19+ cells (PD [CD19+] population) in study PB006-01-03

	PB006 N=142	EU-Tysabri N=147	US-Tysabri N=148
$AUEC_{0-12w}$ baseline-adjusted (10^6/L*h)	n=142	n=147	n=148
Arithmetic mean (SD)	481948 (218317)	481715 (237973)	500108 (275663)
CV (%)	45.3	49.4	55.1
Geometric mean (geometric CV%)	422346 (64.8)	416278 (65.3)	417321 (78.7)
E_{max} (10^6/L)	n=142	n=147	n=148
Arithmetic mean (SD)	865 (334)	870 (326)	876 (350)
CV (%)	38.6	37.5	39.9
Geometric mean (geometric CV%)	808 (38.1)	813 (38.4)	811 (41.9)
E_{max} baseline-adjusted (10^6/L)	n=142	n=147	n=148
Arithmetic mean (SD)	613 (265)	615 (254)	612 (273)
CV (%)	43.3	41.2	44.6
Geometric mean (geometric CV%)	562 (43.9)	568 (42.2)	552 (51.2)
$t_{max, E}$ (h)	n=142	n=147	n=148
Median (min-max)	336.62 (6.00-1011.82)	336.83 (6.00-1848.28)	337.48 (48.00-1680.45)

$AUEC_{0-12w}$=area under the effect time curve from time zero to 12 weeks, E_{max}=maximum effect, CV=coefficient of variation, N=number of subjects, n=number of subjects with data available, PD=pharmacodynamic, SD=standard deviation, t_{max}, E=time to E_{max}.
Note: $AUEC_{0-12w}$ and E_{max} of baseline-adjusted CD19+ cell counts were calculated as the area above zero following administration of 3 mg/kg natalizumab until CD19+ values returned and crossed zero for the first time after Emax and the maximal change from baseline of CD19+ measurements, respectively

E_{max} adjusted for baseline of CD19+ and t_{max} were secondary PD parameters. For E_{max} the changes from baseline were similar in the 3 treatment groups (mean of 612 to 615 x 10^6/L across groups). Median t_{max} was approximately 337 hours in all groups.

B3. CD34+

The CD34+ profiles over time were similar in all treatment groups.

After administration of 3 mg/kg PB006, mean absolute values for CD34+ increased from a predose value of 2.92x10^6/L to 4.43x10^6/L after 6 hours. Mean CD34+ values further increased to reach a maximum on Day 15 (10.18x10^6/L). After this maximum, mean CD34+ values decreased and returned to values similar to predose values by Day 78.

After administration of 3 mg/kg EU-approved Tysabri, mean absolute values for CD34+ increased from a predose value of 2.93x10^6/L to 4.57x10^6/L after 6 hours. Mean CD34+ values further increased to reach a maximum on Day 15 (10.03x10^6/L). After this maximum mean CD34+ values decreased and returned to values similar to predose values by Day 78.

After administration of 3 mg/kg US-licensed Tysabri, mean absolute values for CD34+ increased from a predose value of 2.65x10^6/L to 4.17 10^6/L after 6 hours. Mean CD34+ values further increased to reach a maximum on Day 15 (9.57x10^6/L). After this maximum mean CD34+ values decreased and returned to values similar to predose values by Day 85.

$AUEC_{0-t}$, E_{max}, and $t_{max,E}$ for CD34+ were secondary PD endpoints, and a descriptive evaluation was performed. A summary of these PD parameters for CD34+ is provided in below table.

Table 21: Summary Statistics of PD Parameters for CD34+ (PD Set [Other])

	Treatment	3 mg/kg IV PB006	3 mg/kg IV Tysabri EU	3 mg/kg IV Tysabri US
Parameter (unit)	**Statistic**	**N=145**	**N=148**	**N=145**
$AUEC_{0-t}$ (10^6/L*h)	Mean (SD)	12,591 (6564)	12,632 (7207)	12,206 (7022)
	CV (%)	52.1	57.1	57.5
	Min-Max	3723-44,794	3371-43,862	3157-50,905
	Geom mean (gCV%)	11,182 (51.3)	10,958 (57.1)	10,604 (57.0)
$t_{max,E}$ (h)	Median	340.03	341.94	338.48
	Min-Max	48.00-1007.00	6.00-1345.00	48.00-1874.08
E_{max} (10^6/L)	Mean (SD)	12.5 (6.59)	12.6 (6.84)	11.8 (6.53)
	CV (%)	52.6	54.3	55.4
	Min-Max	3.73-37.7	3.09-43.4	3.05-46.5
	Geom mean (gCV%)	11.1 (51.5)	11.0 (55.9)	10.3 (55.1)

AUEC=area under the effect curve (above threshold effect); CD34+=Cluster of Differentiation 34 activation; CV=coefficient of variation; gCV=coefficient of variation (geometric mean); Geom=geometric; IV=intravenous; Max=maximum; Min=minimum; N=number of subjects; PD=pharmacodynamic(s); Tysabri EU=EU-approved Tysabri; Tysabri US=US-licensed Tysabri

The mean $AUEC_{0-t}$ and E_{max} and median $t_{max,E}$ were similar for the 3 treatments.

B4. sVCAM

The sVCAM profiles over time were similar in all treatment groups.

After administration of 3 mg/kg PB006, the mean absolute values for sVCAM decreased from a predose value of 725,662 µg/L to 592,104 µg/L after 6 hours. A minimum mean value was reached on Day 8 (344,803 µg/L). After this minimum, sVCAM returned to values similar to the predose value by Day 57 (745,579 µg/L).

After administration of 3 mg/kg EU-approved Tysabri, the mean absolute values for sVCAM decreased from a predose value of 751,493 µg/L to 620,966 µg/L after 6 hours. A minimum mean value was reached on Day 8 (366,531 µg/L). After this minimum, sVCAM returned to values similar to the predose value by Day 57 (784,801 µg/L).

After administration of 3 mg/kg US-licensed Tysabri, the mean absolute values for sVCAM decreased from a predose value of 743,345 µg/L to 610,690 µg/L after 6 hours. A minimum mean value was

reached on Day 8 (355,386 µg/L). After this minimum, sVCAM returned to values similar to the predose value by Day 57 (769,688 µg/L).

$AUEC_{base\_neg}$, E_{min} and t_{min} of sVCAM were secondary PD endpoints, and a descriptive evaluation was performed.

Table 22: Summary Statistics of PD Parameters for sVCAM (PD Set [Other])

	Treatment	3 mg/kg IV PB006	3 mg/kg IV Tysabri EU	3 mg/kg IV Tysabri US
Parameter (unit)	**Statistic**	**N=145**	**N=148**	**N=145**
$AUEC_{base\ neg}$ (µg/L*h)	Mean (SD)	-331,097 (203,511)	-293,641 (209,432)	-308,654 (191,012)
	CV (%)	-	-	-
	Min-Max	-1,095,420-294,164	-995,285-287,038	-1,307,922-221,398
	Geom mean (gCV%)	NC	NC	NC
t_{min} (h)	Median	168.00	168.00	168.00
	Min-Max	24.00-843.43	46.35-1011.82	48.00-838.00
E_{min} (µg/L)	Mean (SD)	320 (75.9)	343 (94.1)	335 (94.1)
	CV (%)	23.7	35.7	28.1
	Min-Max	168-548	115-1474	145-882
	Geom mean (gCV%)	311 (24.0)	330 (27.5)	323 (26.9)

AUECbase_neg= area below the individual baseline value minus the area above the individual baseline in case of time intervals for which the curve exceeded the baseline value; CV=coefficient of variation; gCV=coefficient of variation (geometric mean); Geom=geometric; IV=intravenous; Max=maximum; Min=minimum; N=number of subjects; NC=not calculated; PD=pharmacodynamic(s); sVCAM=soluble vascular cell adhesion molecule; Tysabri EU=EU-approved Tysabri; Tysabri US=US-licensed Tysabri

In an exploratory manner, geometric mean ratios with 90% and 95% CIs were calculated for $AUEC_{base\_neg}$ and E_{min} using the same methods as described for the primary PD parameters. The results were not assessed by pre-specified similarity margins. Results are presented for the 95% CIs in the table below.

Table 23: Statistical analysis of the PD endpoint sVCAM (PD [other] population) in study PB006-01-03

Parameter	Treatment	n	Geometric LS Means	Pairwise comparison		
				Pair	Ratio	95% CI
$AUEC_{base\_neg}$ of VCAM (µg/L*h)	PB006	145	-331097	PB006 vs EU-Tysabri	1.1276	0.9700, 1.2852
	EU-Tysabri	148	-293641	PB006 vs US-Tysabri	1.0727	0.9220, 1.2234
	US-Tysabri	145	-308654	EU-Tysabri vs US-Tysabri	0.9514	0.8014, 1.1013
E_{min} of VCAM (µg/L)	PB006	145	320	PB006 vs EU-Tysabri	0.9320	0.8654, 0.9986
	EU-Tysabri	148	343	PB006 vs US-Tysabri	0.9558	0.8872, 1.0245
	US-Tysabri	145	335	EU-Tysabri vs US-Tysabri	1.0256	0.9573, 1.0939

$AUEC_{base\_neg}$=area below the individual baseline value minus the area above the individual baseline in case of time intervals for which the curve exceeded the baseline value, CI=confidence interval, Emin=minimum effect, LS=Least scare, n=number of subjects with data available, PD=pharmacodynamic.

B5. sMAdCAM

The sMAdCAM profiles over time were similar in all treatment groups.

After administration of 3 mg/kg PB006, the mean absolute values for sMAdCAM decreased from a predose value of 20,158 µg/L to 18,422 µg/L after 6 hours. A minimum mean value was reached on Day 29 (4146 µg/L). After this minimum, sMAdCAM returned to values similar to the predose value by Day 71 (20,094 µg/L).

After administration of 3 mg/kg EU-approved Tysabri, the mean absolute values for sMAdCAM decreased from a predose value of 19,778 µg/L to 18,330 µg/L after 6 hours. A minimum mean value was reached on Day 29 (4270 µg/L). After this minimum, sMAdCAM returned to values similar to the predose value by Day 71 (20,173 µg/L).

After administration of 3 mg/kg US-licensed Tysabri, the mean absolute values for sMAdCAM decreased from a predose value of 19,237 µg/L to 17,410 µg/L after 6 hours. A minimum mean value was reached on Day 29 (4023 µg/L). After this minimum, sMAdCAM returned to values similar to the predose value by Day 71 (18,813 µg/L).

$AUEC_{base\_neg}$, E_{min} and t_{min} for sMAdCAM were secondary PD endpoints, and a descriptive evaluation was performed.

Table 24: Summary Statistics of PD Parameters for sMAdCAM (PD Set [Other])

	Treatment	3 mg/kg IV PB006	3 mg/kg IV Tysabri EU	3 mg/kg IV Tysabri US
Parameter (unit)	**Statistic**	**N=145**	**N=148**	**N=145**
$AUEC_{base\_neg}$ (µg/L*h)	Mean (SD)	-16,706 (6351)	-15,746 (7552)	-16,055 (7265)
	CV (%)	-	-	-
	Min-Max	-36,638- -1399	-43,197- -2306	-45,333- -2945
	Geom mean (gCV%)	NC	NC	NC
t_{min} (h)	Median	839.70	839.21	839.00
	Min-Max	168.02-1011.63	340.55-1012.40	501.53-1347.53
E_{min} (µg/L)	Mean (SD)	3.69 (1.16)	3.67 (1.32)	3.53 (1.23)
	CV (%)	31.4	36.0	34.8
	Min-Max	1.04-9.04	1.06-9.31	1.15-9.68
	Geom mean (gCV%)	3.51 (32.7)	3.46 (36.0)	3.34 (34.8)

$AUEC_{base\_neg}$= area below the individual baseline value minus the area above the individual baseline in case of time intervals for which the curve exceeded the baseline value; CV=coefficient of variation; gCV=coefficient of variation (geometric mean); Geom=geometric; IV=intravenous; Max=maximum; Min=minimum; N=number of subjects; PD=pharmacodynamic(s); sMAdCAM=soluble mucosal addressin cell adhesion molecule; Tysabri EU=EU-approved Tysabri; Tysabri US=US-licensed Tysabri

In an exploratory manner, geometric mean ratios with 90% and 95% CIs were calculated for $AUEC_{base\_neg}$ (Area below the individual baseline value minus the area above the individual baseline in case of time intervals for which the curve exceeded the baseline value) and E_{min} using the same methods as described for the primary PD parameters. The results were not assessed by pre-specified similarity margins. Results are presented for the 95% CIs in the table below.

Table 25: Statistical analysis of the PD endpoint sMAdCAM (PD [other] population) in study PB006-01-03

Parameter	Treatment	n	Geometric LS Means	Pairwise comparison		
				Pair	Ratio	95% CI
$AUEC_{base\_neg}$ of MAdCAM (µg/L*h)	PB006	145	-16706	PB006 vs EU-Tysabri	1.0609	0.9577, 1.1642
	EU-Tysabri	148	-15746	PB006 vs US-Tysabri	1.0406	0.9388, 1.1423
	US-Tysabri	145	-16055	EU-Tysabri vs US-Tysabri	0.9808	0.8795, 1.0820
E_{min} of MAdCAM (µg/L)	PB006	145	3.69	PB006 vs EU-Tysabri	1.0034	0.9260, 1.0809
	EU-Tysabri	148	3.67	PB006 vs US-Tysabri	1.0438	0.9628, 1.1248
	US-Tysabri	145	3.53	EU-Tysabri vs US-Tysabri	1.0402	0.9597, 1.1208

$AUEC_{base\_neg}$=area below the individual baseline value minus the area above the individual baseline in case of time intervals for which the curve exceeded the baseline value, CI=confidence interval, Emin=minimum effect, LS=Least scare, n=number of subjects with data available, PD=pharmacodynamic.

Ancillary analyses

COVID-19-Impact

Plan

Changes in conduct or analysis of the study due to COVID-19 were not considered at start of the study since the virus was not known by that time.

In Amendment 3 (20 June 2020) of the study protocol, measures were described which were taken to protect subjects after dosing from a COVID-19 infection:

1) Inclusion criteria:
   a. Reduction of maximum inclusion age from 65 years reduced to 54 years before dosing
   b. 2 negative SAS-CoV-2 tests prior dosing

2) Exclusion criteria: History or evidence of SARS-CoV-2 infection in the last month prior screening 1 or having been in confirmed contact with SARS-CoV-2 positive subjects in the last 2 weeks before dosing

3) Inclusion of polymerase chain reaction [PCR] SARS-CoV-2 tests, update of ambulatory visit period and ambulatory activities, and increase of the in-house period

4) Update of benefit/risk assessment of conduct of this study: The national multiple sclerosis society of the USA, as per the website information published on their website, classifies natalizumab as an immunomodulator rather than an immunosuppressant and states that treatment with natalizumab does not involve any greater risk for infection with e.g. SARS-CoV-2. Subjects in the trial also do not belong to potential high-risk groups. The risk assessment for increase of serious infections is classified as both high impact and low possibility.

Conduct & Outcome

Due to the COVID-19 pandemic, the study was temporarily stopped on 16 Mar 2020. After approval of CSP Version 4.0 (dated 20 June 2020), study enrolment was resumed.

Prior to the enrolment halt, the study had been started with 3 sites. Due to COVID-19 related recruitment

issues, 3 additional sites were included at the time enrolment was resumed.

In order to assess the impact of COVID-19 on the safety in this study, study discontinuations and protocol deviations due to COVID-19 were listed and summarized, if possible.

To investigate whether there were differences between subjects completing the study prior to the study stop and subjects completing the study after the study stop, Tables, Figures and Listings (TFLs) for demographics and AEs were also created on 2 subsets of the Safety Set:

- Pre-stop Safety Set: Subjects randomized to study intervention and dosed with study intervention and who completed the study before the study stop due to COVID-19 on 16 Mar 2020.
- Post-stop Safety Set: Subjects randomized to study intervention and dosed with study intervention and who completed the study after the study stop due to COVID-19 on 16 Mar 2020.

Difference between the subjects in baseline characteristics enrolled before and after the study stop due to COVID-19 was limited and might be due to the small number of subjects randomized before the study stop (n=16) when compared to the number of subjects randomized after the study restart (n=434).

Important protocol deviations related to COVID-19 were reported for 5 subjects, 3 are related to compliance with quarantine requirements, and 2 are related to use of medication without consulting the Investigator.

Before the study stop due to COVID-19, 25 TEAEs were reported for 10 subjects (63%) After the study stop due to COVID-19, 790 TEAEs were reported for 293 subjects (68%). Eleven (11) events of COVID-19 were reported by 11 subjects (3%), 5 events by 5 subjects (3%) receiving 3 mg/kg PB006, 2 events by 2 subjects (1%) receiving 3 mg/kg EU-approved Tysabri, and 4 events by 4 subjects (3%) receiving 3 mg/kg US-licensed Tysabri. One (1) event of asymptomatic COVID-19 was reported by a subject receiving US-licensed Tysabri. There was no difference in the nature and severity of the reported TEAEs. No subjects were withdrawn due to COVID-19.

Absorption

Since PB006 is developed as an IV solution, no clinical studies were conducted to evaluate bioavailability of the product.

No specific food-interaction studies were performed.

Distribution

Volume of distribution (Vz) was assessed in the PK/PD study PB006-01-03. The PK results for study PB006-01-03 are presented in more detail in the Bioequivalence section. PB006 had a mean Vz of 1262 ml, Vz (EU-Tysabri) was 1198 ml and Vz (US-Tysabri) was 1164 ml.

Elimination

$t_{1/2}$ and CL were assessed in the PK/PD study PB006-01-03. PB006 had a geometric mean $t_{1/2}$ of 90.3 h, geometric mean $t_{1/2}$ of EU-Tysabri and US-Tysabri were 87.6 h and 87.5 h, respectively. The geometric mean values for CL were 9.72 ml/h for PB006, 9.48 ml/h for EU-Tysabri, and 9.22 for US-Tysabri.

2.6.2.2. Pharmacodynamics

The applicant conducted two pivotal studies to investigate the clinical similarity between PB006 and Tysabri, one single dose PK/PD study in healthy volunteers (PB006-01-03) and one multiple dose efficacy and safety study in patients with RRMS (PB006-03-01). The latter study included PK evaluation at steady state (C_{trough}) but no assessment of PD was conducted in patients.

Additionally, the applicant conducted a pilot study with Tysabri in healthy subjects (Tysabri Pilot-01-01). The purpose of this pilot study was the collection of PK/PD data with 3 different doses of EU-Tysabri to

establish the appropriately sensitive study design features including the dose selection and primary endpoints for the pivotal PK/PD study PB006-01-03.

Besides the comparative PK and efficacy & safety evaluation conducted for this MAA, comparative PD assessed in study PB006-01-03 can be used to further support the clinical similarity assessment of PB006 and Tysabri.

Mechanism of action

The Mechanism of Action of natalizumab is presented in the SmPC of the reference medicinal product EU-Tysabri (and referred to in the Dossier of Tyruko):

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, VCAM-1, and ligands osteopontin, and an alternatively spliced domain of fibronectin, CS-1. Natalizumab blocks the interaction of α4β7 integrin with the 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.

In MS, lesions are believed to occur when activated T-lymphocytes cross the BBB. Leukocyte migration across the BBB involves interaction between adhesion molecules on inflammatory cells and endothelial cells of the vessel wall. The interaction between α4β1 and its targets is an important component of pathological inflammation in the brain and disruption of these interactions leads to reduced inflammation. Under normal conditions, VCAM-1 is not expressed in the brain parenchyma. However, in the presence of pro-inflammatory cytokines, VCAM-1 is upregulated on endothelial cells and possibly on glial cells near the sites of inflammation. In the setting of CNS inflammation in MS, it is the interaction of α4β1 with VCAM-1, CS-1 and osteopontin that mediates the firm adhesion and transmigration of leukocytes into the brain parenchyma and may perpetuate the inflammatory cascade in CNS tissue. Blockade of the molecular interactions of α4β1 with its targets reduces inflammatory activity present in the brain in MS and inhibits further recruitment of immune cells into inflamed tissue, thus reducing the formation or enlargement of MS lesions.

In the presented dossier for the Tyruko MAA, the applicant further describes the Mechanism of Action for natalizumab in the pathogenesis of MS:

VCAM-1 is upregulated on brain microvascular endothelial cells in inflammatory CNS lesions of patients with MS (Benkert et al., 2012). As VCAM-1 is expressed on inflamed cerebrovascular endothelial cells, α4β1 is believed to be the critical target of natalizumab in preventing leukocyte migration into the CNS in MS (Selewski et al., 2010, "Natalizumab (Tysabri)").

MS is a chronic inflammatory demyelinating disease of the CNS. It may take a relapsing-remitting or a chronic progressive clinical course. The immigration of activated T-lymphocytes into the CNS is fundamental to its pathogenesis. During disease development, CD4+ T-cells encounter environmental triggers of unknown kind in the periphery. This, in a widely accepted view, leads to activation of CNS antigen specific CD4+ T-cells in genetically susceptible individuals. These autoreactive T-cells then cross the BBB as effector T helper cells and initiate a chronic autoimmune disease (Benkert et al., 2012).

Leucocyte migration across the BBB involves interaction between adhesion molecules on inflammatory cells and their counter-receptors present on endothelial cells of the vessel wall. The clinical effect of

natalizumab in multiple sclerosis may be secondary to blockade of the molecular interaction of α4β1 integrin expressed by inflammatory cells with VCAM-1 on vascular endothelial cells, and with CS-1 and/or osteopontin expressed by parenchymal cells in the brain. In MS, the rationale for natalizumab therapy is the reduction of leukocyte migration into the CNS by specifically targeting α4β1, or very-late-activation antigen 4 (EMA Tysabri EPAR – Assessment Report – Variation, 2013). VCAM is induced on venular endothelium in inflammation, including in brain in experimental autoimmune encephalitis. Natalizumab, as an antibody to α4β1, blocks lymphocyte emigration into the brain, α4β1 dependent co-stimulation of immune responses, and EAE. Consequently, α4β1 and not α4β7 is the critical target for experimental autoimmune encephalitis (Yu et al., 2013, "How Natalizumab Binds and Antagonizes α4 Integrins").

Primary and Secondary pharmacology

Study Tysabri Pilot-01-01

A randomised, parallel-group, single dose clinical pharmacology pilot study was performed by the applicant in order to characterise the PK and PD profiles of the reference medicinal product EU-Tysabri. Further, the study aimed to assess and compare the Fab-arm exchange of EU-Tysabri, and to assess the safety of EU-Tysabri. The study is briefly summarised below, with a focus on PD of natalizumab.

Study Design

Tysabri Pilot-01-01 was a single-centre, single-dose, randomized, double-blind, 3-arm parallel-group study in 36 healthy male and female subjects. Subjects were randomized to one of 3 dose groups and treated with single doses of EU-Tysabri. The study schedule included 2 screening visits prior to drug administration, a clinic period from Day -1 to approximately 48 hours after drug administration and 11 ambulatory visits, on Day 5, 8, 15, 22, 29, 36, 43, 57, 71, 78, and 85. An additional follow-up visit was performed 6 months after drug administration, to assess potential neurological symptoms which could suggest PML. Safety was monitored throughout the study by repeated clinical and laboratory evaluations. Samples for assessments of PK, PD and immunogenicity were collected up to Day 85.

Healthy male and female subjects aged 18 to 65 years at screening, with a body weight of 50.0 to 110.0 kg, a BMI of 18.5 to 32.0 kg/m^2, and JCV antibody negative were eligible to the study. Randomisation was stratified by body weight class (50.0 - 70.0 kg, 70.1 - 90.0 kg, 90.1 - 110.0 kg). No formal sample size calculation was performed.

Subjects were randomised to three treatment arms and received a single, 60-minute, IV dose of 1, 3, or 6 mg/kg EU-Tysabri (N=12 for each arm).

Blood samples for assessment of PD parameters were collected pre-dose and 6 hours after start of infusion on Day 1, and on Days 2, 5, 8, 15, 22, 29, 36, 43, 57, 71, 78 and 85.

The PD variables included sMAdCAM, sVCAM, α4-integrin %RS, cell counts for CD34+, CD19+, CD3+, CD3+CD8+, CD3+CD4+, CD3-CD16+CD56+, CD10+CD19+. For α4-integrin %RS, sVCAM and sMadCAM, and for all lymphocyte cell counts the following PD parameters were calculated: The PD parameters were $AUEC_{0\text{-}12w}$ and $AUEC_{4\text{-}12w}$, E_{max} and time to Emax ($T_{max,\ E}$). The PD parameters were estimated on the absolute values, using a noncompartmental model. The linear trapezoidal interpolation method for AUEC calculation was used. Per an addendum to the SAP, geometric CV was added to the PD levels and parameter tables, and ANOVA was performed for relative α4-integrin %RS and CD19+ using the SAS procedure for mixed effect models (with treatments as fixed effects).

The analysis of PD levels was based on the safety population, defined as all subjects who received study medication. The analysis of PD parameters was based on the PD population, defined as all subjects who have received the dose of study medication and provided sufficient bioanalytical assessment results to calculate reliable estimates of the PD parameters.

The demographics and baseline characteristics are described in the following table.

Table 26: Demographic and baseline characteristics in study Tysabri Pilot-01-01 (PK population)

	EU-Tysabri 1 mg/kg N=12	EU-Tysabri 3 mg/kg N=12	EU-Tysabri 6 mg/kg N=12
Age (years)			
Mean (SD)	33 (10)	36 (13)	37 (11)
Sex, n (%)			
Male	3 (25)	5 (42)	7 (58)
Female	9 (75)	7 (58)	5 (42)
Race, n (%)			
White	7 (58)	9 (75)	4 (33)
Black or African American	5 (42)	3 (25)	6 (50)
White + Black or African American	0	0	1 (8)
White + Black or African American + Asian	0	0	1 (8)
Weight (kg)			
Mean (SD)	77.0 (11.5)	79.3 (12.1)	75.5 (12.3)
Height (cm)			
Mean (SD)	169 (10)	172 (9)	174 (10)
Body mass index (kg/m^2)			
Mean (SD)	26.8 (2.7)	26.8 (2.5)	25.0 (2.9)
Weight class, n (%)			
50.0-70.0 kg	3 (25)	3 (25)	4 (33)
70.1-90.0 kg	8 (67)	7 (58)	7 (58)
90.1-110.0 kg	1 (8)	2 (17)	1 (8)

N=number of subjects receiving study medication; n=number of subjects in this category; SD=Standard deviation, EU-Tysabri =EU-approved Tysabri. Please note that the PK population was identical to the PD and the safety population.

PD Results

For α4-integrin (%)RS a clear dose response relationship was observed across the tested doses, indicating good dynamic range of the PD marker. The increase in $AUEC_{0\text{-}12w}$ was approximately 1.7-fold for the dose increase from 1 mg/kg to 3 mg/kg, and approximately 1.3-fold for the dose increase from 3 mg/kg to 6 mg/kg (based on geometric mean values). With regard to variability, the geometric CV for $AUEC_{0\text{-}12w}$ ranged from 18.5% (with 1 mg/kg) to 27.7% (with 3 mg/kg). Overall, these results were the prerequisite for setting up the sensitive PD assessment in the pivotal study PB006-01-03 and based on these results, the 3 mg/kg dose was selected.

CD19+ B-cell counts increased in a sensitive and dose-dependent way upon natalizumab exposure. A clear dose response relationship was observed across the tested doses, indicating good dynamic range of the PD marker. The increase in $AUEC_{0\text{-}12w}$ change from baseline was approximately 1.8-fold for the dose increase from 1 to 3 mg/kg, and approximately 1.4-fold for the dose increase from 3 to 6 mg/kg (based on geometric mean values). The variability was high, with geometric CV being between 50.6% (for 6 mg/kg) and 101.4% (for 1 mg/kg). Based on these results, the 3 mg/kg dose was selected as a sensitive dose for the pivotal PK/PD study PB006-01-03.

CD34+ cell counts increased in a dose dependent way across the tested doses, indicating good dynamic range of the PD marker. The increase for $AUEC_{0\text{-}12w}$ was approximately 1.7-fold for the dose increase from 1 to 3 mg/kg, and approximately 1.5-fold for the dose increase from 3 to 6 mg/kg (based on geometric mean values). Variability was high, with geometric CV between 44.0% (for 1 mg/kg) and 68.7% (for 3 mg/kg).

For sVCAM, a clear dose response relationship was observed across the tested doses, indicating good dynamic range of the PD marker. The increase in $AUEC_{0\text{-}12w}$ below baseline effect was approximately 2.0-

fold for the dose increase from 1 to 3 mg/kg, and approximately 1.3-fold for the dose increase from 3 to 6 mg/kg (based on geometric mean values). With regard to variability, geometric CVs ranged between 33.9 and 71.2%.

Also for sMAdCAM, a clear dose response relationship was observed across the tested doses, indicating good dynamic range of the PD marker. The increase in $AUEC_{0\text{-}12w}$ below baseline effect was approximately 1.7-fold for the dose increase from 1 to 3 mg/kg, and approximately 1.6-fold for the dose increase from 3 to 6 mg/kg (based on geometric mean values). With regard to variability, geometric CVs ranged between 38.9 and 54.8%.

The additionally tested PD parameters CD3+, CD3+CD4+, CD3+CD8+, CD3-CD16+56+, and CD10+CD19+ cells were found to not be dose dependent and thus not sensitive to detect differences in efficacy. These parameters were hence not included in the pivotal PK/PD study PB006-01-03.

2.6.3. Discussion on clinical pharmacology

The clinical pharmacology programme for the natalizumab biosimilar candidate PB006 comprises data from two studies: the bulk of data for the biosimilarity exercise in PK and PD was generated in the pivotal phase 1 PK/PD study PB006-01-03, which investigated biosimilarity between PB006 and both EU- and US-sourced Tysabri in healthy subjects (N=453). Further, trough concentrations and immunogenicity were assessed in a pivotal phase 3 study ("efficacy study" PB006-03-01).

The primary objective of the pivotal PK/PD study was the demonstration of similarity in the PK and PD profiles. In this study, one primary PK endpoint as well as two co-primary PD endpoints were used. The study design and study population were appropriate. It is noted that for the scope of this MAA, the comparison of the biosimilar candidate and EU-Tysabri is considered of higher regulatory significance than the comparison with US-Tysabri.

The analytical methods have been adequately validated; all issues identified have been addressed by the applicant and are considered solved.

A population of healthy subjects was included which is appropriate to detect potential differences between the two treatments since variability is minimised and the mode of action is the same in healthy subjects and patients. In addition, inclusion of only healthy volunteers who were shown to be negative for anti-JCV antibodies as a measure to minimise the risk for PML, is in line with the scientific advice.

Stratification was performed according to the body weight class (50 kg to 65 kg, >65 kg to 80 kg, >80 kg to 92 kg) to ensure balance across the study arms, because according to Tysabri EPAR, body weight was found to influence the natalizumab disposition.

The study protocol was amended at several time points, with extensive changes to its design, including the primary endpoints, sample size, study population, conduct of the study (introduction of a pooling criterion to allow pooling of results of EU- and US-Tysabri for PD evaluation), and evaluation of study results. Some changes were introduced in response to the worldwide SARS-CoV-2 outbreak (including the inclusion of three additional sites), which took place during the early stages of the study and led to a temporary study halt.

Amendments to the primary PD endpoints were made. In the original study protocol, $AUEC_{4\text{-}12wk}$ of α4-integrin RS was specified. This endpoint was agreed during EMA scientific advice. Use of the partial $AUEC_{4\text{-}12wk}$ was reasoned with rapid and strong induction of the marker immediately following administration of natalizumab. Since receptor saturation was high (near max saturation levels) until around the 4 week mark, exclusion of the first 4 weeks leads to increased sensitivity of the marker. However, the endpoint was replaced by $AUEC_{0\text{-}12wk}$ α4-integrin RS in the final amendment, for reasons

of harmonisation across regulatory regions. $AUEC_{4\text{-}12wk}$ α4-integrin RS was planned as an additional supportive analysis. In the original study protocol, $AUEC_{0\text{-}t}$ and E_{max} of CD19+ B-cells were specified. However, these endpoints were downgraded to secondary endpoints with the first amendment. With the final amendment, $AUEC_{0\text{-}12wk}$ of CD19+ cells was reintroduced.

While the addition of a co-primary PD marker acting downstream of the receptor was advised during EMA scientific advice and is endorsed, no scientific justification of the CD19+ B-cells in terms of PD was provided with the documentation, and a discussion of the relevance of the marker was requested from the applicant. In said discussion, the applicant acknowledged that the CD19+ B-cell count does not qualify as a surrogate endpoint. Since the applicant does not aim to derive clinical relevance from the PD characterisation and the clinical efficacy assessment is informed by data generated in a dedicated efficacy study in a patient population, the omission to provide an adequate justification for the clinical relevance of the selected PD endpoint does not impede assessment of biosimilarity and the concern is therefore not further pursued.

In the initial submission, the additional pre-specified analysis of receptor saturation $AUEC_{4\text{-}12wk}$ of α4-integrin %RS was missing and the descriptive results presented were apparently based on the safety set, instead of the pre-specified RS/RO main analysis $AUEC_{4\text{-}12wk}$ set. The applicant was asked to provide the correct analysis and no concerns arise from the submitted data. Also the unpooled sensitivity analyses for AUEC0-12 α4-integrin RS were requested and the data provided raised no concerns.

One methodological concern pertains to potentially jeopardised trial integrity, as any potential data-driven planning of important design aspects (choice of PD endpoints, option for pooling of reference data, etc) may have a non-negligible impact on trial outcome interpretation. Due to the extensive character of the amendments relatively late into the study, a description/overview of the chronological sequence of relevant trial milestones was submitted by the applicant. Measures taken to reflect the late changes in the trial in the randomisation process were described by the applicant. Although many changes were made during study conduct, no severe impact on study integrity could be identified.

Biosimilarity in PK of PB006 and EU-authorised and US-sourced Tysabri was demonstrated following single dose IV infusion of 3 mg/kg natalizumab, as the 90% CIs for the ratio of geometric LS means for the primary PK endpoint $AUC_{0\text{-}inf}$ of total natalizumab of PB006 and EU- (and US-) sourced Tysabri were completely within the acceptance interval of 0.8 – 1.25. This result was supported by the results presented for the secondary endpoints regarding total natalizumab ($AUC_{0\text{-}t}$, C_{max}), and unexchanged natalizumab ($AUC_{0\text{-}inf}$, $AUC_{0\text{-}t}$, C_{max}, t_{max}). T_{max} of total natalizumab supported biosimilarity between PB006 and EU-Tysabri, but a difference was noted for the US-sourced comparator. This however does not raise concerns, given the higher regulatory significance of the comparison with EU-Tysabri. The primary and secondary PK endpoints as well as the equivalence margin (80-125%) used are in line with the "Guideline on similar biological medicinal products containing monoclonal antibodies – non-clinical and clinical issues" (EMA/CHMP/BMWP/403543/2010).

For PB006 and EU-Tysabri, the ratio of geometric LS means for the primary PK endpoint $AUC_{0\text{-}inf}$ of total natalizumab was 0.9864 (90% CI: 0.9410, 1.0340), for $AUC_{0\text{-}t}$ of total natalizumab 0.9840 (90% CI: 0.9387, 1.0316), and for C_{max} 0.9565 (90% CI: 0.9215, 0.9929). Therefore, bioequivalence can be concluded from the results of the primary PK endpoint. The results of the PK secondary analyses support the results of the primary PK analysis.

Demographic and baseline characteristics (mean age, body weight, height, BMI) were balanced between the three treatment arms. Slightly more men than women were recruited in the PB006 group (males 53%) compared to both Tysabri groups (EU-Tysabri: 46.4% males, US-Tysabri: 47.3% males). Race of participants and body weight classes were well balanced between treatment arms.

In study PB006-03-01 after repeated administration, the C_{trough} concentrations were similar in the PB006 and Tysabri groups at all post-baseline timepoints for both FAS and PP populations.

Certificate of analyses for the biosimilar candidate batch No. P79303A, EU-Tysabri batch 1424913 and batch No. 1425561, US-Tysabri batch No. SP0118 used in the clinical trial PB006-01-03 have been provided. As the regulatory decision regarding the biosimilarity is based on the comparability between the biosimilar candidate PB006 and EU-Tysabri, for which certificates of analysis contained all the relevant parameters, the issue is not further pursued.

For the demonstration of biosimilarity in PD, two co-primary endpoints were used: $AUEC_{0\text{-}12wk}$ α4-integrin % RS and $AUEC_{0\text{-}12wk}$ of the baseline-adjusted CD19+ cell fraction. Both co-primary endpoints were met, as the 95% CIs for the ratio of geometric LS means for the co-primary PD endpoints $AUEC_{0\text{-}12wk}$ α4-integrin %RS and $AUEC_{0\text{-}12wk}$ baseline-adjusted CD19+ were completely within a predefined interval of 0.8 – 1.25.

For PB006 and EU-Tysabri, the ratio of geometric LS means for $AUEC_{0\text{-}12wk}$ α4-integrin % RS was 1.0142 (95% CI: 0.9667, 1.0641). The ratio for the comparison against pooled EU- and US-Tysabri was 0.9933 (95% CI: 0.9523, 1.0362).

For PB006 and EU-Tysabri, the ratio of geometric LS means of $AUEC_{0\text{-}12wk}$ of baseline-adjusted CD19+ was 1.0163 (95% CI: 0.8787, 1.1754). The ratio for the comparison against pooled EU- and US-Tysabri was 1.0159 (95% CI: 0.8955, 1.1525).

Although the EMA/CHMP scientific advice recommended to update the primary endpoint to the partial $AUEC_{4\text{-}12W}$ of the α4-integrin receptor saturation to use the most sensitive part of the curve to detect differences between the reference product and the biosimilar candidate as primary endpoint for evaluating biosimilarity, the applicant did not follow the scientific advice.

The EMA/CHMP scientific advice recommended to include additional true PD markers (e.g., lymphocyte count measurements, lymphocyte subset analysis, sVCAM-1 concentration measurements, sMADCAM concentrations) to provide further supportive PD evidence, as α4-integrin RS over time is not a true PD marker since it merely reflects occupancy of the receptor rather than translation into an effect. Thus, secondary endpoints measured in study PB006-01-03, i.e., E_{max}, t_{max} of baseline adjusted CD19+, $AUEC_{base\_neg}$, E_{min}, t_{min} of sVCAM and of sMAdCAM, and $AUEC_{0\text{-}t}$, E_{max}, t_{max} of CD34+ are considered adequate.

A mean maximum response of α4-integrin %RS of 92.6% was observed more rapidly after administration of 3 mg/kg PB006 (24 hours) as compared to administration of 3 mg/kg Tysabri EU (93.2% on Day 5 (96 hours)) and of 3 mg/kg Tysabri US (93.1% on Day 5 (96 hours)).

The use of 95% CI for primary PD parameters is considered adequate. However, the used equivalence margins (80-125%) for assessment of PD endpoints were criticised during EMA scientific advice provided prior to study initiation as being too wide. Also, no sound scientific justification for the acceptance ranges was found in the documentation. The applicant was therefore asked to provide a justification for the equivalence margins of 0.8 – 1.25 used for all PD endpoints. In the responses, the applicant acknowledged that none of the PD endpoints are sufficiently associated with a clinical endpoint and hence did not provide a clinical justification of the pre-specified acceptance margin. Given that in the developmental programme of Tyruko efficacy data were generated which are considered adequate to enable assessment of biosimilarity, this lack of clinical justification does not impede conclusions on biosimilarity, and the issue is therefore not further pursued.

The secondary PD endpoints (E_{max} and t_{max} of baseline-adjusted CD19+, $AUEC_{base\_neg}$, E_{min}, t_{min} of sVCAM and sMAdCAM, $AUEC_{0\text{-}t}$, E_{max}, t_{max} of CD34+) were overall supportive of biosimilarity between PB006 and EU- (and US-)Tysabri. With the exception of sVCAM $AUEC_{base\text{-}neg}$ (ratio: 1.1276; 95% CI: 0.9700,

1.2852), the 95% CIs of the PB006 and EU-Tysabri ratios were contained within a 0.8 – 1.25 interval for all secondary parameters.

2.6.4. Conclusions on clinical pharmacology

From the presented data on PK and PD, biosimilarity between the biosimilar candidate PB006 and EU-Tysabri can be concluded.

2.6.5. Clinical efficacy

2.6.5.1. Dose response study

Not applicable

2.6.5.2. Main study(ies)

Antelope: Efficacy and Safety of the Biosimilar Natalizumab PB006 in Comparison to Tysabri in Patients with Relapsing-Remitting Multiple Sclerosis (RRMS)

Methods

This study was conducted at 48 study centers in 7 countries (Belarus, Croatia, Georgia, Moldova, Poland, Serbia, and Ukraine).

- **Study Participants**

In total, 531 patients were screened for enrolment into the study.

The main Inclusion Criteria were

- Male and female patients (age ≥18 to 60 years), with relapsing remitting MS (RRMS), defined by the 2010 revised McDonald criteria.
- At least 1 documented relapse within the previous year and either ≥1 GdE T1-weighted brain lesions or ≥9 T2-weighted brain lesions at Screening.
- Kurtzke Expanded Disability Status Scale (EDSS) score from 0 to 5 (inclusive) at Screening.

Patients who exhibited any of the following exclusion criteria were not eligible for admission into the study (list incomplete):

- Manifestation of MS other than RRMS
- Relapse within the 30 days prior Screening and until administration of the first dose of study drug.
- Prior treatment with natalizumab, alemtuzumab, ocrelizumab, daclizumab, rituximab, cladribine, or other B- and T-cell targeting therapies.
- Prior total lymphoid irradiation or bone marrow or organ transplantation.
- Any prior treatment within the following time period prior to Screening:
    - 30 days: systemic corticosteroids or interferon-β or glatiramer acetate

- 2 months: fingolimod, any other sphingosine-1-phosphate receptor modulator (e.g., siponimod). any tumour necrosis factor-alpha (TNF-α) inhibitors
- 2 months: dimethyl fumarate
- 3.5 months: Tysabri
- 12 months: immunosuppressive therapy for indications other than MS (e.g., cytarabine, azathioprine, methotrexate, cyclophosphamide, cyclosporine, cladribine)

- Any prior treatment with mitoxantrone.
- Active infections requiring oral or parenteral antibiotic treatment within 2 weeks prior to Screening.
- Increased risk of opportunistic infections; exclusion determination to be made after consultation with the Medical Monitor.
- Patients with JCV index >1.5 at Screening.
- Past or current PML diagnosis.
- Presence of malignancies or neoplastic diseases; past history of malignancies within 5 years prior to Screening (except basal cell and in situ squamous cell carcinomas of the skin that have been excised and resolved).
- History or known presence of recurrent or chronic infection other than recurring urinary tract infections (i.e., hepatitis A, B, or C, human immunodeficiency virus, tuberculosis).
- Clinically relevant, severe cardiac or pulmonary diseases, uncontrolled hypertension, or poorly controlled diabetes.
- Severe renal function impairment as defined by serum creatinine values >120 µmol/L.
- Elevated liver markers
- Decreased WBCs at Screening
- Any investigational drug within 3 months prior to enrollment or within 5 times of its half-life, whichever was longer.
- Unable to undergo MRI scans due to claustrophobia or metallic implants incompatible with MRI.
- Unable to receive gadolinium-enhancing (GdE)-based MRI-contrast agents due to history of hypersensitivity to Gd-based contrast agents or severe renal insufficiency.
- Has a known contraindication and (or) hypersensitivity to any of the constituents of the study drug or comparator drugs, including their excipients.

The onset of the following diseases or conditions presented a valid reason for interrupting or discontinuing treatment: PML, JCV granule cell neuronopathy, opportunistic infections, liver injury, hypersensitivity (not including infusion-related reactions), encephalitis, meningitis, acute retinal necrosis, low lymphocyte count or suicidal ideation/suicidal behaviors

- **Treatments**

Treatments Administered

The test drug, PB006, biosimilar natalizumab, is a concentrate for solution for IV infusion. PB006 is provided in an alternative formulation to Tysabri, based on well-established excipients and containing the same concentration of natalizumab as the reference (comparator) product.

The comparator product, Tysabri (natalizumab), is a concentrate for solution for IV infusion.

Each 15 mL vial of concentrate contained 300 mg natalizumab (20 mg/mL). Both study drugs were to be diluted with 100 mL sodium chloride solution (0.9%), resulting in a natalizumab concentration of approximately 2.6 mg/mL for administration. The diluted solution was to be infused intravenously over 1 hour at a rate of approximately 2 mL/minute (115 mL total infusion volume).

Eligible patients were randomly assigned to 1 of 2 treatment groups in a 1:1 ratio to receive intravenous infusions every 4 weeks of either PB006 or Tysabri at a dose of 300 mg starting at Visit 1 (Week 0) through Visit 12 (Week 44), for a total of 12 infusions. Patients re-randomized and switched from Tysabri to PB006 at Week 24 still received a total of 12 infusions (6 infusions of Tysabri and 6 infusions of PB006).

Table 27: Identity of Investigational Products

Study Drug	Formulation	Strength/Dose
PB006	20 mg/mL concentrate for solution for IV infusion	2.6 mg/mL IV solution infused over 1 hour at 2 mL/minute
Tysabri	20 mg/mL concentrate for solution for IV infusion	2.6 mg/mL IV solution infused over 1 hour at 2 mL/minute

- **Objectives and endpoints**

The goal of the study was to support the demonstration of similarity between PB006 and Tysabri in RRMS patients. Efficacy, safety, immunogenicity, and PK of PB006 versus Tysabri were assessed.

Similarity between PB006 and Tysabri was to be assessed in the primary endpoint.

Table 28: objectives and endpoints

Objectives	Endpoints
Primary	
Evaluate and compare the cumulative number of new active lesions over 24 weeks	Cumulative number of new active lesions over 24 weeks
Secondary	
Evaluate and compare the cumulative number of new active lesions over 48 weeks	Cumulative number of new active lesions over 48 weeks
Evaluate and compare the cumulative number of new gadolinium-enhancing (GdE) T1-weighted lesions over 24 and 48 weeks	Cumulative number of new GdE T1-weighted lesions over 24 and 48 weeks
Evaluate and compare the number of patients without new GdE T1-weighted lesions over 24 and 48 weeks	Number of patients without new GdE T1-weighted lesions over 24 and 48 weeks
Evaluate and compare the cumulative number of new/enlarging T2-weighted lesions over 24 and 48 weeks	Cumulative number of new/enlarging T2-weighted lesions over 24 and 48 weeks
Evaluate and compare the number of patients without new/enlarging T2-weighted lesions over 24 and 48 weeks	Number of patients without new/enlarging T2-weighted lesions over 24 and 48 weeks
Evaluate and compare the number of persistent lesions after 24 and 48 weeks treatment with PB006 or Tysabri	Number of persistent lesions after 24 and 48 weeks
Evaluate and compare the annualized relapse rates and changes in Expanded Disability Status Scale (EDSS) after 24 and 48 weeks	Annualized relapse rate after 24 and 48 weeks Change from baseline in EDSS after 24 and 48 weeks
Evaluate and compare local and systemic adverse events (AEs) and serious adverse events (SAEs) after 24 and 48 weeks	Number of local and systemic AEs and SAEs after 24 and 48 weeks

Evaluate and compare the immunogenic profile (incidence rate of ADA [anti-natalizumab] and persistent antibodies) after 24 and 48 weeks and after switching	Incidence rate of ADA and persistent antibodies after 24 and 48 weeks and after switching
Evaluate and compare the immunogenic profile (incidence rate of neutralizing antibodies) after 24 and 48 weeks and after switching	Incidence rate of neutralizing antibodies after 24 and 48 weeks and after switching
Evaluate and compare natalizumab trough concentration (Ctrough) over time	Natalizumab Ctrough over time
Evaluate and compare the safety profile (physical examination, vital sign measurements, and clinical laboratory tests) over 24 and 48 weeks	Safety profile (physical examination, and change from baseline in vital sign measurements and clinical laboratory tests) over 24 and 48 weeks

Efficacy assessments included evaluation of lesions (assessed by MRI), and relapse rate and disability status (assessed by the Kurtzke EDSS).

For the primary endpoint, the applicant selected an equivalence margin for the mean difference of 2.1 lesions to ensure that 50% of the treatment effect based on the lower bound of the 95% CI of the pooled effect size estimated in a controlled trial of natalizumab for RMS (Miller et al., 2003) would be preserved.

Assessment of lesions was performed using MRI with contrast agents. Brain MRI scans to provide radiologic response data for assessing lesions were to be performed at each site. All MRI scans were to be assessed by the MRI central reading center, with the exception of the brain MRI scans for the PML Follow-up Visits, which were to be assessed locally, only.

A macrocyclic Gd-based contrast agent (gadobutrol, gadoteric acid, or gadoteridol) was to be administered as an IV infusion of 0.1 mmol/kg.

If PML was clinically suspected from Visit 1 through the End-of-Study Visit or Early Discontinuation Visit, the local radiologist was to alert the Investigator, who was to schedule an Unscheduled Suspected PML Visit with the patient within 3 days. MRI scans were to be reviewed immediately by the central reading center. The results of the review were to be communicated to the site, Sponsor, and CRO immediately.

If PML was suspected after the End-of-Study Visit or Early Discontinuation Visit, a PML Follow-up Visit was to be scheduled.

A relapse was defined as the appearance of a new neurological abnormality or worsening of previously stable or improving pre-existing neurological abnormality, separated by at least 30 days from onset of a preceding clinical demyelinating event. The abnormality had to be present for at least 24 hours and have occurred in the absence of fever (<37.5 °C) or infection.

In the event of a relapse, the study-related MRI scans were to be obtained before corticosteroid therapy was initiated. If this could not be done, the MRI scan was to be taken 14 days or more after the last corticosteroid dose. MRI scans were to be reviewed by the MRI central reading center.

The Kurtzke EDSS, commonly used to evaluate the degree of neurologic impairment in MS, is an ordinal clinical rating scale ranging from 0 (normal neurologic examination) to 10 (death due to MS) in half-point increments. Based on a standard neurological examination, the 7 functional systems (plus "other") are rated. These ratings are then used in conjunction with observations and information concerning gait and use of assistive devices to rate the EDSS. EDSS ratings were to be performed by independent examining neurologists.

Safety assessments included AEs (adverse events), physical examinations, ECG, vital signs, laboratory evaluations, Columbia-Suicide Severity Rating Scale, anti-natalizumab antibodies, and anti-JCV antibodies.

- **Sample size**

A total of 230 evaluable patients (115 in each group), i.e., patients who complete the 24-week treatment period without relevant major protocol deviations and for whom sufficient postbaseline MRI data are available, were seen required to achieve 90% power for the equivalence assessment with respect to the cumulative number of new active lesions over 24 weeks treatment, assuming a common standard deviation of 4.0 lesions and no difference between both groups. To account for potential dropouts and non-evaluable patients of up to 10%, approximately 260 patients were planned to be randomized to either Tysabri or PB006. The sample size calculation was based on data published by Miller et al., 2003, which showed a mean number of cumulative new active lesions of 1.0 (±2.6) in the pooled natalizumab groups (3 mg/kg and 6 mg/kg) versus 9.7 (±27.4) in the placebo group in patients with either RRMs or secondary progressive MS. An equivalence margin of 2.1 lesions was chosen to ensure that 50% of the treatment effect, based on the lower bound of the 95% CI of the pooled effect size estimated in Miller et al., 2003, was preserved.

- **Randomisation and Blinding (masking)**

Investigators were required to register all patients who sign the inform consent form (ICF) in the eCRF with the date the ICF was signed. The Investigator/designee was planned to obtain a patient number using an electronic data capture (EDC) system. Patients who did not meet eligibility criteria were to be registered as screen failures in the eCRF along with the date and reason for screen failure. Patients who meet all eligibility requirements and who have signed an ICF were planned to be randomly assigned (using the eCRF Randomization Form) to one of the two treatment groups in a 1:1 ratio. Randomization was planned to be stratified by the following factors at Screening:

• Absence/presence of GdE lesions (0, >0)

• Presence of T2 lesions (≤15, >15)

• JCV status for safety (negative, positive)

The randomization of eligible patients was to be done dynamically using Medidata Randomization and Trial Supply Management integrated with the EDC (Rave). Some further details of the dynamic randomization were provided in a separate Randomisation Plan, which refers to the "Rave RTSM and EDC Integration Specification" (which is not accessible via the submitted dossier).

At Week 24, the 130 patients in the Tysabri group were planned to be re-randomized. Approximately 34 patients were to be randomized (switched) to PB006.

For a subset of patients (n=62), stratification data used for randomization was incorrectly entered at the study sites and was corrected after finalization of the CSR Version 1.0. 25 sites across all participating countries were affected. The errors were distributed across all three stratification factors: absence/presence of GdE lesions (20 errors), presence of T2 lesions (16 errors), and JCV status (38 errors). The applicant provided a separate memo describing all relevant details regarding these errors, including a root cause analysis. As a corrective measure, additional sensitivity analysis of the primary analysis using corrected strata was planned to be performed.

This study was planned to run double-blinded with regard to the study drug. An unblinded pharmacist/ designee was planned to be responsible for maintaining accountability, blinding, and dispensing the study drugs according to the handling instructions. Study center personnel, with the exception of the unblinded

pharmacist/designee, were planned to remain blinded to the identity of the study drug until the database was locked and the study was unblinded.

To maintain investigator blinding, the treatment code was not be broken except in medical emergencies when the appropriate management of the patient required knowledge of the treatment randomization. In these cases, the patient was supposed to receive all appropriate medical care. Prior to any unblinding, the Investigator was supposed to contact the Medical Monitor to discuss options. The unblinding procedure was to be done through the interactive Web response system. As soon as possible and without revealing the patient's study drug assignment (unless important to the safety of patients remaining in the study), the Investigator was to notify the Sponsor if the blind was broken for any reason and the Investigator was unable to contact the Sponsor prior to unblinding.

The Sponsor was allowed to break the code for Serious AEs (SAE) that were unexpected and believed to be causally related to study drug, and that potentially required expedited reporting to regulatory authorities. In such cases, the minimum number of Sponsor personnel were to be unblinded. Treatment codes were not to be broken for the planned analyses of data until all decisions on the evaluability of the data from each individual patient had been made and documented and databases had been locked.

- **Statistical methods**

Analysis sets

Safety population

Patients who received at least 1 (complete or partial) infusion of the study drug were to be included in the Safety Population (SAF). Patients in this group were to be analyzed as treated.

Safety-switch population

Patients who were included in the SAF Population and received at least 1 infusion of the study drug after the timepoint of re-randomization, independent of whether they switched or not, were to be included in the Safety-Switch Population (SSW). Patients in this group were to be analyzed as treated after re-randomization, also considering treatment before re-randomization.

Full analysis set

The Full Analysis Set (FAS) Population was to include all patients who were randomized and received at least 1 (complete or partial) infusion of the study drug. Patients were to be analyzed according to the treatment group to which they were randomized.

Per-Protocol population

Only patients participating in this study who completed the 24-week treatment period without major protocol deviations that may have influenced the analysis of the primary endpoint and for whom sufficient post-baseline MRI data were available (including baseline, Week 24 and at least 1 out of the 3 other scheduled MRI visits) were to be included in the PP Population. The final decision on the PP-Population was to be made in the blinded data review meeting before database lock for the final analysis of the primary endpoint.

In addition, a pre-COVID PP Population defined as all PP patients, including those who were excluded from PP only due to major deviations related to COVID-19, was planned.

General statistical considerations

Data collected in this study were to be presented in subject data listings and summary tables.

Descriptive statistics (number of patients with non-missing values, mean, standard deviation, median, minimum, and maximum) were to be presented for continuous variables.

Frequency distributions (counts and percentages) were to be presented for categorical variables.

Demographics (age, sex, race, ethnicity, and child-bearing potential) and baseline characteristics (weight, height, and BMI) were to be summarized by treatment group.

Medical history (conditions that ended before the date of screening) and current medical conditions (those that started before and were ongoing at screening) were to be coded using the MedDRA v23.0 and tabulated by System Organ Class and Preferred Term and by treatment group. MS disease history data were to be summarized.

Use of prior medications (stopped before treatment started) and concomitant medications (ongoing at treatment start or started after first study treatment) were coded and tabulated by treatment group on the Anatomical Therapeutic Chemical 2, ATC 4, and preferred name levels.

MS-related treatment history was planned to be tabulated. Study drug exposure data was also planned to be summarised descriptively. Natalizumab trough concentration in serum were planned to be summarized descriptively per time point in the FAS and PP population.

Primary efficacy analysis

The primary analysis was to be based on the PP Population. The primary endpoint was the cumulative number of new active lesions over 24 weeks. Similarity between PB006 and Tysabri was to be assessed based on the following set of hypotheses:

$$H0: |\mu_{PB006} - \mu_{Tysabri}| > 2.1 \text{ vs. } H1: |\mu_{PB006} - \mu_{Tysabri}| \leq 2.1$$

The term μ_x denotes the cumulative number of new active lesions over 24 weeks in the respective treatment group. Data were to be analyzed using a negative binomial generalized linear model with fixed effects for the treatment group and stratification factors with log link on the PP Population. Parameters were to be estimated using a maximum likelihood approach and back-transformed to the original scale. Similarity was to be tested based on the corresponding 95% CI. An equivalence margin for the mean difference of 2.1 lesions was chosen and prespecified to ensure that 50% of the treatment effect based on the lower bound of the 95% CI of the assumed effect size (estimate derived from literature data) would be preserved.

Sensitivity analyses for primary efficacy comparisons

Analysis in the Full Analysis Set

As one sensitivity analysis, the primary analysis was to be repeated for the FAS Population. For this purpose, data from early discontinuation MRIs (i.e., End-of-Study Visits that occurred before or after the protocol-scheduled timepoint) were to be incorporated into the derived cumulative endpoint (i.e., all MRI results at all previous visits until the specific timepoint were to be summed and used for analysis). The cumulative sum of measurements from prior timepoints was to be used for analysis at other timepoints with missing MRI results. For intermittent missing MRI results (i.e., missed MRI visits but with MRI data collected at subsequent visits), no imputation was to be done. This process was to ensure that lesions from missed MRI screening visits were identified at later MRI screenings.

To assess the effect of missing values in this study, one further sensitivity analysis based on the FAS Population was to be carried out using a multiple imputation method. One hundred data sets were to be created using a linear regression model with predictive variables including treatment, all stratification variables, sex, age, height, and weight at baseline, and the number of relapses the year prior to Screening. All available data, including early discontinuation data, were to be used for analysis. Only post-baseline timepoints with completely missing MRI data and no subsequent MRI screening (monotone missing) were to be imputed. No imputation was to be done for baseline measurements. Imputation was

to be done only for patients with baseline and at least 1 post-baseline MRI result. Sensitivity analyses were to be performed for the primary endpoint using the negative binomial model described in the SAP.

Analysis with corrected stratification information

As a corrective measure of the stratification error (described and assessed above) an additional sensitivity analysis of the primary analysis was to be performed using corrected data for the stratification variables. For this purpose, a separate negative binomial regression model was fitted based on an updated data set for the PP patient set. For the multiple imputation sensitivity analysis described above corrected stratification data was used.

Analysis excluding patient data from Site 7002

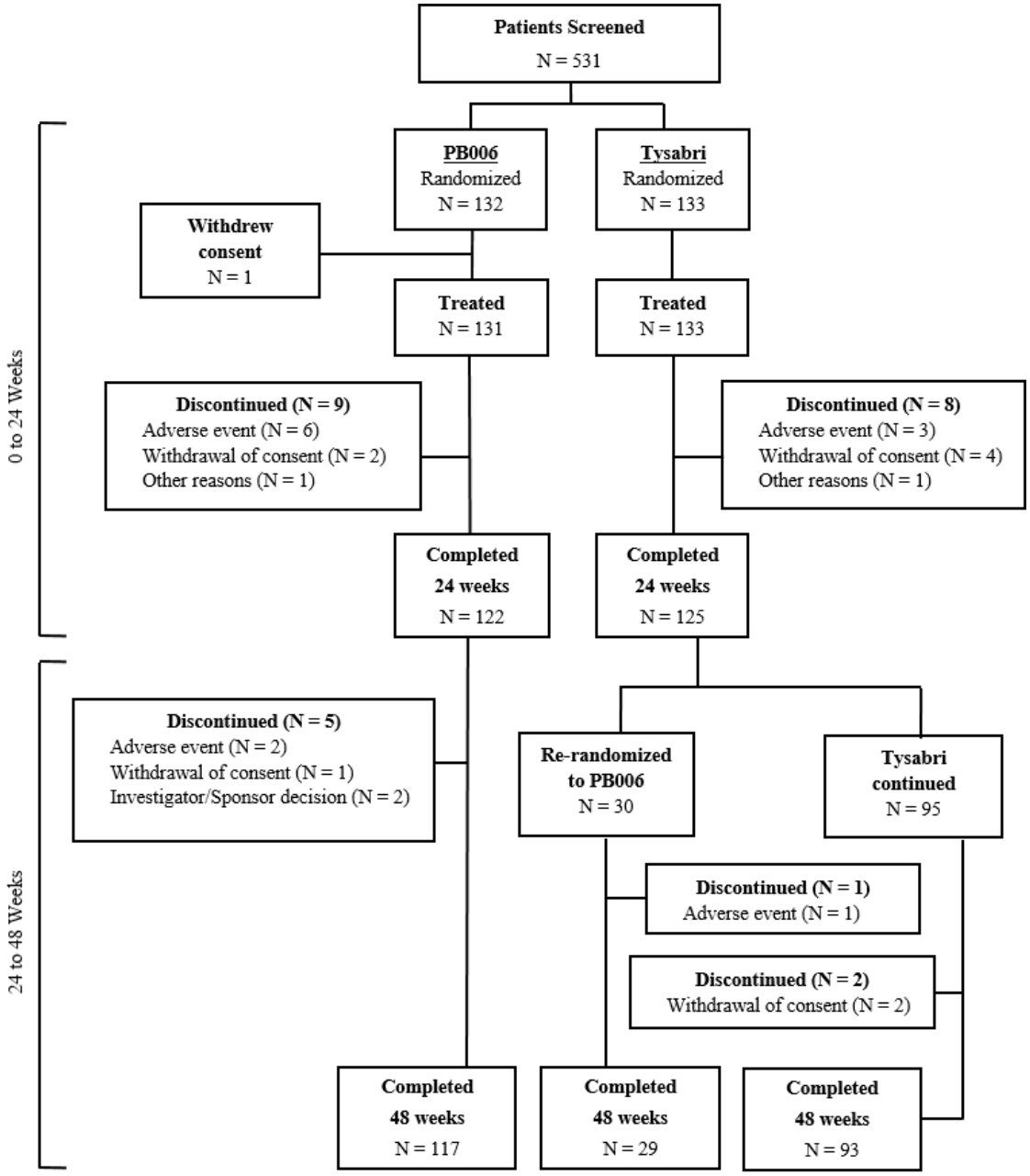
The primary efficacy analysis was planned to be re-run as a sensitivity analysis by excluding all patient data from site 7002. Separate model fitting was carried out in the PP and the FAS sets.

Results

- **Participant flow**

A total of 531 patients were screened for enrollment into the study. Of these, 266 (50.1%) patients were considered screening failures, primarily due to failure to meet inclusion or exclusion criteria (242 [45.6%] patients). A total of 265 patients were randomized. One patient in the PB006 group withdrew consent prior to receiving study drug. Thus, 264 patients overall were treated with study drug (131 and 133 patients in the PB006 and Tysabri groups, respectively).

Figure 6: Participant flow chart over Antilope Study



A total of 247 (93.6%) patients completed the 24-week Primary Treatment Period: 122 (93.1%) in the PB006 group and 125 (94.0%) in the Tysabri group. Study drug was prematurely discontinued in the 24-week Primary Treatment Period in 17 (6.4%) patients overall, with 9 (6.9%) patients in the PB006 group and 8 (6.0%) in the Tysabri group. Six (4.6%) patients in the PB006 group prematurely discontinued study drug due to an AE, compared with 3 (2.3%) patients in the Tysabri group. One patient in the Tysabri group experienced an AE leading to study drug withdrawal at the Week 24 visit, but completed the 24-week Primary Treatment Period. The patient is therefore not included in the 3 patients who discontinued due to an AE. In the AE summary prior to Week 24, this patient was included in the 4 patients who discontinued due to an AE because the event occurred during the 24-week Primary Treatment Period.

Premature discontinuation of study drug due to withdrawal of consent during the 24-week Primary Treatment Period was reported for 6 (2.3%) patients (2 [1.5%] patients in the PB006 group and 4

[3.0%] patients in the Tysabri group). Two (0.8%) patients discontinued the study due to other reasons (1 [0.8%] patient in each treatment group).

At Week 24, all 125 patients in the Tysabri group who had completed the 24-week primary treatment period were re-randomized. Of those in the Tysabri group who were re-randomized, 95 patients remained on Tysabri and 30 patients were switched to PB006. Thus, the total number treated with PB006 at any time during the study was 161 patients.

Table 29: Patient Disposition – Completion by Primary Randomization (FAS Population)

	PB006 (N=131) n (%)	Tysabri (N=133) n (%)	Total (N=264) n (%)
Completed primary period (24 weeks)	122 (93.1)	125 (94.0)	247 (93.6)
Study drug discontinued in primary period – Reason:	9 (6.9)	8 (6.0)	17 (6.4)
Adverse Event	6 (4.6)	3 (2.3)	9 (3.4)
Other	3 (2.3)	5 (3.8)	8 (3.0)
Withdrawn from study in primary period (24 week) – Reason:	9 (6.9)	8 (6.0)	17 (6.4)
Patient withdrawal of consent	2 (1.5)	4 (3.0)	6 (2.3)
Investigator / Sponsor decision	0	0	0
Adverse Events	6 (4.6)	3 (2.3)	9 (3.4)
COVID-19 Related	0	0	0
Pregnancy	0	0	0
Non-Compliance	0	0	0
Lost to Follow-up	0	0	0
Lack of Efficacy	0	0	0
Other, Specify	1 (0.8)	1 (0.8)	2 (0.8)
Re-randomized at Week 24		125 (94.0)	125 (47.3)
-to remain on Tysabri		95 (71.4)	95 (36.0)
-to switch to PB006		30 (22.6)	30 (11.4)
Completed study (48 weeks)	117 (89.3)	122 (91.7)	239 (90.5)
Study drug discontinued – Reason:	14 (10.7)	11 (8.3)	25 (9.5)
Adverse Event	8 (6.1)	4 (3.0)	12 (4.5)
Other	6 (4.6)	7 (5.3)	13 (4.9)
Withdrawn from study – Reason:	14 (10.7)	11 (8.3)	25 (9.5)
Patient withdrawal of consent	3 (2.3)	6 (4.5)	9 (3.4)
Investigator / Sponsor decision	2 (1.5)	0	2 (0.8)
Adverse Events	8 (6.1)	4 (3.0)	12 (4.5)
COVID-19 Related	0	0	0
Pregnancy	0	0	0
Non-Compliance	0	0	0
Lost to Follow-up	0	0	0
Lack of efficacy	0	0	0
Other, Specify	1 (0.8)	1 (0.8)	2 (0.8)

n = Number of patients with the specified event/reason; N = Number of patients in FAS; FAS = Full Analysis Set.
The 48-week summary comprises the full study period, including the first 24 weeks. Note: End of Study eCRF page was only filled-in during the treatment period.

A total of 239 patients completed the 48-week study: 117 patients in the PB006 group, 29 patients in the Tysabri switched to PB006 group, and 93 patients in the Tysabri (continued at Week 24) group. Of the 122 patients in the PB006 group who had completed the primary treatment period, 117 (95.9%) patients completed the study; 2 (1.6%) patients discontinued due to an AE, 2 (1.6%) patients discontinued due to Investigator/Sponsor decision, and 1 (0.8%) patient withdrew consent. Of the 30 patients in the Tysabri switched to PB006 group, 29 (96.7%) patients completed the study, and 1 (3.3%) patient discontinued due to an AE. Of the 95 patients who remained in the Tysabri group after Week 24,

93 (97.9%) patients completed the study and 2 (2.1%) patients discontinued (both due to withdrawal of consent).

- **Recruitment**

Study initiation date was 30 OCT 2019 (First Patient First Visit).

Study completion date was 10 MAR 2021 (Last Patient Last Visit).

The date of the last patient completing the PML Follow-up Visit (24 weeks after last dosing) was on 23 AUG 2021.

- **Conduct of the study**

The Clinical Study Report (CSR) for this study was issued on 10-DEC-2021 and later on two CSR addenda were issued (dated 07 FEB 2022 and 12 MAY 2022). Later, the identification of incorrect stratification at baseline led to preparation of a new version of the CSR (Amendment 1; Version 2.0) issued on 30-JUL-2022, which is considered the final CSR for this MAA.

The most important amendments to the initial CSR were the following:

Amendment 1 (04 SEP 2019) introduced the following changes: two additional sampling times (4 weeks and 28 weeks) were added for anti-natalizumab antibody testing.

Amendment 2 (05 FEB 2020) introduced the following changes:

- The study design and other relevant section were updated to include switching a group of patients from Tysabri to PB006 at Week 24 to evaluate and compare the immunogenic profiles of those on Tysabri only with those who switched – including re-randomization at Week 24
- The numbers of study sites and countries was updated.
- Magnetic Resonance Imaging: Addition of requirements for MRIs performed at Baseline, at an Early Discontinuation Visit, and for unscheduled visits if PML is suspected.
- Primary Endpoint: Data analysis details were added.

Amendment 3 (15 JUL 2020) introduced the following changes:

- Sub-Section 4.1 Overall Study Design: Removal of text reflecting that secondary endpoints are evaluated only once at the end of the study, due to the shift of the primary analysis timepoint.
- Sub-Section 9.7 Primary and Final Analysis was revised to reflect a shift in the primary analysis timepoint.
- Sub-Section 10.4.2 Database Management and Quality Control: Removal of statement that database was to be locked for the primary and then for the final analysis, together with all other endpoints (at Visit 13, Week 48).

Changes in conduct or analysis of the study due to COVID-19 are discussed in a separate section.

Changes Following Study Unblinding/Database Lock and Post-hoc Analyses:

The PB006-03-01 database interim lock occurred on 30 APR 2021. Errors were discovered in the study source data after the database lock, and are described below.

- Minor protocol deviations about incorrect stratification factor use during randomization were not registered in the clinical trial management system (CTMS) for 8 patients due to oversight.

The missing minor protocol deviations were recorded, Study Data Tabulation Model (SDTM) was re-run, and an updated protocol deviation listing was provided after the final database lock on 21 OCT 2021.

- After interim database lock, it was discovered that a protocol deviation for one patient was incorrectly recorded in CTMS. The minor protocol deviation was declassified, SDTM was re-run, and an updated protocol deviation listing was provided after the final database lock on 21 OCT 2021.
- Sponsor review of TFLs identified an inconsistency in the recording of the action taken with the study drug for AEs that caused patients to end treatment (9 patients). The database was unlocked and the appropriate fields were corrected.
- It was discovered after database lock that some of the reasons for end of treatment phase were not consistent with the AEs that were recorded as causing treatment to be stopped for 3 patients. The database was unlocked and the primary reason for withdrawal was corrected.

For errors 1 and 2 above, no database unlock was required and there was no impact on the statistical analysis of the interim lock. Corrected and missing protocol deviations were included in the final database lock on 21 OCT 2021.

For errors 3 and 4 above, there was no relevant impact on the statistical analyses. The database was unlocked to make corrections. Summary table for discontinuation reasons were updated for the final database lock on 21 OCT 2021.

A second database unlock occurred on 11 JUL 2022 to register additional minor protocol deviations related to incorrect stratification factors (see below).

Summary of Changes Between Clinical Study Report Version 1 and Version 2

After finalization of the initial CSR (Version 1.0, dated 10 DEC 2021), 2 CSR addenda were prepared (dated 07 FEB 2022 and 12 MAY 2022). On 07 JUN 2022, the identification of incorrect stratification at baseline affecting 62 patients resulted in the performance of an additional sensitivity analysis of the primary analysis using correct strata. The Sponsor and Contract Research Organisation (CRO) determined that a CSR amendment should be prepared to present the results from the additional sensitivity analysis, as well as all information from the CSR addenda 1 and 2. Additional changes resulting from Quality Control and Sponsor reviews prior to finalization are included in this CSR Amendment 1 (Version 2.0).

A history of the main changes from CSR Version 1.0 (10 DEC 2021) to this CSR Amendment 1 (Version 2.0) is presented below.

CSR Addendum 1 (07 FEB 2022):

Results from the initial analysis of immunogenicity included in the CSR Version 1.0 were limited to timepoints considered most relevant for immunogenicity assessment (baseline [Week 0], Week 8, Week 24, Week 32, and the End-of-Study/Week 48 or Early Discontinuation Visit). The CSR addendum 1 presented the analysis of the immunogenicity results for all timepoints, including Week 4, Week 16, and Week 28, as well as any changes resulting from the analysis of the data from the additional timepoints.

CSR Addendum 2 (11 MAY 2022):

A data integrity issue at a clinical site (7002) was discovered after finalization of the initial CSR. In order to assess the impact of this issue on the validity of the primary endpoint results, a sensitivity analysis of the primary analysis was conducted, excluding patients from site 7002. Additionally, on request by the Rapporteurs, the analyses for EDSS endpoints was re-run excluding all patients from site 7002.

CSR 2.0 (30 JUL 2022)

This CSR Version 2.0 integrates all changes from the CSR addenda 1 and 2 presented above, as well as additional changes described below.

1. After the initial database lock on 30 APR 2021, it was discovered that for 26 patients, stratification data was incorrectly entered into the eCRF at randomization. This was corrected post-randomization. While the primary analysis of the primary endpoint was based on the stratification data used for randomization, an additional sensitivity analysis was performed based on the corrected stratification factors for the 26 patients, in order to assess the impact of the erroneous stratification, as described in the SAP. The corresponding results were reported in CSR Version 1.0.

After finalization of CSR Version 1.0 (on 10 DEC 2021), 36 further cases of incorrect stratification (total of 62 patients) were discovered. Thus, the sensitivity analysis was re- run based on the corrected stratification factors for the 62 patients. The eCRFs were not corrected for all patients because eCRF data had been locked and sites were closed before the discrepancy was first identified on 07 JUN 2022. Therefore, eCRF data are not consistent with NeuroRX and/or Q2 Solutions data for 38 patients (eCRFs were corrected for 24 patients). The CSR Version 2.0 includes the results from the sensitivity analysis of all 62 patients with corrected stratification factors.

2. At the request of the applicant, a statement was added to clarify the definition of "negative" and "positive" for anti-JCV antibodies according to the final result provided on the Q2 Solutions laboratory report.

3. At the request of the applicant, text was revised to describe the number of patients with confirmed positive ADAs at baseline rather than patients who were negative for ADAs at baseline. The change is due to the number of false positive screening assay results (which are then reported as ADA negative upon confirmatory assay), leading to a lower than expected number of patients who are negative for ADAs at baseline.

After database lock (on 30 APR 2021), it was determined that for 26 out of 265 randomized patients (approximately 10% of the patient population), at least 1 of the 3 stratification factors (absence/presence of GdE lesions, presence of T2 lesions, JCV status) was entered incorrectly into the eCRF by investigational sites at Randomization. A root cause analysis and impact assessment was performed and revealed that the immediate risk for imbalanced strata was considered low. Moreover, since the issue was only identified in its totality at the end of the study after database lock, no corrective and preventive action could be implemented within the study itself, other than verifying the correctness of the final status. Since the study was blinded and the randomization was performed according to the entered strata, there was no impact on the data integrity of the study. The pre-specified primary analysis according to the SAP with the IWRS strata was following the intention to treat principle.

On 07 JUN 2022, after finalization of the CSR Version 1.0, it was discovered that the incorrect stratification was not limited to the 26 cases identified in the original MTF (19 MAY 2021). A total of 62 cases (at 25 sites across all participating countries) were affected. A discrepancy was identified in the percentage of patients who were negative for JCV at baseline (67.0% of patients versus 59.8% of patients). The outcome of additional investigations by the Sponsor and the CRO study statistician revealed that at least 1 stratification factor was provided incorrectly during randomization for the 62 affected patients. The errors were distributed across 3 stratification factors: absence/presence of GDE lesions (20 errors), presence of T2 lesions (16 errors), and JCV status (38 errors), with 7 cases where 2 stratification factors were entered incorrectly and 3 cases where all 3 factors were incorrectly provided. With approximately 23% of the patient population affected by the stratification errors and a potential risk of imbalanced strata across treatments, a thorough root-cause analysis and impact assessment was performed.

As corrective measures, minor protocol deviations were registered for all cases, and further training in randomization procedures was performed. The eCRFs were not corrected for all patients because eCRF data had been locked for more than a year when the issue was identified on 07 JUN 2022. Therefore, eCRF data are not consistent with NeuroRX and/or Q2 Solutions data for 38 patients (eCRFs were corrected for 24 patients). Also, an additional sensitivity analysis of the primary analysis using correct strata, as per Q2 Solutions and NeuroRX data, was performed.

Since the study was double-blinded and the randomization was performed according to the entered strata, there was no impact on the data integrity of the study. The pre-specified primary analysis according to the SAP with the IWRS strata was following the ITT principle. The root cause analysis and impact assessment revealed that even with 23% of patients mis-stratified, the imbalance of strata was low and there was no significant impact on the primary analysis. The sensitivity analysis of the primary analysis using the corrected strata confirmed the primary analysis.

Major protocol deviations were reported for 72 patients overall, with 36 (27.5%) patients in the PB006 group and 36 (27.1%) patients in the Tysabri group. The majority of deviations were: study procedure or assessment (18 [13.7%] patients in the PB006 group and 15 [11.3%] patients in the Tysabri group), patient visit completion or timing (10 [7.6%] patients in the PB006 group and 7 [5.3%] patients in the Tysabri group), and inclusion/exclusion criteria (5 [3.8%] patients in the PB006 group and 8 [6.0%] patients in the Tysabri group). One (4.0%) major protocol deviation of SAE reporting in the PB006 group was reported in Poland and 1 (2.1%) major protocol deviation of randomization procedure in the Tysabri group was reported in Ukraine.

Major protocol deviations related to COVID-19 were reported for 8 (6.1%) patients in the PB006 group and 5 (3.8%) patients in the Tysabri group. The majority of deviations in all countries were related to patient visit completion or timing (7 [5.3%] patients in the PB006 group and 5 [3.8%] patients in the Tysabri group). One (0.8%) patient in Ukraine had a major protocol deviation of study procedure or assessment.

- **Baseline data**

The demographic and baseline characteristics were similar between treatment groups for the FAS Population. The FAS Population was comprised of 162 (61.4%) female patients and 102 (38.6%) male patients, similarly distributed between treatment groups. Most (143 [88.3%]) female patients were of childbearing potential. The mean (SD) age of patients was 36.8 (9.05) years in the PB006 group and 36.6 (9.73) years in the Tysabri group. Race was White (100.0% for both groups), and mean (SD) BMI was 24.1 (4.88) kg/m2 in the PB006 group and 24.2 (4.54) kg/m2 in the Tysabri group. At baseline, about half (140 [53.0%]) of patients had no GdE lesions; the remaining 124 (47.0%) patients had >0 GdE lesions. The majority of patients had >15 T2 lesions (255 [96.6%] patients) and a negative JCV status (158 [59.8%] patients) (for details on corrected stratification factors, please see below).

Table 30: Baseline and Demographic Characteristics by Primary Randomization (FAS Population)

	PB006 (N=131)	Tysabri (N=133)	Total (N=264)
Absence/presence of GdE lesions, n (%)			
0	68 (51.9)	72 (54.1)	140 (53.0)
>0	63 (48.1)	61 (45.9)	124 (47.0)
Presence of T2 lesions, n (%)			
≤15	4 (3.1)	5 (3.8)	9 (3.4)
>15	127 (96.9)	128 (96.2)	255 (96.6)
JCV status for safety, n (%)			
Negative	80 (61.1)	78 (58.6)	158 (59.8)
Positive	51 (38.9)	55 (41.4)	106 (40.2)
Age (years)			
n	131	133	264
Mean	36.8	36.6	36.7
SD	9.05	9.73	9.38
Median	35.0	37.0	36.0
Min/Max	18/57	20/ 59	18/59
Sex, n (%)			
Female	84 (64.1)	78 (58.6)	162 (61.4)
Male	47 (35.9)	55 (41.4)	102 (38.6)
Race, n (%)			
American Indian or Alaska Native	0	0	0
Black or African American	0	0	0
Asian	0	0	0
White	131 (100)	133 (100)	264 (100)
Native Hawaiian or Other Pacific Islander	0	0	0
Not Reported	0	0	0
Other	0	0	0
Ethnicity, n (%)			
Hispanic or Latino	0	0	0
Not Hispanic or Latino	131 (100)	133 (100)	264 (100)
Not Reported	0	0	0
Unknown	0	0	0
Childbearing potential, n (%)			
Yes	73 (86.9)	70 (89.7)	143 (88.3)
No	11 (13.1)	8 (10.3)	19 (11.7)
Weight (kg)			
n	131	133	264
Mean	70.0	70.6	70.3
SD	16.14	15.65	15.87
Median	67.7	70.0	68.9
Min/Max	45/138	43/121	43/138
Height (cm)			
n	131	133	264
Mean	170.0	170.4	170.2
SD	8.41	8.61	8.50
Median	168.0	168.0	168.0
Min/Max	154/195	154/194	154/195
Body Mass Index (kg/m^2)			
n	131	133	264
Mean	24.1	24.2	24.2
SD	4.88	4.54	4.70
Median	23.0	23.5	23.3
Min/Max	17/43	16/41	16/43

Abbreviations: N = Number of patients in population/treatment group; FAS = Full Analysis Set; GdE = Gadolinium-Enhancing; JCV = John Cunningham Virus; SD = Standard Deviation.

Note: Percentages of childbearing potential are based on number of female patients.

Note: Corrected levels of stratification factors are summarized in this table.

The frequency of patients per strata after correction of stratification factors is shown below.

Table 31: Frequency of stratification factor (FAS)

	PB006 N=131	Tysabri N=133	Total N=264
Absence/presence of GdE lesions, n (%)			
0	68 (51.9%)	72 (54.1%)	140 (53.0)
>0	63 (48.1%)	61 (45.9%)	124 (47.0)
Presence of T2 lesions, n (%)			
≤15	4 (3.1%)	5 (3.8%)	9 (3.4)
>15	127 (96.9%)	128 (96.2%)	255 (96.6)
JCV status for safety, n (%)			
Negative	80 (61.1%)	78 (58.6%)	158 (59.8)
Positive	51 (38.9%)	55 (41.4%)	106 (40.2)

For the COVID-19-confirmed patients (n=22) in the SAF Population, all parameters were similar to the FAS, SAF, PP, and SSW Populations.

Similar demographic and baseline characteristics were reported for the PP Population and the SSW Population. Demographic and baseline characteristics for the SAF Population were identical to the FAS Population.

Medical History

Similar proportions of patients in both treatment groups had any medical history other than MS (93 [71.0%] patients in the PB006 group and 94 [70.7%] patients in the Tysabri group). The medical history reported for at least 5% of patients in either treatment group were myopia, retinal vascular disorder, depression, hypertension, osteochondrosis, and astigmatism, reported by 18 (13.7%) 14 (10.7%), 9 (6.9%), 7 (5.3%), 7 (5.3%), and 4 (3.1%) patients, respectively, in the PB006 group, and in 17 (12.8%), 11 (8.3%), 5 (3.8%), 6 (4.5%), 6 (4.5%), and 7 (5.3%) patients, respectively, in the Tysabri group. Distribution of all other conditions was low and generally similar across treatment groups.

Similar results were reported for the PP Population and the SSW Population.

Similar proportions of patients in each treatment group had any current conditions (131 [100%] patients in the PB006 group and 133 [100%] patients in the Tysabri group). Current medical conditions (≥5% of patients in either treatment group) that occurred in similar proportions of patients in both treatment groups included MS, myopia, retinal vascular disorder, depression, and osteochondrosis (occurring in 131 [100%], 17 [13.0%], 14 [10.7%], 8 [6.1%], and 7 [5.3%] patients, respectively, in the PB006 group, and 133 [100%], 17 [12.8%], 11 [8.3%], 5 [3.8%], and 6 [4.5%] patients, respectively, in the Tysabri group). Distribution of all other conditions was low and generally similar across the treatment groups.

Similar current medical conditions were reported for the PP Population, and the SSW Population.

Multiple Sclerosis Disease History

The mean (SD) time since diagnosis for patients in the PB006 group (5.34 [4.655] years) was similar to patients in the Tysabri group (5.32 [4.800] years). The mean (SD) time since most recent relapse for patients in the PB006 group (5.08 [2.860] months) was slightly shorter than for patients in the Tysabri group (5.88 [3.038] months).

The number of relapses in the 1 year prior to Screening was similar between treatment groups. Most patients had 1 relapse (86 [65.6%] and 91 [68.4%] patients in the PB006 and Tysabri groups, respectively) or 2 relapses (37 [28.2%] and 38 [28.6%] patients in the PB006 and Tysabri groups, respectively) in the year prior to Screening. The mean (SD) number of relapses in the year prior to Screening (1.4 [0.68] for the PB006 group and 1.4 [0.57] for the Tysabri group) and the mean (SD) baseline EDSS (3.4 [1.07] for the PB006 group and 3.2 [1.21] for the Tysabri group) were similar for both groups.

Multiple Sclerosis-Related Treatment History

Similar numbers of patients in both treatment groups previously received any medication for MS (46 [35.1%] patients in the PB006 group and 44 [33.1%] patients in the Tysabri group). The most frequently administered prior medications were interferon beta-1a (17 [13.0%] and 10 [7.5%] patients in the PB006 and Tysabri groups, respectively), interferon beta-1b (11 [8.4%] and 12 [9.0%] patients in the PB006 and Tysabri groups, respectively), dimethyl fumarate (13 [9.9%] and 9 [6.8%] patients in the PB006 and Tysabri groups, respectively), glatiramer acetate (10 [7.6%] and 9 [6.8%] patients in the PB006 and Tysabri groups, respectively), and laquinimod (3 [2.3%] and 6 [4.5%] patients in the PB006 and Tysabri groups, respectively). All other MS medications were received by ≤1.5% of patients in either treatment group.

- **Numbers analysed**

See participant flow in the prior subsection

- **Outcomes and estimation**

Primary Efficacy Analysis

The primary efficacy endpoint was the cumulative number of new active lesions over 24 weeks. The primary analysis was based on the PP Population, while the FAS Population was analyzed as sensitivity analysis. Results are presented for the PP Population in Table 32.

The difference in new active lesions between Tysabri and PB006 was derived using a negative binomial model with log link. The point estimate was back-transformed to the original scale for similarity assessment. The point estimate (standard error [SE]) for the exponentiated difference between Tysabri and PB006 was 0.17 (0.397). The null hypothesis was rejected, as the 90% and 95% CI for the difference Tysabri-PB006 were within the specified margins (-2.1; 2.1). Thus, the primary endpoint was met.

Table 32: Cumulative Number of New Active Lesions Over 24 Weeks - Primary Analysis (PP Population)

Cumulative number of new active lesions over 24 weeks	Estimate (SE)	90% Confidence Interval	95% Confidence Interval
Least square means			
Exponentiated Difference Tysabri-PB006	0.17 (0.397)	[-0.488; 0.819]	[-0.613; 0.944]

N = Number of patients in population/treatment group; PP = Per-Protocol; SE = Standard Error.

Note: Stratification factors as registered in IWRS were used for the analysis.

Sensitivity Analyses of the Primary Efficacy Endpoint

Analysis Based on the Full Analysis Set

The sensitivity analysis of the primary analysis based on the FAS Population is presented in Table 33. As for the primary analysis, the difference in new active lesions between Tysabri and PB006 was derived using a negative binomial model with log link. The point estimate was back-transformed to the original scale for similarity assessment. The point estimate (SE) for the exponentiated difference between Tysabri and PB006 was 0.23 (0.430). The null hypothesis was rejected, as the 90% and 95% CIs for the difference Tysabri-PB006 were within the specified margins (-2.1; 2.1). Thus, the primary endpoint was confirmed.

The stratification factor absence/presence of GdE lesions was found to have an influence on the cumulative number of new active lesions (with the p-value being <0.0001) and in contrast, treatment, presence of T2 lesions, and JCV status did not have an influence on the number of new active lesions (with corresponding p-values being >0.05).

Table 33: Cumulative number of New Active Lesions Over 24 Weeks - Sensitivity Analysis (FAS Population)

Cumulative number of new active lesions over 24 weeks	Estimate (SE)	90% Confidence Interval	95% Confidence Interval
Least square means			
Exponentiated Difference Tysabri-PB006	0.23 (0.430)	[-0.474; 0.940]	[-0.609; 1.075]

Abbreviations: N = Number of patients in population/treatment group; FAS = Full Analysis Set; IWRS = Interactive Web Response System; SE = Standard Error.
Note: Stratification factors as registered in IWRS were used for the analysis.

Corrected Stratification Variables

For a subset of patients (n=26), stratification data used for randomization was incorrectly entered at the study sites and was corrected post-randomization. These cases were reported as minor protocol deviations. While the primary analysis of the primary endpoint was based on the stratification data used for randomization, an additional sensitivity analysis was performed based on the corrected stratification factors in order to assess the impact of the erroneous stratification, as described in the SAP. This initial sensitivity analysis (n=26) was reported in CSR V1.0 (10 DEC 2021).

On 07 JUN 2022, after finalization of the CSR Version 1.0, it was discovered that the incorrect stratification was not limited to the 26 cases identified in the original CSR V1.0. A total of 62 cases (at 25 sites across all participating countries) were affected. The errors were distributed across 3 stratification factors: absence/presence of GdE lesions (20 errors), presence of T2 lesions (16 errors), and JCV status (38 errors). The eCRFs were not corrected for all patients because eCRF data had been locked for more than a year when the issue was identified on 07 JUN 2022. Therefore, eCRF data are not consistent with NeuroRX and/or Q2 Solutions data for 38 patients (eCRFs were corrected for 24 patients). For all newly identified cases, minor protocol deviations were registered. As a corrective measure, additional sensitivity analysis of the primary analysis using corrected strata, as per Q2 Solutions and NeuroRX data, was performed.

The sensitivity analysis of the primary analysis using corrected stratification variables for all 62 patients is presented in Table 34 for the PP Population. The point estimate (SE) for the exponentiated difference between Tysabri and PB006 was 0.06 (0.083). The null hypothesis was rejected, as the 90% and 95% CIs for the difference Tysabri-PB006 were within the specified margins (-2.1; 2.1). Thus, the primary endpoint was confirmed to be met using corrected stratification variables.

The stratification factor absence/presence of GdE lesions was found to have an influence on the cumulative number of new active lesions (p-value <0.0001). In contrast, treatment, presence of T2 lesions, and JCV status did not have an influence on the number of new active lesions (with corresponding p-values being >0.05).

Table 34: Cumulative Number of New Active Lesions Over 24 Weeks by Corrected Stratification Variables - Sensitivity Analysis (PP Population)

Cumulative number of new active lesions over 24 weeks	Estimate (SE)	90% Confidence Interval	95% Confidence Interval
Least square means			
Exponentiated Difference Tysabri-PB006	0.06 (0.083)	[-0.073; 0.199]	[-0.099; 0.225]

N = Number of patients in population/treatment group; PP = Per-Protocol; SE = Standard Error.

Multiple Imputation

Sensitivity analysis of the primary efficacy endpoint using multiple imputation is presented for the FAS Population in Table 35. All available data, including early discontinuation data, was used. The difference in new active lesions between Tysabri and PB006 was derived using a negative binomial model with log link. The point estimate was back-transformed to the original scale for similarity assessment. The point

estimate (SE) for the exponentiated difference between Tysabri and PB006 was -0.22 (0.300). The null hypothesis was rejected, as the 90% and 95% CIs for the difference Tysabri-PB006 were within the specified margins (-2.1; 2.1). Thus, the sensitivity analysis showed that the results obtained in the primary analysis are robust.

The stratification factor of absence/presence of GdE lesions was found to have an influence on the cumulative number of new active lesions (p-value <0.0001). In contrast, treatment, presence of T2 lesions, and JCV status were found to not have an influence on the number of new active lesions (with corresponding p-values being >0.05).

A sensitivity analysis of the primary analysis using multiple imputation for the pre-COVID PP Population was not performed, as there was no missing data to impute for the population.

Table 35: Cumulative Number of New Active Lesions Over 24 Weeks With Multiple Imputation – Sensitivity Analysis (FAS Population)

Cumulative number of new active lesions over 24 weeks	Estimate (SE)	90% Confidence Interval	95% Confidence Interval
Least square means			
Exponentiated Difference Tysabri-PB006	-0.22 (0.300)	[-0.717; 0.269]	[-0.812; 0.363]

N = Number of patients in population/treatment group; FAS = Full Analysis Set; MI = Multiple Imputation; SE = Standard Error.
Note: The analysis is based on 100 imputed datasets. The imputation is done using linear regression model with predictive variables including treatment, all stratification variables, sex, age, height, and weight at baseline, and number of relapses the year prior to Screening.
Note: Corrected stratification factors were used for the multiple imputation sensitivity analysis.

Data Integrity Impact Assessment on the Primary Efficacy Analysis

The primary efficacy analysis was re-run as a sensitivity analysis by excluding all patient data from site 7002. In the following, the results of the primary efficacy analyses are compared with the corresponding results of the impact assessment sensitivity analyses.

A comparison of the primary efficacy analysis (Table 32) and the corresponding impact assessment sensitivity analysis are shown in Table 36. The impact assessment results were similar to the original primary analysis results. With site 7002 patient data excluded, the CIs were larger due to the lower sample size, but still well within the pre-specified margins (-2.1; 2.1).

Table 36: Cumulative Number of New Active Lesions Over 24 Weeks – Primary Analysis (PP Population) Versus Impact Assessment Sensitivity Analysis

Cumulative number of new active lesions over 24 weeks	**Estimate (SE)**	**90% Confidence Interval**	**95% Confidence Interval**
Primary analysis (Table 32)			
Least square means Exponentiated Difference Tysabri-PB006	0.17 (0.397)	[-0.488; 0.819]	[-0.613; 0.944]
Impact assessment sensitivity analysis (excluding all patients from site 7002)			
Least square means Exponentiated Difference Tysabri-PB006	0.13 (0.447)	[-0.607; 0.862]	[-0.748; 1.003]

PP = Per-Protocol; SE = Standard Error.

A comparison of the sensitivity analysis based on the FAS and the corresponding impact assessment sensitivity analysis was conducted. The impact assessment results were similar to the original results for

the analysis based on the FAS. With site 7002 patient data excluded, CIs were larger due to the lower sample size, but still well within the pre-specified margins (-2.1; 2.1) (Table 37).

Table 37: Cumulative Number Of New Active Lesions Over 24 Weeks (FAS) Versus Impact Assessment Sensitivity Analysis

Cumulative number of new active lesions over 24 weeks	Estimate (SE)	90% Confidence Interval	95% Confidence Interval
Sensitivity analysis (Table 34)			
Least square means Exponentiated Difference Tysabri-PB006	0.23 (0.430)	[-0.474; 0.940]	[-0.609; 1.075]
Impact assessment sensitivity analysis (excluding all patients from site 7002)			
Least square means Exponentiated Difference Tysabri-PB006	0.20 (0.475)	[-0.581; 0.981]	[-0.731; 1.131]

FAS = Full Analysis Set; SE = Standard Error.

A comparison of the sensitivity analysis based on corrected stratification factors (Table 34) and the corresponding impact assessment sensitivity analysis are shown in Table 38. The impact assessment results were similar to the original results for the corrected stratification variables. With site 7002 patient data excluded, CIs were larger due to the lower sample size, but still well within the pre-specified margins (-2.1; 2.1).

Table 38: Cumulative Number of New Active Lesions Over 24 Weeks by Corrected Stratification Variables (PP population) Versus Impact Assessment Sensitivity Analysis

Cumulative number of new active lesions over 24 weeks	Estimate (SE)	90% Confidence Interval	95% Confidence Interval
Corrected stratification variables (Table 34)			
Least square means Exponentiated Difference Tysabri-PB006	0.06 (0.083)	[-0.073; 0.199]	[-0.099; 0.225]
Impact assessment sensitivity analysis (excluding all patients from site 7002)			
Least square means Exponentiated Difference Tysabri-PB006	0.07 (0.088)	[-0.079; 0.212]	[-0.107; 0.239]

PP = Per-Protocol; SE = Standard Error.
[a] Based on corrected stratification for the 62 patients identified with incorrect stratification at baseline.

Summary of the Impact Assessment Analyses

An internal quality assessment at site 7002 revealed serious deviations from the GCP source data handling requirements. However, there were no indications of fraud or a deliberate falsification of data. Study patients were genuine, and the trial execution was supported by available source data. Original source data were on file for several essential data points, e.g., MRIs, lab data reports.

The exclusion of data for all 17 patients from site 7002 did not change the outcome of the primary endpoint analysis. The predefined equivalence criteria were met, and similarity of clinical efficacy was demonstrated. In total, 7 quality audits and 8 quality assessment visits were conducted at 8 investigator sites across countries. No other data integrity observations were identified at other participating study sites. As such, this data integrity issue was considered to be limited to site 7002.

According to the applicant, the validity of the study results and the conclusions on the overall study outcome remain unchanged.

Secondary Efficacy Analysis

Cumulative Number of New Active Lesions Over 48 Weeks

The mean cumulative number of new active lesions was slightly lower in the PB006 group than in the Tysabri group at all timepoints up to Week 48. The mean (SD) cumulative number up to Week 48 was 1.5 (3.72) in the PB006 group, compared to 2.3 (5.68) in the Tysabri group. The results from the PP analysis were similar, with mean cumulative numbers being identical to the FAS for all timepoints.

The average number of new active lesions per MRI scan was lower in the PB006 group than in the Tysabri group at all timepoints up to Week 48. The average number up to Week 48 was 0.31 in the PB006 group compared to 0.48 in the Tysabri group. The results from the PP analysis were similar.

Compared to Week 8, a greater percentage of patients in the PB006 and Tysabri groups had no new active lesions at Week 48, with similar percentages in both treatment groups (116 [98.3%] patients in the PB006 group and 88 [94.6%] patients in the Tysabri group). The percentage of patients with 1 to 3 new active lesions similarly decreased in both treatment groups by Week 48 (2 [1.7%] patients in the PB006 group and 2 [2.2%] patients in the Tysabri group). No patients in the PB006 group had 4 or more new active lesions. In the Tysabri group, 4 to 6 new active lesions, 10 to 12 new active lesions, and >12 new active lesions each occurred in 1 (1.1%) patient; no patients had 7 to 9 new active lesions. The results from the PP analysis were similar.

Cumulative Number of New GdE T1-weighted Lesions Over 24 and 48 Weeks

The mean cumulative number of new GdE T1-weighted lesions was slightly lower in the PB006 group than in the Tysabri group at all timepoints up to Week 48. The mean (SD) cumulative number up to Week 24 was 0.3 (1.01) in the PB006 group compared to 0.4 (1.25) in the Tysabri group, and up to Week 48 was 0.3 (1.02) in the PB006 group compared to 0.4 (1.39) in the Tysabri group. The results from the PP analysis were similar.

The average number of new GdE T1-weighted lesions per MRI scan was lower in the PB006 group than in the Tysabri group at all timepoints up to Week 48. The average number up to Week 48 was 0.06 in the PB006 group compared to 0.09 in the Tysabri group. The results from the PP analysis were similar.

Number of Patients Without New GdE T1-weighted Lesions Over 24 and 48 Weeks

The percentage of patients without new GdE T1-weighted lesions was similar in the PB006 and Tysabri groups at 24 weeks (109 [83.2%] and 105 [78.9%] patients in the PB006 and Tysabri groups, respectively) and at 48 weeks (105 [80.2%]) and (80 [77.7%] patients in the PB006 and Tysabri groups, respectively). The percentage of patients with at least 1 new GdE T1-weighted lesion was similar in both treatment groups over 24 weeks (17 [13.0%] and 22 [16.5%] patients in the PB006 and Tysabri groups, respectively) and over 48 weeks (17 [13.0%] and 16 [15.5%] patients in the PB006 and Tysabri groups, respectively). The results from the PP analysis were similar.

Cumulative Number of New/Enlarging T2-weighted Lesions Over 24 and 48 Weeks

The mean cumulative number of new/enlarging T2-weighted lesions was slightly lower in the PB006 group than in the Tysabri group at all timepoints up to Week 48. The mean (SD) cumulative number up to Week 24 was 1.5 (3.79) in the PB006 group compared to 2.0 (4.12) in the Tysabri group, and up to Week 48 was 1.6 (3.90) in the PB006 group compared to 2.4 (5.79) in the Tysabri group. The results from the PP analysis were similar, with mean cumulative numbers being nearly identical to the FAS for all timepoints.

Number of Patients Without New/Enlarging T2-weighted Lesions Over 24 and 48 Weeks

The percentage of patients without new/enlarging T2-weighted lesions was similar in the PB006 and Tysabri groups at 24 weeks (75 [57.3%] and 72 [54.1%] patients in the PB006 and Tysabri groups, respectively). At 48 weeks, a slightly greater percentage of patients in the PB006 group were without new/enlarging T2-weighted lesions (71 [54.2%] patients) compared with the Tysabri group (52 [50.5%] patients). The percentage of patients with at least 1 new/enlarging T2-weighted lesion was similar in both treatment groups over 24 weeks (51 [38.9%] patients in the PB006 group and 55 [41.4%] patients in the Tysabri group) and over 48 weeks (51 [38.9%] patients in the PB006 group and 44 [42.7%] patients in the Tysabri group).

The results from the PP analysis were similar over 24 and 48 weeks.

Number of Persistent Lesions After 24 and 48 Weeks

The mean cumulative number of persistent lesions was similar in the PB006 and Tysabri groups at all timepoints up to Week 48. The mean (SD) number of persistent lesions up to Week 24 was 0.5 (2.46) and 0.4 (2.92) in the PB006 and Tysabri groups, respectively, and up to Week 48 was 0.5 (2.55) and 0.6 (3.35) in the PB006 and Tysabri groups, respectively.

For the PP Population, a slightly higher mean cumulative number of persistent lesions was reported in the PB006 group than in the Tysabri group at all timepoints up to Week 48. The mean (SD) number of persistent lesions up to Week 24 was 0.5 (2.60) and 0.2 (0.78) in the PB006 and Tysabri groups, respectively, and up to Week 48 was 0.6 (2.69) and 0.2 (0.87) in the PB006 and Tysabri groups, respectively.

Annualized Relapse Rate After 24 and 48 Weeks

Over 24 weeks, a similar percentage of patients in the PB006 group and Tysabri group had 1 relapse (12 [9.2%] and 9 [6.8%] patients in the PB006 and Tysabri groups, respectively), with a similar mean (SD) follow-up time for both groups (0.445 [0.0772] and 0.447 [0.0717] years in the PB006 and Tysabri groups, respectively). No patients in either group had 2 or more relapses at 24 weeks. The ARR was similar over 24 weeks for the PB006 group (0.206) compared with the Tysabri group (0.152).

Over 48 weeks, a similar percentage of patients in the PB006 group and Tysabri group had 1 relapse (18 [13.7%] and 12 [11.7%] patients in the PB006 and Tysabri groups, respectively), with a similar mean (SD) follow-up time for both groups (0.877 [0.1865] and 0.878 [0.1902] years in the PB006 and Tysabri groups, respectively). One (0.8%) patient in the PB006 group had 2 relapses; no patients in either group had 3 or more relapses at 48 weeks. The ARR was similar over 48 weeks for the PB006 group (0.174) compared with the Tysabri group (0.133). The results from the PP analysis were similar.

Table 39: Annualized Relapse Rate by Primary Randomization (FAS Population)

Annualized Relapse Rate	PB006 (N=131)	Tysabri (N=133)
...Over 24 weeks		
Number of patients, n (%)	131 (100)	133 (100)
Number of relapses per patient, n (%)		
1	12 (9.2)	9 (6.8)
2	0	0
≥3	0	0
Total number of relapses	12	9
Follow-up time (years)		
n	131	133
Mean	0.445	0.447
SD	0.0772	0.0717

Median	0.460	0.460
Min/Max	0.00/0.50	0.00/0.49
Total follow-up time (years)	58.29	59.39
Annualized relapse rate (relapses/year)	0.206	0.152
...Over 48 weeks		
Number of patients, n (%)	131 (100)	103 (100)
Number of relapses per patient, n (%)		
1	18 (13.7)	12 (11.7)
2	1 (0.8)	0
≥3	0	0
Total number of relapses	20	12
Follow-up time (years)		
n	131	103
Mean	0.877	0.878
SD	0.1865	0.1902
Median	0.920	0.920
Min/Max	0.06/1.07	0.08/1.09
Total follow-up time (years)	114.91	90.42
Annualized relapse rate (relapses/year)	0.174	0.133

n = Number of patients with the specified event/reason; N = Number of patients in population/treatment group; FAS = Full Analysis Set; SD = Standard Deviation.
Note: For time points after Week 24, patients who switch from Tysabri to PB006 are excluded from this table.
Note: For timepoint over 48 weeks, patients who switch from Tysabri to PB006 at Week 24 are excluded from this table.

Change from Baseline in Expanded Disability Status Scale After 24 and 48 Weeks

At baseline, mean (SD) EDSS scores were similar between treatment groups (3.36 [1.065] in the PB006 group and 3.20 [1.206] in the Tysabri group). The mean (SD) change from baseline was minimal and similar in both treatment groups at 24 weeks (-0.03 [0.211] in the PB006 group and 0.00 [0.354] in the Tysabri group) and at 48 weeks (-0.14 [0.536] in the PB006 group and -0.05 [0.443] in the Tysabri group). The results from the PP analysis were similar.

Table 40: EDSS Scores by primary randomization (FAS population)

	PB006 (N=131)		Tysabri (N=133)		Total (N=264)	
	Observed Value	Change from Baseline	Observed Value	Change from Baseline	Observed Value	Change from Baseline
Baseline						
n	131		133		264	
Mean	3.36		3.20		3.28	
SD	1.065		1.206		1.139	
Median	3.50		3.50		3.50	
Min/Max	1.5/5.0		1.0/5.0		1.0/5.0	
Week 24						
n	122	122	125	125	247	247
Mean	3.37	-0.03	3.18	0.00	3.28	-0.02
SD	1.126	0.211	1.258	0.354	1.196	0.292
Median	3.50	0.00	3.50	0.00	3.50	0.00
Min/Max	1.0/5.5	-1.0/1.0	1.0/6.5	-1.0/2.0	1.0/6.5	-1.0/2.0
Week 48						
n	117	117	93	93	210	210
Mean	3.24	-0.14	3.12	-0.05	3.19	-0.10
SD	1.203	0.536	1.322	0.443	1.255	0.498
Median	3.50	0.00	3.50	0.00	3.50	0.00
Min/Max	1.0/6.0	-2.5/1.0	0.0/6.5	-1.5/1.5	0.0/6.5	-2.5/1.5

N = Number of patients in population/treatment group; FAS = Full Analysis Set; SD = Standard Deviation.
Note: For timepoints after Week 24, patients who switch from Tysabri to PB006 are excluded from this table.

The data integrity impact evaluation for site 7002 showed that EDSS subscores were subject to backdating in the source data for some patients. Therefore, a conservative approach was chosen and the EDSS data were re-analyzed excluding the data from site 7002. The results excluding the 17 patients from site 7002 are shown below.

Table 41: Change from baseline in EDSS after 24 and 48 weeks, excluding patients from site 7002, by primary randomization (FAS population)

	PB006 N=122		**EU-Tysabri N=125**	
	Observed value	**Change from baseline**	**Observed value**	**Change from baseline**
Baseline				
n	122		125	
Mean	3.39		3.26	
SD	1.034		1.175	
Median	3.50		3.50	
Min/Max	1.5/5.0		1.0/5.0	
Week 24				
n	114	114	118	118
Mean	3.38	-0.04	3.24	0.00
SD	1.102	0.218	1.236	0.364
Median	3.50	0.00	3.50	0.00
Min/Max	1.0/5.5	-1.0/1.0	1.0/6.5	-1.0/2.0
Week 48				
n	110	110	86	86
Mean	3.25	-0.15	3.19	-0.05
SD	1.182	0.552	1.302	0.460
Median	3.50	0.00	3.50	0.00
Min/Max	1.0/6.0	-2.5/1.0	0.0/6.5	-1.5/1.5

FAS=full analysis set, Max=maximum, Min=minimum, N=Number of patients in treatment group, n=number of patients with data available, SD=standard deviation.
Note: For time points after Week 24, patients who switched from EU-Tysabri to PB006 were excluded from this table.

By this analysis, very similar results as in the overall analysis were obtained.

Pharmacokinetic Results

Natalizumab C_{trough} was similar in the PB006 and Tysabri groups at all post-baseline timepoints. Mean (SD) natalizumab C_{trough} was 26804.75 ng/mL (12949.541) and 25010.49 ng/mL (12557.895) at Week 8 in the PB006 and Tysabri groups, respectively, and gradually increased in both groups at each timepoint, with natalizumab C C_{trough} trough of 39097.58 ng/mL (16801.710) and 38432.86 ng/mL (16495.407) in the PB006 and Tysabri groups at Week 48, respectively. The results from the PP analysis were similar.

Table 42: Natalizumab Total Trough Concentration (ng/mL) by Primary Randomization (FAS Population)

Concentration (ng/mL)	PB006 (N=131)	Tysabri (N=13
Week 8		
n	118	125
Mean	26804.75	25010.49
SD	12949.541	12557.895
CV (%)	48.311	50.211
Geometric Mean	22270.65	18784.89
Median	26150.00	25400.00

Min/Max	90.2/72500.0	61.5/61700.0
n BLQ	8	2
Week 16		
n	117	122
Mean	33872.92	32543.28
SD	18151.190	14636.925
CV (%)	53.586	44.977
Geometric Mean	29159.08	27939.96
Median	32300.00	30500.00
Min/Max	522.0/143000.0	930.0/74100.0
n BLQ	6	4
Week 24		
n	117	121
Mean	36853.93	35617.65
SD	15292.389	16049.669
CV (%)	41.495	45.061
Geometric Mean	32973.78	30553.77
Median	35300.00	34200.00
Min/Max	3530.0/90500.0	156.0/99800.0
n BLQ	5	4
Week 32		
n	115	94
Mean	37450.04	36865.81
SD	16877.010	19756.050
CV (%)	45.065	53.589
Geometric Mean	31836.24	27586.41
Median	36600.00	34750.00
Min/Max	95.0/82600.0	78.8/109000.0
n BLQ	4	1
Week 48		
n	110	91
Mean	39097.58	38432.86
SD	16801.710	16495.407
CV (%)	42.974	42.920
Geometric Mean	33716.63	34706.02
Median	36850.00	37200.00
Min/Max	64.2/98200.0	5490.0/100000.
n BLQ	5	2

N = Number of patients in population/treatment group; BLQ = below lower limit of quantification; FAS = Full Analysis Set; SD = Standard Deviation; CV = Coefficient of Variation.

Note: For timepoints after Week 24, patients who switch from Tysabri to PB006 are excluded from this table.

- **Ancillary analyses**

COVID-19 Impact

Plan

Changes in conduct or analysis of the study due to Covid-19 could not be considered at start of the study since the virus was not known by that time. In the documentation of statistical methods (Appendix 6.1.9), a pre-COVID PP Population was described, defined as all PP patients, including those who were excluded from PP only due to major deviations related to COVID-19.

In order to assess the impact of COVID-19 on safety and efficacy of the study the following was done:

• Study discontinuations and protocol deviations due to COVID-19 were summarized

• Demography data for confirmed COVID-19 patients were summarized

• Protocol deviations due to COVID-19 were summarized by country

• A sensitivity analysis was considered to be performed on the pre-COVID PP population. Multiple imputation should be performed for this population. Sensitivity analysis should be done for the primary endpoint using imputed data for this population.

Conduct & Outcome

At the time COVID-19 was identified in 2019 and declared a pandemic by the World Health Organization on 11 March 2020, the PB006-03-01 study was still enrolling and treating patients, with the last patient randomized on 4 May 2020.

The frequency of reviews for data quality assurance was increased after the declaration of the pandemic in March 2022 to be able to identify and respond to the potential problems.

Major protocol deviations related to COVID-19 for the FAS were reported for 8 (6.1%) patients in the PB006 group and 5 (3.8%) patients in the Tysabri group. The majority of deviations in all countries were related to patient visit completion or timing.

COVID-19 was one of the most commonly reported treatment-emergent adverse events (in total 24 events due to COVID-19). One patient discontinued due to a treatment-emergent adverse event of COVID-19.

For COVID-19-confirmed patients (n=22) in the SAF Population, all parameters were similar to the FAS, SAF, PP, and SSW Populations. A sensitivity analysis of the primary analysis using multiple imputation for the pre-COVID PP Population was not performed, as there was no missing data to impute for the population.

- **Summary of main efficacy results**

The following tables summarise the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the biosimilarity assessment (see later sections).

Table 43: Summary of efficacy for trial PB006-03-01

Title: Antelope: Efficacy and Safety of the Biosimilar Natalizumab PB006 in Comparison to Tysabri in Patients with Relapsing-Remitting Multiple Sclerosis (RRMS)		
Study identifier	PB006-03-01 EudraCT Number: 2018-004751-20	
Design	This was a Phase 3 multicenter, double-blind, active-controlled, randomized, parallel-group study to assess the similarity in efficacy, safety, and immunogenicity of biosimilar natalizumab PB006 compared to European Union-approved Tysabri in patients with RRMS. Eligible patients were randomly assigned to 1 of the 2 treatment groups in a 1:1 ratio to receive intravenous infusions every 4 weeks of either PB006 or Tysabri at a dose of 300 mg starting at Visit 1 (Week 0) through Visit 12 (Week 44), for a total of 12 infusions. The primary efficacy endpoint, cumulative number of new active lesions over 24 weeks, was evaluated after 24 weeks. Based on requirements by the FDA a subset of 30 patients was switched after 24 weeks of treatment with Tysabri to treatment with PB006 for the remaining treatment period, to rule out any major safety risks in terms of hypersensitivity, immunogenicity or other reactions, potentially associated with such a switch.	
	Duration of main phase:	48 weeks of treatment
	Duration of Run-in phase:	not applicable
	Duration of Extension phase:	not applicable
Hypothesis	Equivalence	

Treatments groups	PB006		Treatment: PB006 Duration: 48 weeks Number randomized: 132
	Tysabri		Treatment: Tysabri Duration: 48 weeks Number randomized: 133
Endpoints and definitions	Primary endpoint		Cumulative number of new active lesions over 24 weeks
	Secondary endpoint		Cumulative number of new active lesions over 48 weeks
	Secondary endpoint		Cumulative number of new GdE T1-weighted lesions over 24 and 48 weeks
	Secondary endpoint		Number of patients without new GdE T1-weighted lesions over 24 and 48 weeks
	Secondary endpoint		Cumulative number of new/enlarging T2-weighted lesions over 24 and 48 weeks
	Secondary endpoint		Number of patients without new/enlarging T2-weighted lesions over 24 and 48 weeks
	Secondary endpoint		Number of persistent lesions after 24 and 48 weeks
	Secondary endpoint		ARR after 24 and 48 weeks
	Secondary endpoint		Change from baseline in EDSS after 24 and 48 weeks
Database lock	21 OCT 2021		
Results and Analysis			
Analysis description	**Primary Analysis**		
Analysis population and time point description	**Per-Protocol Population**: Only patients participating in this study who completed the 24-week treatment period without major protocol deviations that may have influenced the analysis of the primary endpoint and for whom sufficient post-baseline MRI data were available (including baseline, Week 24 and at least 1 out of the 3 other scheduled MRI visits) were to be included in the Per-Protocol (PP) Population. The final decision on the PP Population was to be made in the blinded data review meeting before database lock for the final analysis of the primary endpoint. **Full Analysis Set Population:** The Full Analysis Set (FAS) Population was to include all patients who were randomized and received at least 1 (complete or partial) infusion of the study drug. Patients were to be analyzed according to the treatment group to which they were randomized. Time point: 24 weeks		
Descriptive statistics and estimate variability	Treatment group	PB006	Tysabri
	Number of subjects	111	118
	Cumulative number of new active lesions over 24 weeks (PP population) mean	1.4	1.9
	SD	3.73	3.97
Effect estimate per comparison	Primary endpoint	Comparison groups	Tysabri – PB006
		Exponentiated difference	0.17
		95% CI	-0.613; 0.944
		P-value	Not applicable

Descriptive statistics and estimate variability	Treatment group	PB006	Tysabri
	Number of subjects	126	127
	Cumulative number of new active lesions over 24 weeks (FAS population) mean	1.4	1.9
	SD	3.62	3.98
Effect estimate per comparison	Primary endpoint	Comparison groups	Tysabri – PB006
		Exponentiated difference	0.23
		95% CI	-0.609; 1.075
		P-value	Not applicable
Notes	Summary of reasons for drop-outs in primary period (24 week): • Study drug discontinued in primary period – Reasons: – Adverse Event – Other • Withdrawn from study in primary period – Reasons: – Subject withdrawal of consent – Adverse Events – Other, Specify		
Analysis description	**Secondary analysis**		
Analysis population and time point description	**Full Analysis Set Population**: The Full Analysis Set (FAS) Population was to include all patients who were randomized and received at least 1 (complete or partial) infusion of the study drug. Patients were to be analyzed according to the treatment group to which they were randomized. Time point: 48 weeks		
Descriptive statistics and estimate variability	Treatment group	PB006	Tysabri
	Number of subjects	131	133
	Cumulative number of new active lesions over 48 weeks, by visit and by primary randomization (FAS population)		
	… up to Week 8		
	n	125	128
	Mean	1.3	1.7
	SD	3.14	3.09
	Median	0.0	0.0
	Min/Max	0/25	0/16
	… up to Week 16		
	n	124	128
	Mean	1.4	1.8
	SD	3.54	3.42
	Median	0.0	0.0
	Min/Max	0/30	0/17
	… up to Week 20		
	n	126	128
	Mean	1.4	1.8
	SD	3.57	3.42

	Median	0.0	0.0
	Min/Max	0/30	0/17
	… up to Week 24		
	n	126	127
	Mean	1.4	1.9
	SD	3.62	3.98
	Median	0.0	0.0
	Min/Max	0/30	0/29
	… up to Week 48		
	n	122	96
	Mean	1.5	2.3
	SD	3.72	5.68
	Median	0.0	0.0
	Min/Max	0/30	0/39
Notes	Summary of reasons for drop-outs (48 week): • Study drug discontinued – Reasons: – Adverse Event – Other • Withdrawn from study – Reasons: – Subject withdrawal of consent – Investigator / sponsor decision – Adverse Events – Other, Specify		
Descriptive statistics and estimate variability	Treatment group	PB006	Tysabri
	Number of subjects	131	133
	Cumulative number of new GdE T1-weighted lesions over 24 and 48 weeks, by visit and by primary randomization (FAS population)		
	… up to Week 8		
	n	125	128
	Mean	0.2	0.3
	SD	0.78	1.19
	Median	0.0	0.0
	Min/Max	0/6	0/11
	… up to Week 16		
	n	124	128
	Mean	0.3	0.4
	SD	0.97	1.22
	Median	0.0	0.0
	Min/Max	0/8	0/11
	… up to Week 20		
	n	126	128
	Mean	0.3	0.4
	SD	1.01	1.22

	Median	0.0	0.0
	Min/Max	0/8	0/11
	… up to Week 24		
	n	126	127
	Mean	0.3	0.4
	SD	1.01	1.25
	Median	0.0	0.0
	Min/Max	0/8	0/11
	… up to Week 48		
	n	122	96
	Mean	0.3	0.4
	SD	1.02	1.39
	Median	0.0	0.0
	Min/Max	0/8	0/11
Descriptive statistics and estimate variability	Treatment group	PB006	Tysabri
	Number of subjects	131	133
	Number (%) of patients without new GdE T1-weighted lesions over 24 and 48 weeks (FAS population)		
	Over 24 weeks	n=131	n=133
	Number (%) of patients without new GdE T1-weighted lesions	109 (83.2)	105 (78.9)
	Number (%) of patients with at least one new GdE T1-weighted lesion	17 (13.0)	22 (16.5)
	Number (%) of patients without sufficient MRI data	5 (3.8)	6 (4.5)
	Over 48 weeks	n=131	n=103
	Number (%) of patients without new GdE T1-weighted lesions	105 (80.2)	80 (77.7)
	Number (%) of patients with at least one new GdE T1-weighted lesion	17 (13.0)	16 (15.5)
	Number (%) of patients without sufficient MRI data	9 (6.9)	7 (6.8)
Descriptive statistics and estimate variability	Treatment group	PB006	Tysabri
	Number of subjects	131	133
	Cumulative number of new/enlarging T2-weighted lesions over 24 and 48 weeks, by visit and by primary randomization (FAS population)		
	… up to Week 8		
	n	125	128
	Mean	1.3	1.8
	SD	3.34	3.28

	Median	0.0	0.0
	Min/Max	0/26	0/16
	… up to Week 16		
	n	124	128
	Mean	1.4	1.9
	SD	3.72	3.59
	Median	0.0	0.0
	Min/Max	0/31	0/17
	… up to Week 20		
	n	126	128
	Mean	1.5	1.9
	SD	3.75	3.59
	Median	0.0	0.0
	Min/Max	0/31	0/17
	… up to Week 24		
	n	126	127
	Mean	1.5	2.0
	SD	3.79	4.12
	Median	0.0	0.0
	Min/Max	0/31	0/29
	… up to Week 48		
	n	122	96
	Mean	1.6	2.4
	SD	3.90	5.79
	Median	0.0	0.0
	Min/Max	0/31	0/39
Descriptive statistics and estimate variability	Treatment group	PB006	Tysabri
	Number of subjects	131	133
	Number (%) of patients without new/enlarging T2-weighted lesions over 24 and 48 weeks (FAS population)		
	Over 24 weeks	n=131	n=133
	Number (%) of patients without new/enlarging T2-weighted lesions	75 (57.3)	72 (54.1)
	Number (%) of patients with at least one new/enlarging T2-weighted lesion	51 (38.9)	55 (41.4)
	Number (%) of patients without sufficient MRI data	5 (3.8)	6 (4.5)
	Over 48 weeks	n=131	n=103
	Number (%) of patients without new/enlarging T2-weighted lesions	71 (54.2)	52 (50.5)

	Number (%) of patients with at least one new/enlarging T2-weighted lesion	51 (38.9)	44 (42.7)
	Number (%) of patients without sufficient MRI data	9 (6.9)	7 (6.8)
Descriptive statistics and estimate variability	Treatment group	PB006	Tysabri
	Number of subjects	131	133
	Number of persistent lesions over 24 and 48 weeks, by visit and by primary randomization (FAS population)		
	… up to Week 8		
	n	125	128
	Mean	0.3	0.3
	SD	1.59	1.34
	Median	0.0	0.0
	Min/Max	0/16	0/14
	… up to Week 16		
	n	124	128
	Mean	0.4	0.4
	SD	1.97	2.38
	Median	0.0	0.0
	Min/Max	0/19	0/26
	… up to Week 20		
	n	126	128
	Mean	0.4	0.4
	SD	2.28	2.90
	Median	0.0	0.0
	Min/Max	0/22	0/32
	… up to Week 24		
	n	126	127
	Mean	0.5	0.4
	SD	2.46	2.92
	Median	0.0	0.0
	Min/Max	0/23	0/32
	… up to Week 48		
	n	122	96
	Mean	0.5	0.6
	SD	2.55	3.35
	Median	0.0	0.0
	Min/Max	0/23	0/32
Descriptive statistics and estimate variability	Treatment group	PB006	Tysabri
	Number of subjects	131	133
	ARR after 24 and 48 weeks (FAS population)		

	Over 24 weeks				
	Number of patients	131		133	
	Number of relapses per patient, n (%)				
	1	12 (9.2%)		9 (6.8%)	
	2	0		0	
	≥3	0		0	
	Total number of relapses	12		9	
	Follow-up time (years)				
	N	131		133	
	Mean	0.445		0.447	
	SD	0.0772		0.0717	
	Median	0.460		0.460	
	Min/Max	0.00/0.50		0.00/0.49	
	Total follow-up time (years)	58.29		59.39	
	ARR (relapses/year)	0.206		0.152	
	Over 48 weeks				
	Number of patients	131		103	
	Number of relapses per patient, n (%)				
	1	18 (13.7%)		12 (11.7%)	
	2	1 (0.8%)		0	
	≥3	0		0	
	Total number of relapses	20		12	
	Follow-up time (years)				
	n	131		103	
	Mean	0.877		0.878	
	SD	0.1865		0.1902	
	Median	0.920		0.920	
	Min/Max	0.06/1.07		0.08/1.09	
	Total follow-up time (years)	114.91		90.42	
	ARR (relapses/year)	0.174		0.133	
Descriptive statistics and estimate variability	Treatment group	PB006		Tysabri	
	Number of subjects	131		133	
	Change from baseline in EDSS after 24 and 48 weeks, by primary randomization (FAS population)	**Observed value**	**Change from baseline**	**Observed value**	**Change from baseline**
	Baseline				
	n	131		133	
	Mean	3.36		3.20	
	SD	1.065		1.206	

	Median	3.50		3.50	
	Min/Max	1.5/5.0		1.0/5.0	
	Week 24				
	n	122	122	125	125
	Mean	3.37	-0.03	3.18	0.00
	SD	1.126	0.211	1.258	0.354
	Median	3.50	0.00	3.50	0.00
	Min/Max	1.0/5.5	-1.0/1.0	1.0/6.5	-1.0/2.0
	Week 48				
	n	117	117	93	93
	Mean	3.24	-0.14	3.12	-0.05
	SD	1.203	0.536	1.322	0.443
	Median	3.50	0.00	3.50	0.00
	Min/Max	1.0/6.0	-2.5/1.0	0.0/6.5	-1.5/1.5

2.6.6. Discussion on clinical efficacy

Design and conduct of clinical studies

The applicant conducted a pivotal efficacy and safety study, PB006-03-01, to compare PB006 to the EU reference product Tysabri.

This was a randomized, double-blind, active-controlled, parallel-group study to compare the efficacy, safety and immunogenicity of PB006 versus EU-Tysabri, and to demonstrate similarity between PB006 and EU-Tysabri in patients with RRMS.

In general, the study design followed the Scientific Advice received from CHMP in 2017.

The inclusion criteria define patients with RRMS. The criteria in this study are less stringent than in the SmPC of the originator (e.g., only one third of patients had 2 or more relapses in the year prior to screening). However, the eligibility criteria do resemble those of the pivotal phase 3 studies of the originator and thus, the selected study population is regarded sufficiently sensitive for the comparative efficacy assessment. The exclusion criteria are found in line with the contraindications, special warnings, and precautions for use of the reference product, Tysabri EU, and with the scientific advice received.

The dose of 300 mg, dosing frequency (once every 4 weeks), route and method of administration are in line with the SmPC of the reference product and are thus, appropriate. The number of doses (12) over a study period of 44 weeks is also considered adequate.

The primary endpoint, cumulative number of new active lesions over 24 weeks, and secondary endpoints, i.e., cumulative number of new active lesions over 48 weeks, cumulative number of new GdE T1-weighted lesions over 24 and 48 weeks, number of patients without new GdE T1-weighted lesions over 24 and 48 weeks, cumulative number of new/enlarging T2-weighted lesions over 24 and 48 weeks, number of patients without new/enlarging T2-weighted lesions over 24 and 48 weeks, number of persistent lesions after 24 and 48 weeks, ARR after 24 and 48 weeks and change from baseline in EDSS after 24 and 48 weeks are considered adequate to assess the clinical similarity between the biosimilar candidate and the reference product because they examine the effect on brain lesion activity and measure the delay of the disability, respectively. Moreover, the efficacy endpoints are in line with the "Guideline on clinical investigation of medicinal products for the treatment of Multiple Sclerosis" (EMA/CHMP/771815/2011, Rev. 2), the efficacy endpoints studied in the clinical trials which supported

the MA of the reference product, Tysabri EU and the "Guideline on similar biological medicinal products containing interferon beta" (EMA/CHMP/BMWP/652000/2010).

The primary endpoint was also endorsed during the Scientific Advice in 2017. For this endpoint, the applicant defined an equivalence margin of 2.1 lesions based on the lower bound of the 95% CI of the pooled effect size estimated in a controlled trial of natalizumab for RMS published in 2003. This is problematic in two ways.

First, the methodological considerations for the derivation of the margin are based on a single study, because only one appropriate reference for the derivation of the equivalence margin in the agreed primary endpoint could be sourced. The relatively small sample size in that study (approximately 70 patients per treatment arm) and the fact that in the lack of further data no meta-analysis was possible, introduce uncertainty about the actual effect size of natalizumab over placebo in the selected endpoint. Thus, a more conservative planning of the equivalence margin would have been desirable. Secondly, the mean number of new active lesions over 6 months with natalizumab was only approximately 1 in the cited publication by Miller et al. In light of this low number in new lesions observed with the comparator, the equivalence margin of 2.1 is considered rather wide for the efficacy similarity assessment from a therapeutic perspective, which was derived solely based on methodological considerations on the large effect size of natalizumab with 8.7 lesions (95% CI: 4.14 to 13.35) in the publication of Miller et al. Further, the reference article is dated in 2003. During the last years, the therapeutic management of RMS has evolved towards a more intensive management of inflammatory activity so it is highly questionable that 2.1 lesions within 24 weeks could be considered as no relevant from a therapeutic RMS management perspective. However, the actual difference that was observed for the primary endpoint in study PB006-03-01 was close to zero and the 95% CI was narrow and accounted for a difference of less than one lesion (point estimate: 0.17 lesions; 95% CI: -0.613; 0.944). Thus, the primary endpoint would have been met even with a tighter equivalence margin.

It is noted that no biomarker of PD was included in the study. This is striking, since a lot of emphasis has been put on the evaluation of PD markers in the phase 1 study in healthy volunteers. The inclusion of PD markers in the phase 3 study would have allowed to draw conclusions on the robustness and validity of the respective markers by comparison of the response in healthy subjects and patients.

From the methodological view, several issues concerning the randomisation procedure were identified. One of those issues pertains to the applied method of dynamic allocation *per se* and its potential for type-1-error inflation. The applicant provided details on the methodology, but uncertainty still persists as regards a potential inflation due to the specific situation resulting from complex randomisation methodology and erroneous stratification. However, from an assessment perspective it is considered very unlikely that the impact is of a magnitude that altered general study conclusions, and is therefore not considered finally relevant for the conclusion on biosimilarity. Another area for further assessment is about potential implications arising from misinterpretation of stratification data which lead to erroneous stratification during randomisation. However, additional sensitivity analyses corroborated that the influence of stratification errors was negligible and did not change study conclusions.

The definition of the analysis sets is considered adequate. In general, the statistical methods chosen for descriptive as well as inferential analyses are considered suitable. The use of negative binomial regression analysis for the comparative primary efficacy evaluation of lesion count data is endorsed.

Efficacy data and additional analyses

A total of 265 patients were randomized. Of those, 264 patients were treated with study drug (131 and 133 patients in the PB006 and Tysabri groups, respectively). 93.6% of the enrolled patients completed the primary study period of 24 weeks and 90.5% completed the entire study. Discontinuation due to adverse events accounted for 4.5% overall and was twice as abundant in the PB006 group compared to

the Tysabri group. Baseline characteristics as well as medical history (including MS disease history) were well balanced between groups.

Misclassification of the three stratification factors was discovered on two occasions; first, after database lock but still during the study (26 cases) and later after finalization of the CSR for a total of 62 cases. The errors were distributed across all 3 stratification factors (absence/presence of GdE lesions, presence of T2 lesions, and JCV status). A root cause analysis and impact assessment were conducted. According to the applicant, this analysis revealed that even with 23% of patients mis-stratified, the imbalance of strata was low and there was no significant impact on the primary analysis. In some strata, however, the relationship between the two groups flipped and the impact on the analysis is not entirely clear. This issue is discussed further below for the primary efficacy analysis. Given the late discovery of the misclassification of the three stratification factors, the applicant was asked to discuss any impact on the study results. It was affirmed that there was no significant impact of the misclassification of the three stratification factors on the study results. The treatment arms ended up being well-balanced for the corrected strata and the sensitivity analysis performed using the corrected strata confirms the primary analysis. Most importantly, it was affirmed that no inclusion of subjects with JCV index >1.5 had occurred.

The analysis of the primary endpoint was the cumulative number of new active lesions over 24 weeks. The primary analysis was based on the PP Population and demonstrated similarity between the test and reference product based on the pre-specified equivalence margin.

Subsequently, sensitivity analyses were conducted to account for the errors in stratification and the GCP related findings.

The first sensitivity analysis was conducted using corrected stratification variables for all 62 patients, who had been misclassified. From this model, a 95%-CI is obtained showing a width being 20% of the width of the CI resulting from the original model using faulty stratification data. The potential prognostic impact of the stratification variables was not substantially altered by using the corrected stratification factors, and the stratification factor absence/presence of GdE lesions was found to have an influence on the cumulative number of new active lesion in both models. The change in the point estimate and CI was shown to be caused by few influential outlying values. When re-conducting the primary efficacy analysis as a sensitivity analysis by excluding all patient data from site 7002, where the routine quality assessment revealed questionable data integrity, similar results to the original primary analysis were obtained. The CI was larger due to the lower sample size, but still within the pre-specified margins. Finally, after correction for stratification and excluding site 7002, the primary outcome also demonstrated a small 95% CI well within the pre-specified margin. The primary outcome measure was distinctly lower than 1 and positive in all analyses, meaning that the number of new lesions was slightly lower for PB006 than the reference product.

While sensitivity analyses were conducted on the primary efficacy endpoint to account for the impact of stratification correction and exclusion of one study centre, no such data were presented for the secondary endpoints. Upon request, the applicant affirmed that the primary efficacy endpoint as well as the secondary efficacy endpoints based on MRI readings were not affected by the GCP issue encountered at site 7002. EDSS was pointed out as the only efficacy or safety parameter that may have been affected by the inconsistencies encountered at site 7002. The applicant has re-run the analyses for EDSS endpoints excluding all 17 patients from site 7002 demonstrating very similar results as in the overall analysis.

Generally, the result of the primary analysis is supported by the results of the secondary analyses.

Most of the secondary efficacy parameters displayed similar results between treatments. In some parameters, numerically lower numbers were observed for PB006. This was the case for the mean

cumulative number of new active lesions, the number of new active lesions per MRI scan, and new active lesions at Week 48, and the mean cumulative number of new/enlarging T2-weighted lesions.

The number of disease relapse, on the other hand, was larger in the PB006 group compared to the reference product. Over 24 weeks there were 12 relapses in the PB006 group compared to 9 cases in the Tysabri group, corresponding to an ARR of 0.206 and 0.152, respectively. Over 48 weeks the number of relapses was 20 and 12 for PB006 and Tysabri, respectively, and the ARR was 0.174 as compared to 0.133.

Overall, less new active lesions and less T2-weighted lesions were observed after treatment with PB006 as compared to Tysabri. However, this trend was not reflected in the relapse rate, which was higher in the PB006 group as compared to Tysabri, although it is acknowledged that the overall number of relapses over 48 weeks was low in both treatment arms in study PB006-03-01, with 18 (13.7%) of patients having one relapse and 1 patient (0.8%) having two relapses in the PB006 and 12 (11.7%) of patients in the EU-Tysabri group having one relapse. Interpretation is slightly hampered by the fact the 131 and 133 patients began treatment in the PB006 and Tysabri arm, respectively, whereas 131 and 103 patients continued treatment through week 48 due to the fact that 30 patients were switched from Tysabri to PB006 after 24 weeks. For better comparison, the applicant provided ARR data obtained with the originator. According to the Tysabri product information, ARR was 0.281 versus 0.805 for Tysabri and placebo, respectively, after one year. This shows that the relapse rate observed in study PB006-03-01 (0.174 and 0.133 for PB006 and Tysabri, respectively) is in line with the published data on Tysabri.

Further, the proportion of patients who received methylprednisolone as rescue therapy was similar between treatment groups.

EDSS scores were similar between treatment groups. The mean change from baseline was minimal and similar in both treatment groups at 24 weeks and at 48 weeks.

In order to assess the impact of COVID-19 on safety and efficacy of the study the following was done:

• Study discontinuations and protocol deviations due to COVID-19 were summarized

• Demography data for confirmed COVID-19 patients were summarized

• Protocol deviations due to COVID-19 were summarized by country

• A sensitivity analysis was considered to be performed on the pre-COVID PP population. Multiple imputation should be performed for this population. Sensitivity analysis should be done for the primary endpoint using imputed data for this population.

The applicant explained that this sensitivity analysis was not conducted because the pre-COVID PP population and the PP population were identical.

2.6.7. Conclusions on the clinical efficacy

From the presented data on efficacy, biosimilarity between the PB006 and EU-Tysabri can be concluded.

2.6.8. Clinical safety

The assessment of safety in the PB006 clinical program was focused on the comparison of PB006 to Tysabri. Safety data with PB006 were collected in studies PB006-01-02, PB006-01-03 and PB006-03-01. These safety data are presented by study, i.e., no integrated analysis was performed, due to the different study designs, populations and doses administered.

The principal safety data for PB006 and comparative safety data with Tysabri is derived from the pivotal, Phase 1, single-dose study in 450 healthy subjects (PB006-01-03) and the pivotal Phase 3, multiple-dose study in 264 RRMS patients (PB006-03-01) treated for up to 48 weeks. In both studies, safety analyses were based on the SAF, defined as all subjects who received at least one dose of study drug. Subjects were analysed as treated. In the Phase 3 study PB006-03-01, additional safety analyses were performed for the SSW population, defined as patients who received at least one infusion of study drug after the time point of re-randomization.

Similar procedures for assessing safety were followed in the Phase 1 study PB006-01-03 and the Phase 3 study PB006-03-01. In addition to evaluation of AEs, SAEs and AEs leading to discontinuation, adverse events of special interest (AESIs) were defined for study PB006-03-01. Both studies included an assessment of ECG, vital signs and physical examination.

In study PB006-01-03, AEs were reported from start of intervention until the end of study Visit (Day 85). In study PB006-03-01, AEs were reported from screening until Week 48/ end of study or Early Discontinuation visit.

In both studies, safety monitoring included an assessment of neurological symptoms which could be suggestive of PML, and both studies included a PML follow-up visit 6 months after the last dose of study drug to monitor for AEs suggestive of PML.

For study PB006-03-01, PML, JCV granule cell neuronopathy, opportunistic infections, liver injury, hypersensitivity, encephalitis, meningitis, and acute retinal necrosis were defined as AESIs, based on the known safety profile of Tysabri.

2.6.8.1. Patient exposure

Overall, PB006 was administered to 159 healthy subjects as single dose (for 149 of these subjects at 3 mg/kg, and for 10 of these subjects at 300 mg), and to 161 patients with RRMS as multiple doses of 300 mg in 4-weekly intervals.

Tysabri (US-Tysabri or EU-Tysabri) was administered to 337 healthy subjects as single dose (1 to 6 mg/kg), and EU-Tysabri was administered to 133 patients with RRMS as multiple doses of 300 mg in 4-weekly intervals.

Study PB006-01-02

This was a single-dose study with PB006 in healthy male and female subjects. A total of 68 subjects were screened, and 10 of these were enrolled and received a single IV dose of 300 mg PB006 (administered over 60 minutes). All 10 subjects completed the study as per protocol, and comprised the SAF population.

Study PB006-01-03

This was a single dose study with PB006, EU-Tysabri and US-Tysabri in healthy male and female subjects. A total of 453 subjects were randomized (150, 152 and 151 in the PB006, EU-Tysabri and US-Tysabri groups, respectively). Three of the randomized subjects were not dosed with study drug. One subject did not receive study drug due to an error in the pharmacy (only diluent was infused); one subject was not dosed due to a vasovagal reaction prior to dosing; one subject was not dosed due to problems with venous access. Thus, 149, 151 and 150 subjects received a single IV dose of 3 mg/kg PB006, EU-Tysabri and US-Tysabri, respectively, and comprised the SAF population.

On 16 Mar 2020, study enrolment was temporarily held due to COVID-19. At this time 16 subjects completed the study (3 received 3 mg/kg PB006, 5 received 3 mg/kg EU-Tysabri, and 8 received 3

mg/kg US-Tysabri), and were included in the Pre-stop Safety Set. The other 434 subjects completed the study after the study halt or were enrolled after restart of the study and were included in the Post-stop Safety Set.

A total of 438 subjects completed the study, 145, 148 and 145 in the PB006, EU-Tysabri and US-Tysabri groups, respectively. No subject discontinued the study due to an AE).

All subjects in the SAF population received the full dose of study drug. In 4 subjects, the infusion was temporarily interrupted (e.g., due to problems with the flow of infusion; not due to AEs.

For PB006, the lowest dose administered was 156 mg natalizumab and the highest dose administered was 274 mg natalizumab. For EU-Tysabri, the lowest dose administered was 150.3 mg natalizumab and the highest dose administered was 276.1 mg natalizumab. For US- Tysabri, the lowest dose administered was 151.3 mg natalizumab and the highest dose administered was 275.6 mg natalizumab.

Study PB006-03-01

This was a multiple-dose study in RRMS patients. Study drug (PB006 or EU-Tysabri) was administered as 300 mg IV infusion, in 4-week intervals, for a total of 12 infusions.

A total of 265 patients were randomized, 132 patients to treatment with PB006 and 133 patients to treatment with EU-Tysabri. One patient randomized to the PB006 group withdrew consent prior to receiving study drug. Thus, 264 patients overall were treated with study drug, i.e., 131 and 133 patients in the PB006 and EU-Tysabri groups, respectively. After 24 weeks of treatment, 30 patients from the EU-Tysabri group were switched to treatment with PB006 for the remaining treatment period. Thus, the total number of patients treated with PB006 at any time during the study was 161.

Patient disposition by primary randomization was similar for the 2 treatment groups, as summarized for the full analysis set (FAS; identical to the SAF population with regard to patient numbers) in

Table 44: Patient disposition, by primary randomization in study PB006-03-01 (FAS population)

	PB006 N=131 n (%)	**EU-Tysabri N=133 n (%)**	**Total N=264 n (%)**
Completed primary period (24 weeks)	**122 (93.1)**	**125 (94.0)**	**247 (93.6)**
Study drug discontinued in primary period – Reason:	9 (6.9)	8 (6.0)	17 (6.4)
Subject withdrawal of consent	2 (1.5)	4 (3.0)	6 (2.3)
Investigator / sponsor decision	0	0	0
Adverse events	6 (4.6)	3 (2.3)	9 (3.4)
COVID-19 related	0	0	0
Pregnancy	0	0	0
Non-compliance	0	0	0
Lost to follow-up	0	0	0
Lack of efficacy	0	0	0
Other	1 (0.8)	1 (0.8)	2 (0.8)
Re-randomized at week 24		**125 (94.0)**	**125 (47.3)**
-to remain on EU-Tysabri		95 (71.4)	95 (36.0)
-to switch to PB006		30 (22.6)	30 (11.4)
Completed study (48 weeks)	**117 (89.3)**	**122 (91.7)**	**239 (90.5)**
Study drug discontinued – Reason:	14 (10.7)	11 (8.3)	25 (9.5)
Subject withdrawal of consent	3 (2.3)	6 (4.5)	9 (3.4)
Investigator / sponsor decision	2 (1.5)	0	2 (0.8)
Adverse events*	8 (6.1)	4 (3.0)	12 (4.5)
COVID-19 related	0	0	0
Pregnancy	0	0	0
Non-compliance	0	0	0
Lost to follow-up	0	0	0
Lack of efficacy	0	0	0
Other	1 (0.8)	1 (0.8)	2 (0.8)

AE=adverse event, COVID-19=coronavirus disease 2019, eCRF=electronic case report form, FAS=full analysis set, N=Number of patients in treatment group, n=number of patients with specified event/reason.
Note: The 48-week summary comprises the full study period, including the first 24 weeks.
Note: End of Study eCRF page was only filled-in during the treatment period.
*Except for one patient discontinuing due to COVID-19 (unrelated), all AEs leading to discontinuation were assessed as at least possibly related to study drug

A total of 247 patients (93.6%) completed the 24-week primary treatment period, 122 (93.1%) in the PB006 group and 125 (94.0%) in the EU-Tysabri group. The percentage of patients who prematurely discontinued study drug in the 24-week period was similar in the PB006 and EU-Tysabri groups (6.9% versus 6.0%). Six patients (4.6%) in the PB006 group prematurely discontinued due to an AE, compared with 3 patients (2.3%) in the EU-Tysabri group.

At Week 24, all 125 patients in the EU-Tysabri group who had completed the primary treatment period were re-randomized. Of these, 30 patients were switched to treatment with PB006 for the remaining treatment period, and 95 patients remained on EU-Tysabri and were thus treated with EU-Tysabri for the entire study.

Of the 30 patients in the switch group, 29 patients (96.7%) completed the study, and 1 patient (3.3%) discontinued due to AE. Of the 95 patients who remained in the EU-Tysabri group, 93 patients (97.9%) completed the study and 2 patients (2.1%) discontinued (both due to withdrawal of consent).

2.6.8.2. Adverse events

Study PB006-01-02

Treatment-emergent adverse events were reported in 8 subjects (80%), and study drug related treatment emergent AEs (TEAEs) were reported in 6 subjects (60%). In all subjects, TEAEs were mild or

moderate, and no severe TEAEs were reported. No SAEs, fatal AEs or AEs leading to discontinuation occurred in the study.

Most commonly reported TEAEs were in the system organ class (SOCs) general disorders and administration site conditions (80%) and nervous system disorders (50%). On the preferred term (PT) level, most common were headache (5 subjects, 50%), and catheter site pain, fatigue and injection site haematoma (2 subjects each, 20%). All other TEAEs were reported in 1 subject each.

Of the 6 subjects (60%) who experienced at least 1 study drug-related TEAE, the most commonly reported was headache (5 subjects, 50%). The remaining event was fatigue (1 subject, 10%).

Study PB006-01-03

In this single-dose, healthy subject study, AE frequencies were similar across the 3 groups. Approximately two-thirds of subjects in each group reported any TEAE, while in 34-37% of subjects across groups, study drug-related TEAEs occurred. No fatal TEAEs occurred. SAEs were reported for 2 subjects (1.3%; 6 SAEs overall) treated with US-Tysabri, while no SAEs occurred in the other 2 groups. No subject reported TEAEs leading to study drug discontinuation.

Table 45: Overall summary of AEs in study PB006-01-03 (SAF population)

	Number of subjects (%)			
	PB006 3 mg/kg N=149	**EU-Tysabri 3 mg/kg N=151**	**US-Tysabri 3 mg/kg N=150**	**Total N=450**
Any TEAE	103 (69.1)	102 (67.5)	98 (65.3)	303 (67.3)
Any related TEAE	55 (36.9)	52 (34.4)	52 (34.4)	159 (35.3)
Any treatment-emergent SAE	0	0	2 (1.3)	2 (0.4)
Any severe TEAE	0	0	1 (0.7)	1 (0.2)
Any TEAE leading to study drug discontinuation	0	0	0	0
Fatal TEAE	0	0	0	0

SAE=serious adverse event, SAF=safety population, TEAE=treatment-emergent adverse event.

TEAEs occurring in >1% of subjects in any treatment group are presented by SOC and PT in table below.

Table 46: TEAEs by SOC and PT in >1% of subjects in any treatment group in study PB006-01-03 (SAF population)

	Number of subjects (%)			
System organ class Preferred term	**PB006 N=149**	**EU-Tysabri N=151**	**US-Tysabri N=150**	**Total N=450**
Any event	**103 (69.1)**	**102 (67.5)**	**98 (65.3)**	**303 (67.3)**
Nervous system disorders	**48 (32.2)**	**50 (33.1)**	**45 (30.0)**	**143 (31.8)**
Headache	44 (29.5)	44 (29.1)	39 (26.0)	127 (28.2)
Dizziness	4 (2.7)	4 (2.6)	3 (2.0)	11 (2.4)
Paraesthesia	1 (0.7)	3 (2.0)	1 (0.7)	5 (1.1)
Somnolence	0	1 (0.7)	2 (1.3)	3 (0.7)
Migraine	2 (1.3)	0	0	2 (0.4)
General disorders and administration site conditions	**50 (33.6)**	**47 (31.1)**	**44 (29.3)**	**141 (31.3)**
Injection site reaction	32 (21.5)	30 (19.9)	27 (18.0)	89 (19.8)
Fatigue	9 (6.0)	7 (4.6)	3 (2.0)	19 (4.2)
Vessel puncture site reaction	2 (1.3)	4 (2.6)	7 (4.7)	13 (2.9)
Catheter site related reaction	3 (2.0)	5 (3.3)	3 (2.0)	11 (2.4)
Infusion site reaction	6 (4.0)	2 (1.3)	1 (0.7)	9 (2.0)
Pyrexia	3 (2.0)	1 (0.7)	0	4 (0.9)

System organ class Preferred term	Number of subjects (%) PB006 N=149	EU-Tysabri N=151	US-Tysabri N=150	Total N=450
Gastrointestinal disorders	**23 (15.4)**	**26 (17.2)**	**28 (18.7)**	**77 (17.1)**
Nausea	6 (4.0)	12 (7.9)	9 (6.0)	27 (6.0)
Diarrhoea	5 (3.4)	4 (2.6)	4 (2.7)	13 (2.9)
Abdominal pain	3 (2.0)	2 (1.3)	7 (4.7)	12 (2.7)
Toothache	3 (2.0)	2 (1.3)	2 (1.3)	7 (1.6)
Infections and infestations	**26 (17.4)**	**25 (16.6)**	**21 (14.0)**	**72 (16.0)**
Nasopharyngitis	8 (5.4)	9 (6.0)	7 (4.7)	24 (5.3)
Upper respiratory tract infection	6 (4.0)	4 (2.6)	2 (1.3)	12 (2.7)
COVID-19	5 (3.4)	2 (1.3)	4 (2.7)	11 (2.4)
Cystitis	0	3 (2.0)	1 (0.7)	4 (0.9)
Ear infection	0	3 (2.0)	0	3 (0.7)
Musculoskeletal and connective tissue disorders	**10 (6.7)**	**21 (13.9)**	**12 (8.0)**	**43 (9.6)**
Back pain	4 (2.7)	5 (3.3)	5 (3.3)	14 (3.1)
Myalgia	4 (2.7)	5 (3.3)	1 (0.7)	10 (2.2)
Arthralgia	0	4 (2.6)	3 (2.0)	7 (1.6)
Respiratory, thoracic and mediastinal disorders	**13 (8.7)**	**10 (6.6)**	**12 (8.0)**	**35 (7.8)**
Oropharyngeal pain	9 (6.0)	5 (3.3)	3 (2.0)	17 (3.8)
Epistaxis	2 (1.3)	0	4 (2.7)	6 (1.3)
Cough	1 (0.7)	3 (2.0)	1 (0.7)	5 (1.1)
Injury, poisoning and procedural complications	**9 (6.0)**	**6 (4.0)**	**13 (8.7)**	**28 (6.2)**
Arthropod bite	4 (2.7)	3 (2.0)	0	7 (1.6)
Skin and subcutaneous tissue disorders	**5 (3.4)**	**7 (4.6)**	**9 (6.0)**	**21 (4.7)**
Metabolism and nutrition disorders	**3 (2.0)**	**5 (3.3)**	**3 (2.0)**	**11 (2.4)**
Decreased appetite	3 (2.0)	4 (2.6)	3 (2.0)	10 (2.2)
Reproductive system and breast disorders	**4 (2.7)**	**1 (0.7)**	**6 (4.0)**	**11 (2.4)**
Dysmenorrhoea	3 (2.0)	1 (0.7)	2 (1.3)	6 (1.3)
Renal and urinary disorders	**2 (1.3)**	**4 (2.6)**	**4 (2.7)**	**10 (2.2)**
Dysuria	2 (1.3)	3 (2.0)	2 (1.3)	7 (1.6)
Psychiatric disorders	**4 (2.7)**	**3 (2.0)**	**2 (1.3)**	**9 (2.0)**
Vascular disorders	**3 (2.0)**	**2 (1.3)**	**4 (2.7)**	**9 (2.0)**
Eye disorders	**3 (2.0)**	**2 (1.3)**	**3 (2.0)**	**8 (1.8)**
Ear and labyrinth disorders	**1 (0.7)**	**4 (2.6)**	**2 (1.3)**	**7 (1.6)**

N=number of subjects in group, n=number of subjects with event, PT=preferred term, SAF=safety population, SOC=system organ class, TEAE=treatment-emergent adverse event.
Sorted by descending frequency in the "total" column.

Most frequently reported were TEAEs in the SOCs nervous system disorders (30-33% across groups) and general disorders and administration site conditions (29-34% across groups).

On the PT level, most frequently reported was headache (26-30% across groups), followed by AEs related to the injection/infusion, with PTs of injection site reaction (18-21% across groups), infusion site reaction (1-4% across groups), vessel puncture site reaction (1-5% across groups), and catheter site related reaction (2-3% across groups).

While on the level of individual PTs the frequencies may have slightly differed between the PB006, the EU-Tysabri and US-Tysabri groups, the overall AE profile was considered to be similar between the 3 groups.

COVID-19 analyses

Eleven AEs of COVID-19 and 1 AE of asymptomatic COVID-19 were reported. All cases were mild with the outcome being recovered. No subjects were withdrawn due to COVID-19. None of the subjects had received a COVID-19 vaccination.

The overall frequency of AEs was similar in the Pre-stop and the Post-stop Safety Set (62.5% versus 67.5% of subjects overall). Generally, the limited number of subjects in the Pre-stop Set (N=16) does not allow a meaningful comparison to the Post-stop Set.

Adverse events related to study drug

TEAEs related to study drug are presented by SOC and PT in table below. The percentage of subjects with related TEAEs was similar in all 3 treatment groups.

Table 47: Study drug-related TEAEs by SOC and PT in study PB006-01-03 (SAF population)

	Number of subjects (%)			
System organ class Preferred term	**PB006** **N=149**	**EU-Tysabri** **N=151**	**US-Tysabri** **N=150**	**Total** **N=450**
Any event	**55 (36.9)**	**52 (34.4)**	**52 (34.7)**	**159 (35.3)**
General disorders and administration site conditions	**32 (21.5)**	**32 (21.2)**	**29 (19.3)**	**93 (20.7)**
Injection site reaction	27 (18.1)	28 (18.5)	24 (16.0)	79 (17.6)
Infusion site reaction	4 (2.7)	1 (0.7)	1 (0.7)	6 (1.3)
Chills	1 (0.7)	2 (1.3)	0	3 (0.7)
Chest discomfort	1 (0.7)	0	1 (0.7)	2 (0.4)
Injection site induration	0	1 (0.7)	1 (0.7)	2 (0.4)
Asthenia	0	0	1 (0.7)	1 (0.2)
Fatigue	0	1 (0.7)	0	1 (0.2)
Malaise	0	0	1 (0.7)	1 (0.2)
Pyrexia	0	1 (0.7)	0	1 (0.2)
Nervous system disorders	**20 (13.4)**	**23 (15.2)**	**18 (12.0)**	**61 (13.6)**
Headache	19 (12.8)	20 (13.2)	16 (10.7)	55 (12.2)
Dizziness	1 (0.7)	2 (1.3)	2 (1.3)	5 (1.1)
Paraesthesia	0	1 (0.7)	1 (0.7)	2 (0.4)
Somnolence	0	1 (0.7)	1 (0.7)	2 (0.4)
Migraine	1 (0.7)	0	0	1 (0.2)
Gastrointestinal disorders	**5 (3.4)**	**10 (6.6)**	**9 (6.0)**	**24 (5.3)**
Nausea	4 (2.7)	8 (5.3)	6 (4.0)	18 (4.0)
Diarrhoea	0	2 (1.3)	1 (0.7)	3 (0.7)
Abdominal pain upper	0	0	1 (0.7)	1 (0.2)
Aphthous ulcer	1 (0.7)	0	0	1 (0.2)
Dry mouth	0	0	1 (0.7)	1 (0.2)
Dyspepsia	0	0	1 (0.7)	1 (0.2)
Gastrointestinal sounds abnormal	0	0	1 (0.7)	1 (0.2)
Mouth ulceration	0	1 (0.7)	0	1 (0.2)
Retching	0	1 (0.7)	0	1 (0.2)
Stomatitis	0	0	1 (0.7)	1 (0.2)

System organ class Preferred term	Number of subjects (%) PB006 N=149	EU-Tysabri N=151	US-Tysabri N=150	Total N=450
Infections and infestations	**4 (2.7)**	**5 (3.3)**	**4 (2.7)**	**13 (2.9)**
Upper respiratory tract infection	1 (0.7)	3 (2.0)	1 (0.7)	5 (1.1)
Oral herpes	1 (0.7)	0	1 (0.7)	2 (0.4)
Bronchitis	1 (0.7)	0	0	1 (0.2)
Fungal infection	0	0	1 (0.7)	1 (0.2)
Gastroenteritis	1 (0.7)	0	0	1 (0.2)
Influenza	1 (0.7)	0	0	1 (0.2)
Nasal herpes	0	0	1 (0.7)	1 (0.2)
Nasopharyngitis	0	1 (0.7)	0	1 (0.2)
Urinary tract infection	0	1 (0.7)	0	1 (0.2)
Metabolism and nutrition disorders	**1 (0.7)**	**2 (1.3)**	**2 (1.3)**	**5 (1.1)**
Decreased appetite	1 (0.7)	2 (1.3)	2 (1.3)	5 (1.1)
Musculoskeletal and connective tissue disorders	**0**	**4 (2.6)**	**0**	**4 (0.9)**
Muscle spasms	0	1 (0.7)	0	1 (0.2)
Muscle tightness	0	1 (0.7)	0	1 (0.2)
Musculoskeletal stiffness	0	1 (0.7)	0	1 (0.2)
Myalgia	0	1 (0.7)	0	1 (0.2)
Respiratory, thoracic and mediastinal disorders	**2 (1.3)**	**1 (0.7)**	**1 (0.7)**	**4 (0.9)**
Oropharyngeal pain	2 (1.3)	1 (0.7)	0	3 (0.7)
Nasal congestion	0	0	1 (0.7)	1 (0.2)
Rhinorrhoea	0	0	1 (0.7)	1 (0.2)
Skin and subcutaneous tissue disorders	**0**	**1 (0.7)**	**3 (2.0)**	**4 (0.9)**
Pruritus	0	0	2 (1.3)	2 (0.4)
Erythema	0	0	1 (0.7)	1 (0.2)
Rash macular	0	0	1 (0.7)	1 (0.2)
Rash maculo-papular	0	0	1 (0.7)	1 (0.2)
Rash morbilliform	0	1 (0.7)	0	1 (0.2)
Injury, poisoning and procedural complications	**1 (0.7)**	**1 (0.7)**	**0**	**2 (0.4)**
Contusion	0	1 (0.7)	0	1 (0.2)
Infusion related reaction	1 (0.7)	0	0	1 (0.2)
Reproductive system and breast disorders	**1 (0.7)**	**0**	**0**	**1 (0.2)**
Dysmenorrhoea	1 (0.7)	0	0	1 (0.2)
Vascular disorders	**1 (0.7)**	**0**	**0**	**1 (0.2)**
Hot flush	1 (0.7)	0	0	1 (0.2)

N=number of subjects in group, n=number of subjects with event, PT=preferred term, SAF=safety population, SOC=system organ class, TEAE=treatment-emergent adverse event.
Sorted by descending frequency in the "total" column.

Most frequently reported were TEAEs in the SOCs general disorders and administration site conditions (19-21% across groups), and nervous system disorders (12-15% across groups).

On the PT level, most frequently reported were AEs related to the injection/infusion, with PTs of injection site reaction (16-19% across groups), infusion site reaction (1-3% across groups) and injection site induration (0-0.7% across groups). Other frequently reported AEs were headache (11-13% across groups) and nausea (3-5% across groups). All other events were reported in no more than 3 subjects per treatment group.

While on the level of individual PTs the frequencies may have slightly differed between the PB006, the EU-Tysabri and US-Tysabri groups, the AE profile of study drug-related TEAEs was overall considered to be similar between the 3 groups.

In the majority of subjects, TEAEs were mild. Mild TEAEs were reported in 65.6% of subjects, with similar frequencies across groups. Moderate TEAEs were reported in 6.0% of subjects, with similar frequencies across groups. Only 1 subject, treated with US-Tysabri, reported severe TEAEs, acute kidney injury and ureterolithiasis. Both were unlikely related to study drug, and the outcome was reported as recovered.

Study PB006-03-01

In this multiple-dose study in RRMS patients, AE frequencies were generally balanced across the 3 groups.

Table 48: Overall summary of AEs, by treatment sequence in study PB006-03-01 (SAF population)

	Number of patients (%)		
	PB006 300 mg N=131	**PB006 after switch from EU-Tysabri 300 mg N=30**	**EU-Tysabri 300 mg N=103**
Any TEAE	85 (64.9)	22 (73.3)	71 (68.9)
Any related TEAE	31 (23.7)	8 (26.7)	22 (21.4)
Any TEAE of grade 3 or higher*	4 (3.1)	0	1 (1.0)
Any treatment-emergent SAE	3 (2.3)	0	2 (1.9)
Any treatment-emergent related SAE	0	0	0
Any TEAE of special interest	6 (4.6)	2 (6.7)	6 (5.8)
Any TEAE leading to temporary study drug interruption	4 (3.1)	2 (6.7)	1 (1.0)
Any TEAE leading to permanent study drug discontinuation	8 (6.1)	1 (3.3)	3 (2.9)
Any TEAE leading to withdrawal from study**	0	0	0
Fatal TEAE	0	0	0

SAE=serious adverse event, SAF=safety population, PML= progressive multifocal leukoencephalopathy, TEAE=treatment-emergent adverse event.
*No grade 4 or 5 AEs were reported.
**A TEAE was considered to be leading to withdrawal from study only if the patient did not proceed to PML follow-up because of this event.

Overall, the percentages of patients with TEAEs and with TEAEs related to study drug were similar across groups. Approximately two-thirds of patients in each group reported any TEAE, while in approximately 25% of patients in each group, study drug-related TEAEs occurred. Few patients experienced TEAEs of Grade 3, AESIs, SAEs, or AEs leading to study drug discontinuation or withdrawal. The frequencies of TEAEs of Grade 3 and of TEAEs leading to discontinuations were numerically higher in the PB006 group than in the other groups. However, these differences were not considered to be clinically meaningful.

Table 49: Overall summary of AEs from Week 24 to 48 in study PB006-03-01 (SSW population)

	Number of patients (%)		
	PB006 continuing 300 mg N=131	**PB006 after switch from EU-Tysabri 300 mg N=30**	**EU-Tysabri continuing 300 mg N=103**
Any TEAE	55 (45.1)	15 (50.0)	42 (44.2)
Any related TEAE	6 (4.9)	3 (10.0)	7 (7.4)
Any TEAE of Grade 3 or higher*	2 (1.6)	0	1 (1.1)
Any treatment-emergent SAE	2 (1.6)	0	2 (2.1)
Any treatment-emergent related SAE	1 (0.8)	0	0
Any TEAE of special interest	1 (0.8)	1 (3.3)	2 (2.1)
Any TEAE leading to temporary study drug interruption	3 (2.5)	2 (6.7)	1 (1.1)
Any TEAE leading to permanent study drug discontinuation	2 (1.6)	0	0
Any TEAE leading to withdrawal from study**	0	0	0
Fatal TEAE	0	0	0

SAE=serious adverse event, SAF=safety population, PML= progressive multifocal leukoencephalopathy, TEAE=treatment-emergent adverse event.
*No Grade 4 or 5 AEs were reported.
**A TEAE was considered to be leading to withdrawal from study only if the patient did not proceed to PML follow-up because of this event.

TEAEs are presented for the overall treatment period of 48 weeks in 2 formats in the clinical study report, 1) by preceding treatment and 2) by treatment sequence.

In the tables by preceding treatment, the PB006 group includes AE data from the 131 patients who were initially randomized to PB006 plus data from the 30 patients who switched from the EU-Tysabri group to PB006 after 24 weeks (for the remaining 24-week treatment period); thus, the number of patients for this group is 161. In addition, AE data are presented for the 133 patients who were randomized to and treated with EU-Tysabri. In this presentation AEs occurring in patients after switching from EU-Tysabri to PB006 were counted for both treatment arms. In order to account for the varying exposure in the 2 groups, event rates per 100 patient years (PY) are included.

In the tables by treatment sequence, AE data are presented for the 131 patients who received only PB006 for the full treatment period, the 30 patients who switched from EU-Tysabri to PB006, and the 103 patients who received only EU-Tysabri for the full treatment period (i.e., not including the patients who switched to PB006 after 24 weeks).

In order to account for the varying exposure in the 3 treatment groups, event rates per 100 PY are included.

In the present report, the focus lies on the evaluation of events by treatment sequence.

Table 50: TEAEs by SOC and PT, by treatment sequence, in >1% of patients in any group in study PB006-03-01 (SAF population)

	PB006 300 mg N=131		**PB006 after switch from EU-Tysabri 300 mg N=30**		**EU-Tysabri 300 mg N=103**	
System organ class Preferred term	**Patients with event n (%)**	**Number of events /rate per 100 PY***	**Patients with event n (%)**	**Number of events /rate per 100 PY***	**Patients with event n (%)**	**Number of events /rate per 100 PY***
Any event	**85 (64.9)**	**221 / 192.34**	**22 (73.3)**	**60 / 219.62**	**71 (68.9)**	**176 / 194.65**
Blood and lymphatic system disorders	**5 (3.8)**	**5 / 4.35**	**0**	**0 / 0**	**4 (3.9)**	**4 / 4.42**
Anaemia	4 (3.1)	4 / 3.48	0	0 / 0	0	0 / 0
Ear and labyrinth disorders	**2 (1.5)**	**2 / 1.74**	**0**	**0 / 0**	**0**	**0 / 0**
Gastrointestinal disorders	**13 (9.9)**	**17 / 14.80**	**0**	**0 / 0**	**12 (11.7)**	**18 / 19.91**

System organ class Preferred term	PB006 300 mg N=131		PB006 after switch from EU-Tysabri 300 mg N=30		EU-Tysabri 300 mg N=103	
	Patients with event n (%)	**Number of events /rate per 100 PY***	**Patients with event n (%)**	**Number of events /rate per 100 PY***	**Patients with event n (%)**	**Number of events /rate per 100 PY***
Diarrhoea	3 (2.3)	3 / 2.61	0	0 / 0	5 (4.9)	5 / 5.53
Nausea	4 (3.1)	4 / 3.48	0	0 / 0	3 (2.9)	3 / 3.32
Constipation	2 (1.5)	2 / 1.74	0	0 / 0	3 (2.9)	4 / 4.42
Vomiting	0	0 / 0	0	0 / 0	2 (1.9)	2 / 2.21
General disorders and administration site conditions	**13 (9.9)**	**14 / 12.18**	**7 (23.3)**	**8 / 29.28**	**5 (4.9)**	**5 / 5.53**
Asthenia	5 (3.8)	5 / 4.35	1 (3.3)	1 / 3.66	1 (1.0)	1 / 1.11
Fatigue	5 (3.8)	5 / 4.35	0	0 / 0	1 (1.0)	1 / 1.11
Pyrexia	1 (0.8)	1 / 0.87	1 (3.3)	1 / 3.66	3 (2.9)	3 / 3.32
Hyperthermia	2 (1.5)	2 / 1.74	1 (3.3)	1 / 3.66	0	0 / 0
Feeling hot	0	0 / 0	2 (6.7)	2 / 7.32	0	0 / 0
Oedema peripheral	1 (0.8)	1 / 0.87	1 (3.3)	1 / 3.66	0	0 / 0
Discomfort	0	0 / 0	1 (3.3)	1 / 3.66	0	0 / 0
Infusion site pain	0	0 / 0	1 (3.3)	1 / 3.66	0	0 / 0
Hepatobiliary disorders	**2 (1.5)**	**2 / 1.74**	**1 (3.3)**	**1 / 3.66**	**1 (1.0)**	**1 / 1.11**
Hyperbilirubinaemia	1 (0.8)	1 / 0.87	1 (3.3)	1 / 3.66	0	0 / 0
Immune system disorders	**0**	**0 / 0**	**1 (3.3)**	**1 / 3.66**	**0**	**0 / 0**
Hypersensitivity	0	0 / 0	1 (3.3)	1 / 3.66	0	0 / 0
Infections and infestations	**39 (29.8)**	**54 / 47.00**	**15 (50.0)**	**19 / 69.55**	**34 (33.0)**	**44 / 48.66**
Nasopharyngitis	11 (8.4)	16 / 13.93	5 (16.7)	5 / 18.30	8 (7.8)	9 / 9.95
COVID-19	11 (8.4)	11 / 9.57	4 (13.3)	4 / 14.64	6 (5.8)	6 / 6.64
Upper respiratory tract infection	2 (1.5)	2 / 1.74	1 (3.3)	1 / 3.66	3 (2.9)	3 / 3.32
Pharyngitis	1 (0.8)	2 / 1.74	1 (3.3)	1 / 3.66	3 (2.9)	4 / 4.42
Pneumonia	3 (2.3)	3 / 2.61	0	0 / 0	1 (1.0)	1 / 1.11
Respiratory tract infection	2 (1.5)	2 / 1.74	1 (3.3)	1 / 3.66	1 (1.0)	1 / 1.11
Urinary tract infection	2 (1.5)	2 / 1.74	0	0 / 0	2 (1.9)	3 / 3.32
Bronchitis	0	0 / 0	1 (3.3)	1 / 3.66	2 (1.9)	3 / 3.32
Cystitis	2 (1.5)	2 / 1.74	0	0 / 0	1 (1.0)	1 / 1.11
Oral herpes	1 (0.8)	1 / 0.87	1 (3.3)	1 / 3.66	1 (1.0)	1 / 1.11
Rhinitis	1 (0.8)	1 / 0.87	1 (3.3)	1 / 3.66	1 (1.0)	1 / 1.11
Respiratory tract infection viral	1 (0.8)	1 / 0.87	1 (3.3)	1 / 3.66	0	0 / 0
COVID-19 pneumonia	0	0 / 0	1 (3.3)	1 / 3.66	0	0 / 0
Laryngitis	0	0 / 0	1 (3.3)	1 / 3.66	0	0 / 0
Pyoderma streptococcal	0	0 / 0	1 (3.3)	1 / 3.66	0	0 / 0
Injury, poisoning and procedural complications	**2 (1.5)**	**4 / 3.48**	**1 (3.3)**	**1 / 3.66**	**3 (2.9)**	**3 / 3.32**
Contusion	0	0 / 0	1 (3.3)	1 / 3.66	1 (1.0)	1 / 1.11
Investigations	**8 (6.1)**	**11 / 9.57**	**2 (6.7)**	**3 / 10.98**	**9 (8.7)**	**11 / 12.17**
Weight decreased	2 (1.5)	2 / 1.74	1 (3.3)	1 / 3.66	3 (2.9)	3 / 3.32
Alanine aminotransferase increased	2 (1.5)	2 / 1.74	0	0 / 0	1 (1.0)	2 / 2.21
Blood pressure increased	1 (0.8)	1 / 0.87	0	0 / 0	2 (1.9)	2 / 2.21
C-reactive protein increased	2 (1.5)	2 / 1.74	0	0 / 0	1 (1.0)	1 / 1.11
Gamma-glutamyltransferase increased	1 (0.8)	1 / 0.87	1 (3.3)	2 / 7.32	0	0 / 0
Metabolism and nutrition disorders	**1 (0.8)**	**1 / 0.87**	**1 (3.3)**	**1 / 3.66**	**2 (1.9)**	**3 / 3.32**
Hyperlipidaemia	0	0 / 0	1 (3.3)	1 / 3.66	0	0 / 0
Musculoskeletal and connective tissue disorders	**17 (13.0)**	**21 / 18.28**	**1 (3.3)**	**1 / 3.66**	**9 (8.7)**	**13 / 14.38**
Back pain	7 (5.3)	7 / 6.09	1 (3.3)	1 / 3.66	3 (2.9)	3 / 3.32
Pain in extremity	2 (1.5)	2 / 1.74	0	0 / 0	4 (3.9)	4 / 4.42
Muscle spasms	2 (1.5)	2 / 1.74	0	0 / 0	1 (1.0)	1 / 1.11
Myalgia	2 (1.5)	2 / 1.74	0	0 / 0	1 (1.0)	1 / 1.11
Neck pain	1 (0.8)	2 / 1.74	0	0 / 0	2 (1.9)	2 / 2.21
Arthralgia	2 (1.5)	2 / 1.74	0	0 / 0	0	0 / 0
Musculoskeletal stiffness	0	0 / 0	0	0 / 0	2 (1.9)	2 / 2.21
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	**2 (1.5)**	**2 / 1.74**	**0**	**0 / 0**	**0**	**0 / 0**
Nervous system disorders	**33 (25.2)**	**48 / 41.78**	**8 (26.7)**	**13 / 47.58**	**24 (23.3)**	**56 / 61.93**

System organ class Preferred term	PB006 300 mg N=131		PB006 after switch from EU-Tysabri 300 mg N=30		EU-Tysabri 300 mg N=103	
	Patients with event n (%)	Number of events /rate per 100 PY*	Patients with event n (%)	Number of events /rate per 100 PY*	Patients with event n (%)	Number of events /rate per 100 PY*
Headache	25 (19.1)	36 / 31.33	4 (13.3)	5 / 18.30	19 (18.4)	47 / 51.98
Dizziness	3 (2.3)	3 / 2.61	2 (6.7)	2 / 7.32	1 (1.0)	2 / 2.21
Hypoaesthesia	2 (1.5)	2 / 1.74	1 (3.3)	1 / 3.66	1 (1.0)	1 / 1.11
Paraesthesia	1 (0.8)	1 / 0.87	1 (3.3)	1 / 3.66	0	0 / 0
Presyncope	1 (0.8)	1 / 0.87	1 (3.3)	1 / 3.66	0	0 / 0
Tension headache	1 (0.8)	2 / 1.74	1 (3.3)	3 / 10.98	0	0 / 0
Psychiatric disorders	**7 (5.3)**	**10 / 8.70**	**4 (13.3)**	**4 / 14.64**	**2 (1.9)**	**2 / 2.21**
Depression	3 (2.3)	3 / 2.61	3 (10.0)	3 / 10.98	1 (1.0)	1 / 1.11
Insomnia	4 (3.1)	6 / 5.22	0	0 / 0	1 (1.0)	1 / 1.11
Sleep disorder	0	0 / 0	1 (3.3)	1 / 3.66	0	0 / 0
Renal and urinary disorders	**1 (0.8)**	**1 / 0.87**	**3 (10.0)**	**4 / 14.64**	**2 (1.9)**	**4 / 4.42**
Leukocyturia	0	0 / 0	1 (3.3)	1 / 3.66	1 (1.0)	1 / 1.11
Dysuria	0	0 / 0	1 (3.3)	1 / 3.66	0	0 / 0
Haematuria	0	0 / 0	1 (3.3)	1 / 3.66	0	0 / 0
Urinary retention	0	0 / 0	1 (3.3)	1 / 3.66	0	0 / 0
Reproductive system and breast disorders	**3 (2.3)**	**3 / 2.61**	**0**	**0 / 0**	**1 (1.0)**	**1 / 1.11**
Respiratory, thoracic and mediastinal disorders	**10 (7.6)**	**11 / 9.57**	**1 (3.3)**	**1 / 3.66**	**4 (3.9)**	**5 / 5.53**
Oropharyngeal pain	5 (3.8)	5 / 4.35	0	0 / 0	3 (2.9)	3 / 3.32
Rhinorrhoea	1 (0.8)	1 / 0.87	1 (3.3)	1 / 3.66	0	0 / 0
Skin and subcutaneous tissue disorders	**8 (6.1)**	**10 / 8.70**	**2 (6.7)**	**2 / 7.32**	**3 (2.9)**	**4 / 4.42**
Urticaria	2 (1.5)	2 / 1.74	0	0 / 0	1 (1.0)	1 / 1.11
Erythema	1 (0.8)	1 / 0.87	1 (3.3)	1 / 3.66	0	0 / 0
Hyperhidrosis	1 (0.8)	1 / 0.87	1 (3.3)	1 / 3.66	0	0 / 0
Pruritus	2 (1.5)	2 / 1.74	0	0 / 0	0	0 / 0
Vascular disorders	**4 (3.1)**	**4 / 3.48**	**1 (3.3)**	**1 / 3.66**	**0**	**0 / 0**
Hypotension	2 (1.5)	2 / 1.74	0	0 / 0	0	0 / 0
Hypertension	0	0 / 0	1 (3.3)	1 / 3.66	0	0 / 0

N=number of patients in group, PT=preferred term, PY=patient year, SAF=safety population, SOC=system organ class, TEAE=treatment-emergent adverse event.
Note: *Patient years calculated as the sum of (last day of follow-up – first day of exposure + 1)/365.25 for all patients in group. Adverse events were summarized according to the randomized study drug sequence.

With regard to AE data by treatment sequence, the percentage of patients with TEAEs was similar in the PB006, switch and the EU-Tysabri groups (64.9, 73.3 and 68.9%), with event rates per 100 PY of 192.34, 219.62 and 194.65, respectively.

Most frequently reported in all groups were TEAEs in the SOCs infections and infestations (29.8, 50.0 and 33.0% in PB006, switch and EU-Tysabri groups, respectively) and nervous system disorders (25.2, 26.7 and 23.3% in PB006, switch and EU-Tysabri groups, respectively). On the PT level, most frequently reported was headache (19.1, 13.3 and 18.4% in PB006, switch and EU-Tysabri groups, respectively), followed by nasopharyngitis (8.4, 16.7 and 7.8% in PB006, switch and EU-Tysabri groups, respectively) and COVID-19 (8.4, 13.3 and 5.8% in PB006, switch and EU-Tysabri groups, respectively). While on the level of individual PTs the frequencies slightly differed between the PB006 and EU-Tysabri groups, sometimes favoring Tysabri and sometimes favoring PB006, the AE profile was overall considered to be similar between the 2 groups. In the switch group, the AE frequencies tended to be higher compared to the other 2 groups. However, when interpreting the data, the limited group size needs to be considered.

A summary of TEAEs after the switch of a subset of patients from EU-Tysabri to PB006 (i.e., time period from Week 24 to 48) is provided for the SSW population. The frequencies of TEAEs were similar in the PB006 continuing, switch, and EU-Tysabri continuing groups (45.1, 50.0 and 44.2%, respectively). Comparing the AE data for patients switching from EU-Tysabri to PB006 versus patients continuing on EU-Tysabri no clinically meaningful differences were observed.

Adverse events related to study drug

Table 51: Study drug-related TEAEs by SOC and PT, by treatment sequence in study PB006-03-01 (SAF population)

System organ class Preferred term	PB006 300 mg N=131		PB006 after switch from EU-Tysabri 300 mg N=30		EU-Tysabri 300 mg N=103	
	Patients with event n (%)	Number of events /rate per 100 PY*	Patients with event n (%)	Number of events /rate per 100 PY*	Patients with event n (%)	Number of events /rate per 100 PY*
Any event	**31 (23.7)**	**53 / 46.13**	**8 (26.7)**	**15 / 54.90**	**22 (21.4)**	**51 / 56.40**
Blood and lymphatic system disorders	**1 (0.8)**	**1 / 0.87**	**0**	**0 / 0**	**2 (1.9)**	**2 / 2.21**
Lymphadenitis	0	0 / 0	0	0 / 0	1 (1.0)	1 / 1.11
Lymphopenia	1 (0.8)	1 / 0.87	0	0 / 0	0	0 / 0
Neutropenia	0	0 / 0	0	0 / 0	1 (1.0)	1 / 1.11
Eye disorders	**0**	**0 / 0**	**0**	**0 / 0**	**1 (1.0)**	**1 / 1.11**
Ocular discomfort	0	0 / 0	0	0 / 0	1 (1.0)	1 / 1.11
Gastrointestinal disorders	**1 (0.8)**	**1 / 0.87**	**0**	**0 / 0**	**4 (3.9)**	**5 / 5.53**
Nausea	1 (0.8)	1 / 0.87	0	0 / 0	2 (1.9)	2 / 2.21
Constipation	0	0 / 0	0	0 / 0	1 (1.0)	2 / 2.21
Vomiting	0	0 / 0	0	0 / 0	1 (1.0)	1 / 1.11
General disorders and administration site conditions	**6 (4.6)**	**6 / 5.22**	**4 (13.3)**	**4 / 14.64**	**3 (2.9)**	**3 / 3.32**
Hyperthermia	2 (1.5)	2 / 1.74	1 (3.3)	1 / 3.66	0	0 / 0
Pyrexia	1 (0.8)	1 / 0.87	0	0 / 0	2 (1.9)	2 / 2.21
Asthenia	2 (1.5)	2 / 1.74	0	0 / 0	0	0 / 0
Fatigue	1 (0.8)	1 / 0.87	0	0 / 0	1 (1.0)	1 / 1.11
Discomfort	0	0 / 0	1 (3.3)	1 / 3.66	0	0 / 0
Feeling hot	0	0 / 0	1 (3.3)	1 / 3.66	0	0 / 0
Infusion site pain	0	0 / 0	1 (3.3)	1 / 3.66	0	0 / 0
Hepatobiliary disorders	**1 (0.8)**	**1 / 0.87**	**1 (3.3)**	**1 / 3.66**	**0**	**0 / 0**
Hyperbilirubinaemia	1 (0.8)	1 / 0.87	1 (3.3)	1 / 3.66	0	0 / 0
Immune system disorders	**0**	**0 / 0**	**1 (3.3)**	**1 / 3.66**	**0**	**0 / 0**
Hypersensitivity	0	0 / 0	1 (3.3)	1 / 3.66	0	0 / 0
Infections and infestations	**6 (4.6)**	**7 / 6.09**	**3 (10.0)**	**3 / 10.98**	**9 (8.7)**	**10 / 11.06**
Cystitis	2 (1.5)	2 / 1.74	0	0 / 0	1 (1.0)	1 / 1.11
Oral herpes	1 (0.8)	1 / 0.87	1 (3.3)	1 / 3.66	1 (1.0)	1 / 1.11
Pharyngitis	1 (0.8)	1 / 0.87	0	0 / 0	2 (1.9)	2 / 2.21
Herpes simplex	1 (0.8)	1 / 0.87	0	0 / 0	1 (1.0)	1 / 1.11
Bronchitis	0	0 / 0	1 (3.3)	1 / 3.66	0	0 / 0
Ear infection	1 (0.8)	1 / 0.87	0	0 / 0	0	0 / 0
Furuncle	0	0 / 0	0	0 / 0	1 (1.0)	1 / 1.11
Laryngitis	0	0 / 0	1 (3.3)	1 / 3.66	0	0 / 0
Sinusitis	0	0 / 0	0	0 / 0	1 (1.0)	1 / 1.11
Tinea versicolour	1 (0.8)	1 / 0.87	0	0 / 0	0	0 / 0
Urinary tract infection	0	0 / 0	0	0 / 0	1 (1.0)	1 / 1.11
Urinary tract infection enterococcal	0	0 / 0	0	0 / 0	1 (1.0)	1 / 1.11
Vulvovaginal candidiasis	0	0 / 0	0	0 / 0	1 (1.0)	1 / 1.11
Injury, poisoning and procedural complications	**0**	**0 / 0**	**1 (3.3)**	**1 / 3.66**	**0**	**0 / 0**
Contusion	0	0 / 0	1 (3.3)	1 / 3.66	0	0 / 0
Investigations	**4 (3.1)**	**7 / 6.09**	**1 (3.3)**	**2 / 7.32**	**3 (2.9)**	**4 / 4.42**
Gamma-glutamyltransferase increased	1 (0.8)	1 / 0.87	1 (3.3)	2 / 7.32	0	0 / 0
Weight decreased	1 (0.8)	1 / 0.87	0	0 / 0	1 (1.0)	1 / 1.11
Alanine aminotransferase increased	1 (0.8)	1 / 0.87	0	0 / 0	0	0 / 0
Aspartate aminotransferase increased	0	0 / 0	0	0 / 0	1 (1.0)	1 / 1.11
Bilirubin conjugated increased	1 (0.8)	1 / 0.87	0	0 / 0	0	0 / 0
Blood triglycerides increased	1 (0.8)	2 / 1.74	0	0 / 0	0	0 / 0
C-reactive protein increased	1 (0.8)	1 / 0.87	0	0 / 0	0	0 / 0
Lymphocyte count increased	0	0 / 0	0	0 / 0	1 (1.0)	1 / 1.11
White blood cell count increased	0	0 / 0	0	0 / 0	1 (1.0)	1 / 1.11

System organ class Preferred term	PB006 300 mg N=131		PB006 after switch from EU-Tysabri 300 mg N=30		EU-Tysabri 300 mg N=103	
	Patients with event n (%)	**Number of events /rate per 100 PY***	**Patients with event n (%)**	**Number of events /rate per 100 PY***	**Patients with event n (%)**	**Number of events /rate per 100 PY***
Musculoskeletal and connective tissue disorders	**4 (3.1)**	**4 / 3.48**	**0**	**0 / 0**	**2 (1.9)**	**3 / 3.32**
Muscle spasms	2 (1.5)	2 / 1.74	0	0 / 0	1 (1.0)	1 / 1.11
Myalgia	1 (0.8)	1 / 0.87	0	0 / 0	1 (1.0)	1 / 1.11
Arthralgia	1 (0.8)	1 / 0.87	0	0 / 0	0	0 / 0
Back pain	0	0 / 0	0	0 / 0	1 (1.0)	1 / 1.11
Nervous system disorders	**10 (7.6)**	**13 / 11.31**	**1 (3.3)**	**1 / 3.66**	**2 (1.9)**	**18 / 19.91**
Headache	7 (5.3)	9 / 7.83	0	0 / 0	2 (1.9)	16 / 17.70
Dizziness	2 (1.5)	2 / 1.74	1 (3.3)	1 / 3.66	1 (1.0)	2 / 2.21
Dysgeusia	1 (0.8)	1 / 0.87	0	0 / 0	0	0 / 0
Trigeminal neuralgia	1 (0.8)	1 / 0.87	0	0 / 0	0	0 / 0
Psychiatric disorders	**2 (1.5)**	**2 / 1.74**	**0**	**0 / 0**	**1 (1.0)**	**1 / 1.11**
Insomnia	2 (1.5)	2 / 1.74	0	0 / 0	1 (1.0)	1 / 1.11
Renal and urinary disorders	**0**	**0 / 0**	**0**	**0 / 0**	**1 (1.0)**	**1 / 1.11**
Leukocyturia	0	0 / 0	0	0 / 0	1 (1.0)	1 / 1.11
Reproductive system and breast disorders	**1 (0.8)**	**1 / 0.87**	**0**	**0 / 0**	**0**	**0 / 0**
Menorrhagia	1 (0.8)	1 / 0.87	0	0 / 0	0	0 / 0
Skin and subcutaneous tissue disorders	**6 (4.6)**	**7 / 6.09**	**2 (6.7)**	**2 / 7.32**	**2 (1.9)**	**3 / 3.32**
Urticaria	2 (1.5)	2 / 1.74	0	0 / 0	1 (1.0)	1 / 1.11
Erythema	1 (0.8)	1 / 0.87	1 (3.3)	1 / 3.66	0	0 / 0
Hyperhidrosis	1 (0.8)	1 / 0.87	1 (3.3)	1 / 3.66	0	0 / 0
Pruritus	2 (1.5)	2 / 1.74	0	0 / 0	0	0 / 0
Alopecia	1 (0.8)	1 / 0.87	0	0 / 0	0	0 / 0
Angioedema	0	0 / 0	0	0 / 0	1 (1.0)	1 / 1.11
Rash	0	0 / 0	0	0 / 0	1 (1.0)	1 / 1.11
Vascular disorders	**3 (2.3)**	**3 / 2.61**	**0**	**0 / 0**	**0**	**0 / 0**
Hypotension	2 (1.5)	2 / 1.74	0	0 / 0	0	0 / 0
Blood pressure fluctuation	1 (0.8)	1 / 0.87	0	0 / 0	0	0 / 0

N=number of patients in group, PT=preferred term, PY=patient year, SAF=safety population, SOC=system organ class, TEAE=treatment-emergent adverse event.
Note: *Patient years calculated as the sum of (last day of follow-up – first day of exposure + 1)/365.25 for all patients in group. Adverse events were summarized according to the randomized study drug sequence.

With regard to AE data by treatment sequence, the percentage of patients with study drug-related TEAEs was similar in the PB006, switch and the EU-Tysabri group (23.7, 26.7 and 21.4%), with event rates per 100 PY of 46.13, 54.90 and 56.40, respectively.

Most frequently reported in both groups were study drug-related TEAEs in the SOCs infections and infestations (4.6, 10.0 and 8.7% in PB006, switch and EU-Tysabri groups, respectively), nervous system disorders (7.6, 3.3 and 1.9% in PB006, switch and EU-Tysabri groups, respectively) and general disorders and administration site conditions (4.6, 13.3 and 2.9% in PB006, switch and EU-Tysabri groups, respectively). On the PT level, most frequently reported was headache (5.3, 0 and 1.9% in PB006, switch and EU-Tysabri groups, respectively), followed by dizziness (1.5, 3.3 and 1.0% in PB006, switch and EU-Tysabri groups, respectively). While on the level of individual PTs the frequencies slightly differed between groups, the AE profile was overall considered to be similar between the 3 groups.

With regard to data after the switch (week 24 to 48), the frequencies of study drug-related TEAEs were similar in the PB006 continuing, switch group and EU-Tysabri continuing groups (4.9, 10.0 and 7.4%, respectively). The total number of events in the 3 groups was 6, 3 and 7, respectively. Comparing the AE profile for patients switching from EU-Tysabri to PB006 versus patients continuing on EU-Tysabri no clinically meaningful differences were observed.

Treatment-emergent adverse events by severity

No TEAEs of CTCAE grade 4 or 5 were reported. The majority of TEAEs was of CTCAE grade 1 or 2. TEAEs of Grade 3 are summarized by SOC and PT, by treatment sequence in table below.

Table 52: TEAEs of CTCAE grade 3, by treatment sequence in study PB006-03-01 (SAF population)

	PB006 N=131		**PB006 after switch from EU-Tysabri N=30**		**EU-Tysabri N=103**	
System organ class Preferred term	**Patients with event n (%)**	**Number of events /rate per 100 PY***	**Patients with event n (%)**	**Number of events /rate per 100 PY***	**Patients with event n (%)**	**Number of events /rate per 100 PY***
Any event	**4 (3.1)**	**4 / 3.48**	**0**	**0 / 0**	**1 (1.0)**	**1 / 1.11**
Investigations	**2 (1.5)**	**2 / 1.74**	**0**	**0 / 0**	**0**	**0 / 0**
Alanine aminotransferase increased	1 (0.8)	1 / 0.87	0	0 / 0	0	0 / 0
Blood triglycerides increased	1 (0.8)	1 / 0.87	0	0 / 0	0	0 / 0
Musculoskeletal and connective tissue disorders	**0**	**0 / 0**	**0**	**0 / 0**	**1 (1.0)**	**1 / 1.11**
Pain in extremity	0	0 / 0	0	0 / 0	1 (1.0)	1 / 1.11
Respiratory, thoracic and mediastinal disorders	**1 (0.8)**	**1 / 0.87**	**0**	**0 / 0**	**0**	**0 / 0**
Nasal septum deviation	1 (0.8)	1 / 0.87	0	0 / 0	0	0 / 0
Skin and subcutaneous tissue disorders	**1 (0.8)**	**1 / 0.87**	**0**	**0 / 0**	**0**	**0 / 0**
Urticaria	1 (0.8)	1 / 0.87	0	0 / 0	0	0 / 0

N=number of patients in group, PT=preferred term, PY=patient year, SAF=safety population, SOC=system organ class, TEAE=treatment-emergent adverse event.
Note: *Patient years calculated as the sum of (last day of follow-up – first day of exposure + 1)/365.25 for all patients in group. Adverse events were summarized according to the randomized study drug sequence.

Treatment-emergent adverse events of CTCAE Grade 3 occurred in few patients (4 patients treated with PB006, 1 patient treated with EU-Tysabri, and no patients in the switch group).

With regard to events in PB006 group, the events alanine aminotransferase (ALT) increased and a nasal septum deviation were both assessed as not related and recovered or recovering. Nasal septum deviation was serious. The event of blood triglycerides increased was assessed as probably related, with the outcome being recovered. The event of urticaria was assessed as probably related, with the outcome being recovered. Study drug was discontinued due to this AE.

2.6.8.3. Serious adverse event/deaths/other significant events

Study PB006-01-03

There were no fatal AEs in this study. SAEs were reported for 2 subjects and were unlikely related to study drug (based on investigator assessment) and were resolved within 1 or 2 days.

Study PB006-03-01

There were no fatal AEs in this study. SAEs were reported for 5 subjects.

All SAEs except for an event of hypotension were not or unlikely related to study drug, based on investigator assessment. Hypotension, experienced by one patient in the PB006 group, was assessed as possibly related to study drug, and the patient discontinued due to this SAE. A female (30-50 years of age) patient had an ongoing medical history of essential hypertension. She received a total of 12 infusions of PB006. During the Week 44 infusion, the patient experienced the SAE of moderate hypotension. (blood pressure decreased to below 70/50 mmHg). After medication, the patient's blood pressure normalized within few minutes, and the event was considered resolved on the same day. The investigator could not rule out infusion-related reaction and considered this event to be an important medical event. The study drug was permanently discontinued the same day, and the patient was

withdrawn from the study on one month later. A causal relationship of the SAE of hypotension to the study drug (PB006) was recorded as possibly related.

Other significant adverse events

Treatment-emergent adverse events leading to discontinuation occurred in 8 patients in the PB006 group and 4 patients in the EU-Tysabri group, and are presented for the entire treatment period in the following table.

Table 53: TEAEs leading to discontinuation in PB006-03-01

Treatment group: PB006		
Preferred term	Relationship to study drug	Outcome
Asthenia	Probably related	Recovered/ resolved
Hyperhidrosis	Probably related	Recovered/ resolved
Blood pressure fluctuation	Probably related	Recovered/ resolved
Dizziness	Probably related	Recovered/ resolved
Ear infection	Possibly related	Recovered/ resolved
Herpes simplex	Possibly related	Recovered/ resolved
Trigeminal neuralgia	Possibly related	Recovered/ resolved
COVID-19	Not related	Recovered/ resolved
Pruritus	Possibly related	Recovered/ resolved
Pruritus#	Possibly related	Recovered/ resolved
Urticaria#	Probably related	Recovered/ resolved
Hypotension	Possibly related	Recovered/ resolved
Urticaria#	Possibly related	Recovered/ resolved
Treatment group: EU-Tysabri		
Urinary tract infection enterococcal	Probably related	Recovered/ resolved
Hypersensitivity#	Related	Recovered/ resolved
Pharyngitis	Possibly related	Recovered/ resolved
Urticaria#	Possibly related	Recovered/ resolved
Angioedema	Possibly related	Recovered/ resolved

#Events occurred on the day of study drug administration.

Except for 1 patient in the PB006 group (who discontinued due to COVID-19), all TEAEs leading to discontinuation were at least possibly related to study drug. Most common TEAEs leading to discontinuation were pruritus and urticaria, which are known and common adverse drug reactions of natalizumab.

Adverse events of special interest

AESIs were defined in the study protocol based information from the Tysabri label, and included PML, JCV granule cell neuronopathy, opportunistic infections, liver injury, hypersensitivity, encephalitis, meningitis, and ARN. AESIs are provided by treatment sequence in the following table.

Table 54: Treatment-emergent AESIs by SOC and PT, by treatment sequence in study PB006-03-01 (SAF population)

	PB006 300 mg N=131		**PB006 after switch from EU-Tysabri 300 mg N=30**		**EU-Tysabri 300 mg N=103**	
System organ class Preferred term	**Patients with event n (%)**	**Number of events /rate per 100 PY***	**Patients with event n (%)**	**Number of events /rate per 100 PY***	**Patients with event n (%)**	**Number of events /rate per 100 PY***
Any event	**6 (4.6)**	**6 / 5.22**	**2 (6.7)**	**2 / 7.32**	**6 (5.8)**	**8 / 8.85**
Immune system disorders	**0**	**0 / 0**	**1 (3.3)**	**1 / 3.66**	**0**	**0 / 0**
Hypersensitivity	0	0 / 0	1 (3.3)	1 / 3.66	0	0 / 0
Infections and infestations	**2 (1.5)**	**2 / 1.74**	**1 (3.3)**	**1 / 3.66**	**5 (4.9)**	**6 / 6.64**
Oral herpes	1 (0.8)	1 / 0.87	1 (3.3)	1 / 3.66	1 (1.0)	1 / 1.11
Herpes simplex	1 (0.8)	1 / 0.87	0	0 / 0	1 (1.0)	1 / 1.11
Herpes zoster	0	0 / 0	0	0 / 0	1 (1.0)	1 / 1.11
Pharyngitis	0	0 / 0	0	0 / 0	1 (1.0)	1 / 1.11
Urinary tract infection	0	0 / 0	0	0 / 0	1 (1.0)	1 / 1.11
Urinary tract infection enterococcal	0	0 / 0	0	0 / 0	1 (1.0)	1 / 1.11
Investigations	**1 (0.8)**	**1 / 0.87**	**0**	**0 / 0**	**0**	**0 / 0**
Alanine aminotransferase increased	1 (0.8)	1 / 0.87	0	0 / 0	0	0 / 0
Skin and subcutaneous tissue disorders	**3 (2.3)**	**3 / 2.61**	**0**	**0 / 0**	**1 (1.0)**	**2 / 2.21**
Urticaria	2 (1.5)	2 / 1.74	0	0 / 0	1 (1.0)	1 / 1.11
Angioedema	0	0 / 0	0	0 / 0	1 (1.0)	1 / 1.11
Pruritus	1 (0.8)	1 / 0.87	0	0 / 0	0	0 / 0

AESI=adverse event of special interest, N=number of patients in group, PT=preferred term, PY=patient year, SAF=safety population, SOC=system organ class, TEAE=treatment-emergent adverse event.
Note: *Patient years calculated as the sum of (last day of follow-up – first day of exposure + 1)/365.25 for all patients in group. Adverse events were summarized according to the randomized study drug sequence.

Most commonly reported were events of herpes (with PTs oral herpes, herpes simplex, herpes zoster) and urticaria. The profile of AESIs was considered to be similar for PB006 and EU-Tysabri.

Anti-JCV antibody status and PML risk evaluation

Throughout clinical development of PB006, the STRATIFY JCV DxSelect assay was used by Polpharma Biologics for screening of study subjects and as risk minimisation measures during the biosimilar clinical studies. In parallel, a new anti-JCV IgG assay was developed (ImmunoWELL JCV IgG assay) for use in clinical practice. Analytical performance of the ImmunoWELL JCV IgG test was validated for its precision, selectivity (interference and cross-reactivity), a potential hook-effect, and robustness (including sample and kit stability). Effects of plasma or serum sample matrix were also evaluated. Provided results confirmed the suitability of the assay for its intended use and the test has CE marking for the qualitative detection of antibodies to JC Virus in human serum or plasma.

Samples collected during the PB006 biosimilar clinical studies PB006-03-01 and PB006-01-03 were used for clinical validation of the ImmunoWELL JCV IgG assay against the STRATIFY JCV DxSelect assay by comparison of the STRATIFY JCV DxSelect test results with ImmunoWELL JCV IgG test results from matching samples (taken at the same time during the clinical studies).

A detailed clinical validation report has been provided. Further information on the design and validation of the ImmunoWELL JCV IgG assay is included in the instructions for use provided with the test kits.

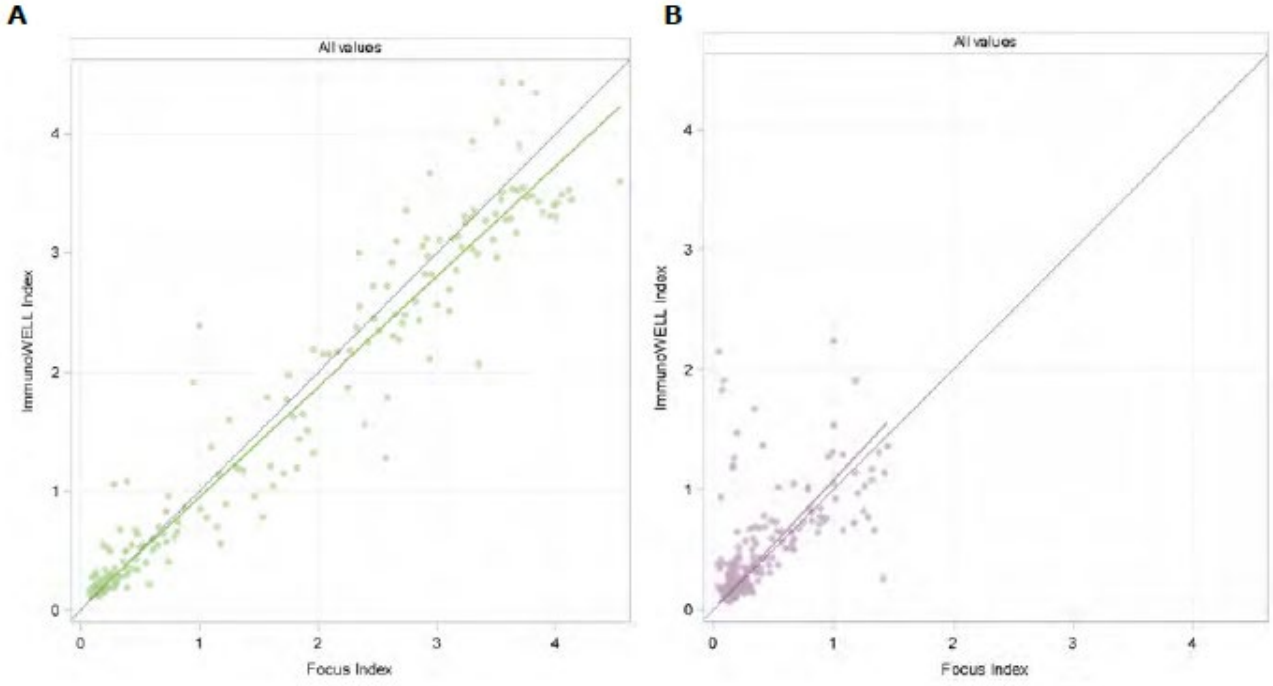
Similar to the STRATIFY JCV DxSelect assay, upper and lower cut-offs were defined for the equivocal zone in a separate screening assay to balance sensitivity and specificity of the ImmunoWELL JCV IgG Test. This resulted in an equivocal zone ranging from 0.25 to 0.50 for the screening assay, similar to the cut-off values for the STRATIFY JCV DXSelect assay (0.20 to 0.40).

Consecutively, a total of 397 unique samples were included in the final clinical performance evaluation. There were 200 healthy subjects evaluated with both the STRATIFY JCV DxSelect and the ImmunoWELL JCV IgG Test. In addition, 197 (RR)MS patients were evaluated with both the current standard assay and the ImmunoWELL JCV IgG Test.

Samples from healthy subjects covered the entire range of index values of the STRATIFY JCV DXSelect test (0 to >4). However, due to the PML risk in MS patients, the index range for patient samples taken during clinical study PB006-03-01 only extended up to 1.5. This condition had previously been agreed with national scientific advice procedures. Despite the restricted index range for patient samples, the previously defined cut-off (0.5) falls well within the range of samples.

The correlation of index values from both assays is shown in the following figure, separately for healthy subjects (A) and MS patients (B).

Figure 7: Correlation of index values from both assays for healthy volunteers (A) and R(R)MS patients



parameter	two-sided 95% confidence intervals from regression analysis					
	A: healthy subjects (full index range)			B: MS patients (index 0 – 1.5)		
	value	lower	upper	value	lower	upper
intercept (target: 0)	0.02	-0.00	0.05	0.01	-0.03	0.04
slope (target: 1)	0.93	0.90	0.96	1.07	0.96	1.26

Note: samples from healthy subjects (panel A) were obtained from study PB006-01-03, samples from patients with relapsing remitting multiple sclerosis (RRMS, panel B) from study PB006-03-01

Further, clinical performance of the ImmunoWELL JCV IgG Test was compared to the STRATIFY JCV DxSelect test in terms of assay sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). The results are summarised in the following table.

Table 55: Assay sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV)

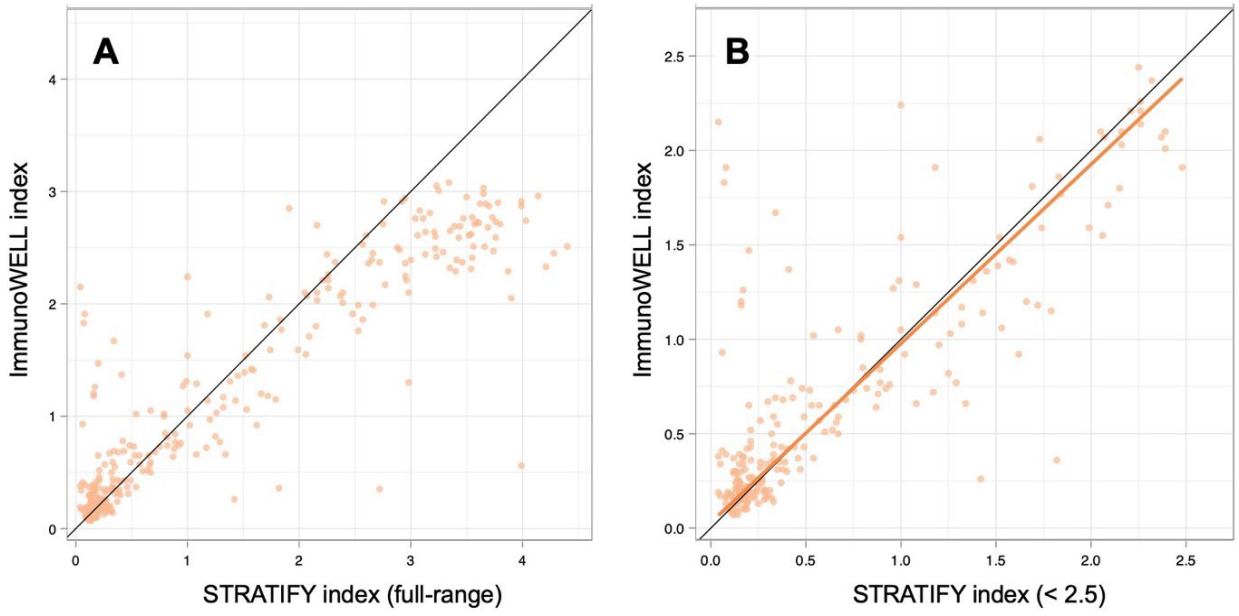
Population	Sensitivity	Specificity	PPV	NPV
Multiple Sclerosis Patients	92% (83-97%)	74% (65-81%)	67% (57-76%)	94% (87-98%)
Healthy Subjects	97% (92-99%)	73% (61-83%)	88% (81-98%)	92% (82-98%)

High concordance was found for assay sensitivity and NPV, whereas PPV had only 67% performance in MS patients. As the assay is supposed to reliably detect anti-JVC antibodies in patients, the values for sensitivity and NPV are the most critical ones, whereas a low PPV does not raise a concern for missing JVC infections in patients. However, in clinical practice the above-described cut-off values for determination of positive/negative results are of relatively minor importance. Rather, risk stratification is currently based on index values between 0.9 and 1.5 in clinical practice. Therefore, the applicant presented further comparative test data from the healthy subject population across the whole index range, and based on a regression analysis it was implied that the ImmunoWELL JCV IgG test might slightly underestimate the index estimated from the STRATIFY JCV DxSelect test (see also Discussion on clinical safety).

Moreover, the applicant provided an analysis with the focus on values in and around the range of 0.9-1.5 using additional data from the analysis of MS patient samples with higher JCV index values.

Data from the combined MS patient data set was used for correlation analysis and a high degree of correlation between the test results was observed at low (<0.9), intermediate (0.9 - 1.5) and high index values (1.5 - 2.5). Similar to previous observations from healthy subject samples, the correlation decreases at very high index values above approx. 2.5 (due to saturation effects/non-linearity of the qualitative ELISA test). This difference is regarded clinically not meaningful as all patients with high index values are in the "high PML risk" category, without further sub-stratification by index value. For regression analysis, the range <2.5 was therefore selected, showing a high degree of correlation in terms of slope and intercept in the clinically relevant index range.

Figure 8: Correlation of index values from ImmunoWELL JCV IgG vs. STRATIFY JCV DxSelect using combined dataset from MS patient samples (PB006-03-01)



parameter	**two-sided 95% confidence intervals from regression analysis**

	value	lower	upper
intercept (target: 0)	0.03	0.00	0.06
slope (target: 1)	0.95	0.89	1.00

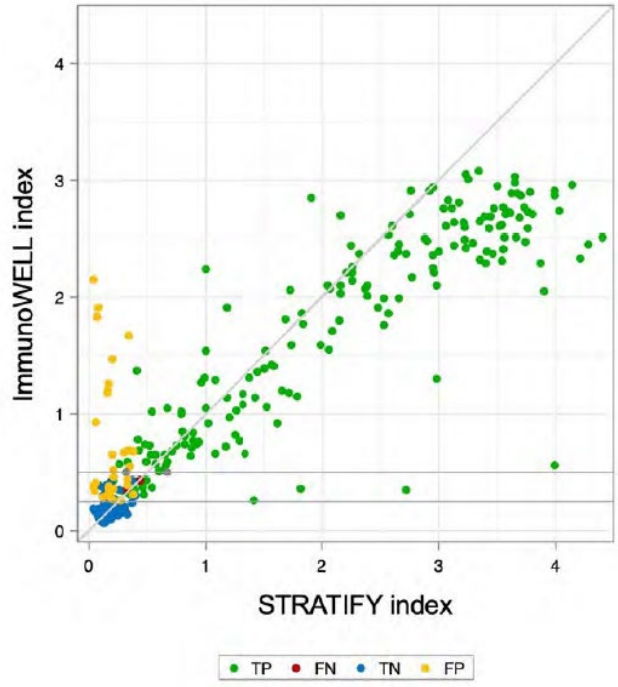
At the relevant threshold values of 0.9 and 1.5, the estimated ImmunoWELL JCV IgG test values for MS patients are 0.882 (90% confidence interval: 0.849 - 0.921) and 1.451 (90% confidence interval: 1.388 - 1.517). The relative differences are 2.0% and 3.3.%, respectively. If a minor offset exists between the two assays, it is expected to be below 0.1 index values.

Table 56: Performance characteristics of ImmunoWELL JCV IgG (combined data set)

MS patient population				
STRATIFY JCV DxSelect value	**ImmunoWELL JVC IgG**			
	estimate	**90% CI**	**abs. difference**	**rel. difference**
0.9	0.882	0.849 - 0.921	-0.01775	-2.0%
1.5	1.451	1.388 - 1.517	-0.04916	-3.3%
2	1.925	1.839 - 2.015	-0.07534	-3.8%
2.5	2.398	2.287 – 2.510	-0.10152	-4.1%

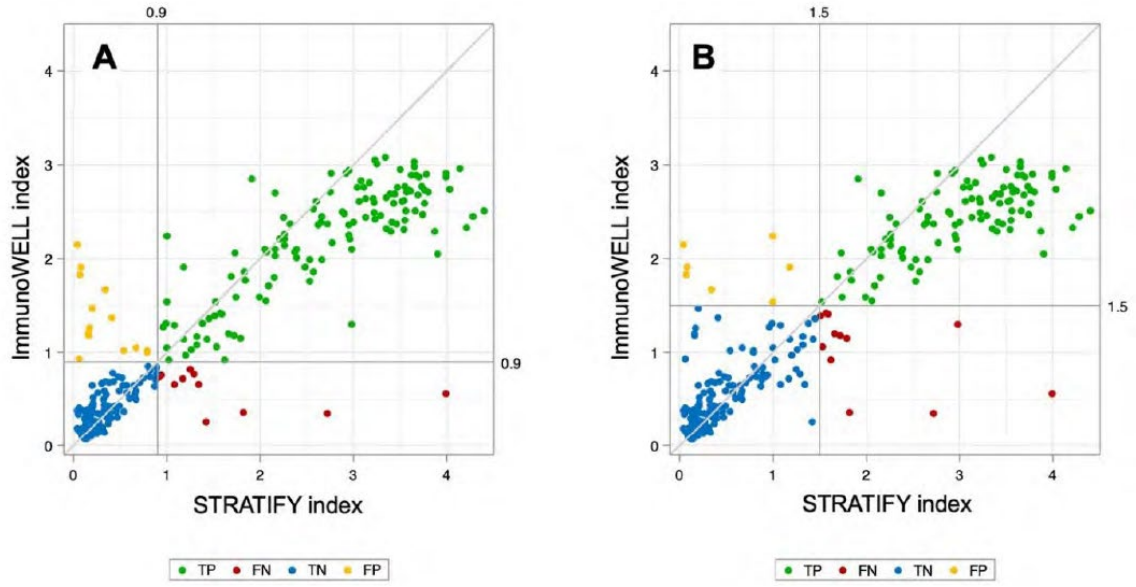
Sub-range analyses with the focus on values in and around the range of 0.9-1.5 were performed to determine sensitivity, specificity, NPV and PPV for the qualitative test results (positive/negative). The combined total data set from MS patient samples described above is shown as an example in the following figure covering the entire index value range with 'true' positives (TP), 'false' positives (FP), 'true' negatives (TN) and 'false' negatives (FN) highlighted by colour.

Figure 9: True and false positives/negatives in combined total MS patient data set



The applicant also assessed the binary agreement between the STRATIFY JCV DxSelect and the ImmunoWELL JCV IgG results at the threshold values of 0.9 and 1.5. Sensitivity in this analysis describes the percent of ImmunoWELL JCV IgG test index values above the threshold within those where STRATIFY JCV DxSelect index values are above the threshold, whereas specificity describes the percent of ImmunoWELL JCV IgG test index values below the threshold within those where STRATFIY index values are below the threshold. For the combined MS patient data set at threshold index values, the results are visualized in the following figure, using the same color coding for 'true' positives, 'true' negatives, 'false' positives and 'false' negatives as above.

Figure 10: Binary agreement at the threshold values of 0.9 (A) and 1.5 (B) in combined MS patient data set



TN/TP = True negative/positive
FN/FP = False negative/positive

Furthermore, the binary agreements are presented using threshold values of 0.8 and 1.4 for the ImmunoWELL JCV IgG test and threshold values of 0.9 and 1.5 for the STRATFIY JCV DxSelect test. Separate tables are shown for the healthy subject population from study PB006-01-03 and for the combined MS patient data set from study PB006-03-01 covering the index range above 1.5 as previously described.

*Table 57: Binary agreement at the threshold values of 0.8/0.9 and 1.4/1.5**

MS patient population (combined dataset)								
	threshold 0.8/0.9*				**threshold 1.4/1.5***			
	STRATIFY JCV Dx Select				**STRATIFY JCV Dx Select**			
ImmunoWELL JCV IgG	Positive		Negative		Positive		Negative	
	N	%	N	%	N	%	N	%
Positive	130	92.86	17	9.83	106	91.38	8	4.06
Negative	10	7.14	156	90.17	10	8.62	189	95.94

Sensitivity	92.9%	91.4%
Specificity	90.2%	95.9%
PPV	88.4%	93.0%
NPV	94.0%	95.0%

Healthy subject population								
	threshold 0.8/0.9*				**threshold 1.4/1.5***			
	STRATIFY JCV Dx Select				**STRATIFY JCV Dx Select**			
ImmunoWELL JCV IgG	Positive		Negative		Positive		Negative	
	N	%	N	%	N	%	N	%
Positive	102	96.23	5	5.62	85	92.39	3	2.91
Negative	4	3.77	84	94.38	7	7.61	100	97.09

Sensitivity	96.2%	92.4%
Specificity	94.4%	97.1%
PPV	95.3%	96.6%

NPV	95.5%	93.5%

* 0.8 and 1.4 threshold values correspond to ImmunoWELL JCV IgG; 0.9 and 1.5 threshold values refer to STRATIFY JCV DxSelect
PPV / NPV = positive / negative predictive values

For both, the combined MS patient populations and the healthy subject population, high sensitivities were observed at the threshold values of 0.9 (92.1% and 94.3%) and 1.5 (89.7% and 91.3%), corresponding to the low numbers of false negatives. Also, the specificity values in this analysis indicate strong agreement with results of 91.9% and above. The revised threshold values of 0.8 and 1.4 generally lead to a minor improvement in sensitivity for the ImmunoWELL JCV IgG test (e.g. from 92.1% to 92.9% at the lower threshold and from 89.7% to 91.4% at the upper threshold for MS patients) and a concomitant minor reduction in specificity (see Discussion on Clinical Safety).

All clinical studies included for safety reasons a PML follow-up visit 6 months after (last) dosing with study drug, to assess new neurological symptoms which could be suggestive of PML. Of note, for the healthy subject studies this visit was not mandatory, and in the time period between the end of study visit and the PML follow-up visit the subjects were not considered as study subjects.

Study PB006-01-02

Screening for anti-JCV antibodies was only performed at screening, and subjects who were JCV-positive were not enrolled in the study. Of the 68 subjects who were screened, 39 were JCV-positive and therefore not eligible for inclusion in the study.

No PML cases were reported in the study and no new neurological symptoms, which could be suggestive for PML were observed on the additional follow-up visit on Day 169 (±1 week) after administration of the study drug.

Study PB006-01-03

Subjects who were JCV-positive were not enrolled in the study. All subjects who were randomized were tested negative for anti-JCV antibodies at Screening 1. On Day 85, 19 subjects tested positive for anti-JCV antibodies, 9 subjects after PB006, 4 subjects after EU-Tysabri, and 6 subjects after US-Tysabri.

No PML cases were reported in the study and no new neurological symptoms that could be suggestive for PML were observed for the subjects who returned for the additional follow-up visit on Day 169.

Study PB006-03-01

While at baseline, no patients presented with a JCV index >1.5, the percentage of patients who were JCV-positive with index >1.5 increased during the treatment period in both groups. An index >1.5 was reported for 4.9 and 4.5% of patients in the PB006 and EU-Tysabri groups, respectively, at Week 24 and for 6.0 and 5.9% of patients in the PB006 and EU-Tysabri groups, respectively, at Week 48.

No cases of PML occurred in this study. For 5 patients, PML was suspected based on MRI findings. PML was not confirmed in subsequent clinical evaluations in any of these patients.

Additionally, investigators were to monitor all patients who had received at least one dose of study drug (including prematurely withdrawn patients) for PML for approximately 6 months after discontinuing natalizumab. Of the 265 patients randomized, 253 subjects completed the PML follow-up visit. No subjects had any signs suggestive of PML at the follow-up visit.

2.6.8.4. Laboratory findings

Natalizumab binds to the α4 subunit of the α4β1-integrin that is highly expressed on the surface of all leukocytes, with the exception of neutrophils.

Study PB006-01-03

In study PB006-01-03, increases from baseline in mean numbers of circulating leukocytes, lymphocytes, eosinophils and monocytes were reported, which were similar in all 3 treatment groups. These increases are consistent with expression of α4β1 on these white-cell subgroups and are a known PD effect of natalizumab [Polman et al. 2006].

Mean numbers of leukocytes, lymphocytes, eosinophils and monocytes at Day 36 and Day 85 (end of study) are summarized in the following table.

Table 58: Mean numbers of leukocytes, lymphocytes, eosinophils and monocytes in study PB006-01-03 (SAF population)

	PB006 N=149	**EU-Tysabri N=151**	**US-Tysabri N=150**
Leukocytes (x 10^9/L)			
Baseline	6.13	6.01	6.16
Day 36	6.97	6.99	7.10
Day 85	5.50	5.60	5.68
Lymphocytes (x 10^9/L)			
Baseline	1.89	1.92	1.91
Day 36	2.89	2.85	2.97
Day 85	1.77	1.79	1.83
Eosinophils (x 10^9/L)			
Baseline	0.16	0.15	0.16
Day 36	0.24	0.23	0.24
Day 85	0.14	0.16	0.16
Monocytes (x 10^9/L)			
Baseline	0.44	0.43	0.45
Day 36	0.53	0.52	0.54
Day 85	0.42	0.41	0.42

For all these parameters the increases were transient, with values returning to or below baseline levels at Day 85. Changes from baseline in neutrophils were small.

In addition, small mean decreases from baseline were observed for haematocrit, erythrocytes, hemoglobin and platelets.

The majority of subjects had at least 1 out-of-range clinical laboratory value. Most of these were minor and considered by the investigator to have no clinical implication.

Out-of-range values were considered to be clinically significant by the investigator for 4 subjects. For these subjects laboratory TEAEs were reported:

- One subject (EU-Tysabri): ALT increased and aspartate aminotransferase (AST) increased, both unlikely related to study drug, outcome recovered.
- One subject (PB006): leukocytosis, unlikely related to study drug, outcome recovered.
- One subject (EU-Tysabri): leukocyturia, unlikely related to study drug, outcome recovered.
- One subject (US-Tysabri): anaemia, unlikely related to study drug, outcome recovering/resolving.

In addition, the laboratory AE haematuria was reported in 1 subject (11031) in the PB006 group.

One subject had a positive pregnancy test on Day 85. On Day 109 the pregnancy was terminated by an induced abortion.

Overall, results for laboratory parameters were similar for PB006, EU-Tysabri and US-Tysabri.

Study PB006-03-01

Hematology results

Notable findings with regard to mean changes from baseline included:

- Increases in eosinophils, with a mean change of 0.15 and 0.17 x 10^9/L from baseline to end of study in PB006 and EU-Tysabri, respectively.
- Increases in leukocytes, with a mean change of 1.73 and 2.06 x 10^9/L from baseline to end of study in PB006 and EU-Tysabri, respectively.
- Increases in lymphocytes, with a mean change of 1.57 and 1.76 x 10^9/L from baseline to end of study in PB006 and EU-Tysabri, respectively.
- Increases in monocytes, with a mean change of 0.11 and 0.12 x 10^9/L from baseline to end of study in PB006 and EU-Tysabri, respectively.
- Small decreases in hemoglobin, with a mean change of -5.3 and -1.5 g/L from baseline to end of study in PB006 and EU-Tysabri, respectively. Larger decreases were reported at other timepoints during the treatment period.

Generally, the changes from baseline were of a similar extent in both treatment groups. Increases in leukocytes, lymphocytes, eosinophils and monocytes and known PD effects of natalizumab; decreases in hemoglobin are known adverse reactions and described in the Tysabri SmPC. No clinically relevant changes from baseline were observed for the other parameters.

At baseline, most patients in both treatment groups had normal hematology values for all parameters. The majority of the changes from baseline for most abnormal hematology parameters were small, not clinically relevant, and similar between the treatment groups. Notable findings were observed for hemoglobin, leukocytes and lymphocytes.

Hemoglobin was reported as low at baseline for 8.4% and 10.5% of patients in the PB006 and EU-Tysabri groups, respectively. At all subsequent timepoints up to Week 40, a larger percentage of patients in both groups presented with low hemoglobin values. In both groups, the largest percentage of patients with low hemoglobin values was reported at Week 32 (with 19.5% in PB006 and 16.1% with EU-Tysabri).

At the end of study, 15.0 and 8.0% of patients in the PB006 and EU-Tysabri groups, respectively, reported low hemoglobin. However, in absolute values decreases in hemoglobin were rather small, with a mean change of -5.3 and -1.5 g/L from baseline to end of study in PB006 and EU-approved Tysabri, respectively (values at baseline were 141.6 g/L in both groups).

The percentage of patients with high leukocyte counts increased for both groups (14.4 and 14.8% in the PB006 and EU-approved Tysabri groups, respectively) at the end of study compared to baseline (3.8 and 5.3% patients in the PB006 and EU-Tysabri groups, respectively). Similarly, the percentage of patients with high lymphocyte counts increased for both groups (25.5 and 23.3% in the PB006 and EU-Tysabri groups, respectively) at the end of study compared to baseline (1.5 and 0% in the PB006 and EU-Tysabri groups, respectively). The largest percentage was reported at Week 40, when 33.0 and 31.5% of patients in the PB006 and EU-Tysabri group, respectively, presented with high lymphocytes.

Hematology values were categorized as within reference range (normal) or outside the reference range (low or high), and shifts in the categories between baseline were analyzed. Parameters showing a shift to levels outside the reference range (normal to low) in ≥10% of patients in both treatment groups included erythrocytes, hemoglobin, and neutrophils/leukocytes. Parameters showing a shift to levels outside the reference range (normal to high) in ≥10% of patients in both treatment groups included leukocytes, lymphocytes, lymphocytes/leukocytes, and neutrophils. Parameters showing a shift to levels outside the reference range (normal to high) in ≥10% of patients in the PB006 group only included

basophils/leukocytes and haematocrit. Parameters showing a shift to levels outside the reference range (normal to high) in ≥10% of patients in the EU-Tysabri group only included eosinophils/leukocytes.

Hematology TEAEs

AEs of anaemia were reported for 4 patients in the PB006 group, and iron deficiency anaemia and normocytic anaemia were reported for 1 patient each in the EU-Tysabri group. Lymphocyte count increased and white blood cell count increased were reported as AE in 1 patient each in the EU-Tysabri group. In addition, neutropenia was reported in 1 patient in the EU-approved Tysabri group, and lymphopenia was reported in 1 patient in the PB006 group.

Serum chemistry results

In general, mean serum chemistry values at baseline were similar for the PB006 and EU-Tysabri groups. The mean changes from baseline were generally small, not clinically relevant, similar between the treatment groups, and did not indicate any clinically meaningful trend in serum chemistry values over the course of the study overall or within either treatment group.

At baseline, most patients in both treatment groups had normal serum chemistry values for most parameters. The majority of the changes from baseline for abnormal serum chemistry parameters were small, not clinically relevant, and similar between the treatment groups. At baseline, both treatment groups had patients with abnormal high cholesterol (40.5% in the PB006 group and 32.3% in the EU-Tysabri group) and abnormal high low density lipoprotein (LDL) cholesterol (35.1% in the PB006 group and 28.6% in the EU-Tysabri group) but no notable changes in either treatment group occurred for those parameters by the end of study (31.6 and 34.4% for cholesterol in the PB006 and EU-Tysabri groups, respectively, and 29.1 and 28.0% for LDL cholesterol in the PB006 and EU-Tysabri groups, respectively).

Chemistry values were categorized as within reference range (normal) or outside the reference range (low or high), and shifts in the categories between baseline were analyzed. Parameters showing a shift to levels outside the reference range (normal to high) in ≥10% of patients in both treatment groups included C-reactive protein, cholesterol, LDL cholesterol, and triglycerides. Parameters showing a shift to levels outside the reference range (normal to high) in ≥10% of patients in the PB006 group only included bilirubin and direct bilirubin. A shift in phosphate to levels outside the reference range (normal to low) in ≥10% of patients was reported in both treatment groups and a shift in high density lipoprotein cholesterol to levels outside the reference range (normal to low) in ≥10% of patients was reported for the Tysabri group only.

Serum chemistry TEAEs

AEs of hyperbilirubinaemia were reported for 1 patient each in the PB006 and the switch groups, and bilirubin conjugated increased was reported for 1 patient in the PB006 group. With regard to liver enzyme elevations, ALT increased was reported for 2 patients in the PB006 group and for 1 patient in the EU-Tysabri group; AST increased was reported for 1 patient in the EU-Tysabri group, and gamma-glutamyl transferase increased was reported for 1 patient each in the PB006 and the switch group. Hyperlipidaemia was reported for 1 patient in the switch group, blood triglycerides increased was reported for 1 patient for the PB006 group and hypertriglyceridemia was reported for 1 patient in the EU-Tysabri group. C-reactive protein increased was reported for 2 patients in the PB006 group and 1 patient in the EU-Tysabri group.

Overall, with regard to clinical laboratory, the results were similar for PB006 and EU-Tysabri. The safety profile of laboratory AEs was similar for PB006 and EU-Tysabri and in line with the published data for Tysabri.

2.6.8.5. Immunological events

Immunogenicity Assays

In the development of the reference product Tysabri, persistent antibodies were associated with a substantial decrease in the effectiveness of natalizumab and an increased incidence of hypersensitivity reactions. Additional infusion-related reactions associated with persistent antibodies included rigors, nausea, vomiting and flushing. At that time, antibodies against natalizumab were detected in approximately 10% of patients in 2-year controlled clinical trials in MS patients and persistent anti-natalizumab antibodies developed in approximately 6% of patients.

The applicant has adopted an electrochemiluminescence immunoassay (ECLIA) bridging assay to screen, confirm and quantify in terms of titer of ~~quantify~~ natalizumab specific antibodies in human serum matrix. The adopted three-tiered approach for determination of ADAs was well described and developed. It is considered state of the art and valid for its intended use.

Further, the applicant presented a qualitative assay for the detection of neutralising ADA's in human serum. The presented assay was well described and established. It was setup correctly and fully validated. Thus it is considered valid for its intended use.

Study PB006-01-02

The ADA test results of study PB006-01-02 indicated that a single IV infusion of 300 mg PB006 induced a treatment-emergent ADA response in 3 out of 10 subjects (30%) of subjects. While this was higher than the ADA incidence reported in the Tysabri studies that were conducted by the originator, this result is consistent with a higher drug tolerance level of the PB006 ADA assay compared to that of the originator's assay. The detected ADA response was not associated with any treatment-related adverse events.

Study PB006-01-03

The immunogenicity of PB006 (N=149 subjects) was evaluated in direct comparison to EU-Tysabri (N=151) and US-Tysabri (N=150) following a single intravenous infusion of 3 mg PB006/kg to healthy volunteers.

There was no detectable difference in ADA or NAb response dynamics in subjects receiving PB006 compared to EU-Tysabri or US-Tysabri:

- The incidence of treatment-emergent ADA following a single intravenous infusion of 3 mg natalizumab/kg to healthy volunteers was similar across all three treatment groups: 87% for PB006 compared to 87% for EU-Tysabri and 92% for US-Tysabri.
- NAb was detected in the majority of ADA positive subjects, again at a similar incidence across the three treatment groups: 84% for PB006 compared to 77% for EU-Tysabri and 87% for US-Tysabri in terms of the total number of treated subjects.
- ADA and NAb titer profiles were indistinguishable across treatment groups

There was no treatment-related difference in the impact of ADA or NAb positive status on PK ($AUC_{0\text{-}inf}$ and C_{max}) or PD (Blood CD19+, α4-integrin receptor saturation, blood CD34+, soluble VCAM-1 and soluble MAdCAM-1).

By all parameters evaluated, the immunogenicity profile of PB006 was indistinguishable from that of EU-Tysabri and US-Tysabri.

Study PB006-03-01

Treatment-emergent ADA

Through Week 24, 79% of subjects in the PB006 treatment group were confirmed positive for treatment-emergent ADA compared to 74% for EU-Tysabri. For PB006, 23% of subjects were classified as transient ADA positive compared to 56% persistent ADA positive; for EU-Tysabri, 19% of subjects were classified as transient ADA positive compared to 55% persistent ADA positive.

Geometric mean maximal (Week 0 to 24) ADA titer for total treatment-emergent ADA positive was 223.6 for PB006 compared to 150.7 for EU-Tysabri. Median ADA titer in both treatment groups was 160.

The peak frequency of ADA positive subjects was at week 8 for PB006 (65% ADA positive) and EU-Tysabri (61% ADA positive), declining progressively thereafter to 11% for PB006 and 10% for EU-Tysabri at week 48. Geometric mean ADA titer increased from the 24- to 48-week timepoints in the diminishing proportion of ADA positive subjects in both treatment groups.

Overall time-course of the ADA response to PB006 mirrored that of the ADA response to EU-Tysabri throughout the 48-week treatment period in terms of ADA frequency, but with a modestly higher geometric mean ADA titer for PB006. As discussed in ISI sections 3.2.5 and 3.2.6, there was no difference in either the drug trough concentrations or efficacy parameters; and results from the comparative single-dose PK study (PB006-01-03) indicated a modestly lower geometric mean ADA titer for PB006 compared to EU-Tysabri or US-Tysabri. While it is unclear if the observed difference in geometric ADA titer observed in the PB006-03-01 study represents a real difference, there was no impact on drug exposure or on efficacy.

NAb

A relatively high proportion (approx. 87%) of ADA positive subjects were also NAb positive, indicating suitable sensitivity / drug tolerance of the NAb assay. NAb titers were also measured to optimize a comparison of the relative magnitude of the humoral immune response to PB006 compared to EU-Tysabri.

At Week 24, in terms of the total number of treated subjects, 69% of subjects in the PB006 treatment group were positive for NAb compared to 66% for EU-Tysabri. Geometric mean maximal (Week 0 to 24) NAb titer was 39.2 for PB006 compared to 32.6 for EU-Tysabri. Median NAb titer for week 0 to 24 was 23.0 for PB006 compared to 26.0 for EU-Tysabri.

Persistent ADA and NAb combined

In the confirmatory efficacy and safety study, PB006-03-01, there was a strong concordance between the ADA and NAb response dynamics in the PB006 treatment group compared to those for subjects treated with EU-Tysabri: at the 24-week treatment timepoint corresponding to the primary efficacy endpoint, the incidence of persistent treatment-emergent ADA was 79% for PB006 compared with 74% for EU-Tysabri, allied to a NAb positive incidence of 69% for PB006 compared with 67% for EU- Tysabri in terms of the total number of treated subjects.

Impact of ADA / NAb on systemic drug concentration

PB006 vs. EU-Tysabri

Because serum drug levels represent the most sensitive indicator of ADA formation at levels that could influence clinical responses, serum total natalizumab trough concentration was compared for the ADA positive vs. ADA negative subpopulations in each treatment group at Week 24 and Week 48. A corresponding analysis for the NAb positive vs. NAb negative subpopulations was also performed.

At week 24, the geometric mean total serum natalizumab trough concentration was similar for ADA positive subjects treated with PB006 (11375.8 ng/mL) or EU-Tysabri (10405.1 ng/mL). For NAb positive

subjects at Week 24, the geometric mean total serum natalizumab trough concentration was slightly higher for the PB006 treatment group (8350.6 ng/mL) compared to the EU- Tysabri treatment group (6957.4 ng/mL).

Serum natalizumab trough concentration was clearly lower in ADA positive subjects compared to ADA negative subjects in the same treatment group: at week 24, the geometric mean total serum natalizumab concentration for ADA positive subjects treated with PB006 was 31% of that for the ADA negative subjects; for EU-Tysabri, the geometric mean total serum natalizumab trough concentration for ADA positive subjects treated with PB006 was 30% of that for the ADA negative subjects. The high overlap of the 95% CIs for serum total natalizumab trough concentration by ADA/NAb category supports the conclusion that there was no difference in the scale of impact of ADA or NAb formation on serum natalizumab concentration following treatment with PB006 or EU-Tysabri for 24 weeks.

At week 48, the lower number of ADA positive (n=13 for PB006; n=9 for EU-Tysabri) or NAb positive (n=8 for PB006; n=4 for EU-Tysabri) subjects hampers the comparison across treatment groups. The geometric mean total serum natalizumab concentration for the ADA positive and NAb positive subjects in all treatment groups were lower than those at week 24, most likely reflecting the higher ADA and NAb titers measured at week 48 compared to week 24.

Impact of ADA / NAb on efficacy endpoints

Overall, there was no treatment-related difference in the impact of ADA or NAb positive status on either the primary (week 24) or secondary (week 48) efficacy endpoints in study PB006-03-01. Although ADA titers increased during the 48-week treatment period, ADA and NAb frequency progressively declined, which could reduce the scale of any negative impact of increasing ADA / NAb titer on clinical efficacy at the treatment group level during continuing treatment. This may explain why the ADA / NAb positive subpopulations showed similar efficacy at week 48 compared to week 24 despite the higher ADA and NAb titers at week 48.

Impact of ADA on clinical safety

Because infusion-related reactions associated with persistent antibody-positivity are a recognized risk for Tysabri, line listings are presented to describe the relationship of hypersensitivity reactions and symptoms corresponding to the SMQ term “anaphylaxis” reported in study PB006-03-01 to ADA positive/negative status and coincident ADA titer.

In subjects treated with PB006, 10 events were reported from 9 subjects; 7 of the events were considered as being possibly or probably related to drug administration. Five of the 7 events were detected in subjects with a coincident classification of “ADA positive”. One event of hypotension was coincident with an ADA titer value of 40960 and study discontinuation.

In subjects treated with EU-Tysabri, 5 events were reported from 4 subjects; 3 of the events were considered as being possibly-related to drug administration.

Summary of immunogenicity results from confirmatory efficacy and safety study in RRMS patients, PB006-03-01

The following two tables summarize the relationship of the treatment-emergent ADA and NAb responses to relevant clinical parameters at Treatment Week 24 and Week 48 respectively.

Table 59: Summary of ADA & NAb response parameters vs. clinical impact in Week 0 to Week 24 of study PB006-03-01 (Safety Analysis Population)

Parameter	**PB006 (N=131)**	**EU-Tysabri (N=133)**
%ADA positive (Week 0 to 24): • Total treatment-emergent • Transient • Persistent	 79% (n=104) 23% (n=30) 56% (n=74)	 74% (n=98) 19% (n=25) 55% (n=73)
Geometric mean maximal (Week 0 to 24) ADA titer for total treatment-emergent ADA positive	223.5 (n=104)	150.7 (n=98)
%NAb positive (Week 0 to 24)	69% (n=90)	66% (n=88)
Geometric mean maximal (Week 0 to 24) NAb titer	39.2 (n=90)	32.6 (n=88)
Geometric mean drug trough concentration at Week 24: • ADA negative • ADA positive • NAb negative • NAb positive	 36155.8 (n=85) 11375.8 (n=37) 21662.0 (n=12) 8350.6 (n=25)	 36200.9 (n=88) 10405.1 (n=37) 24419.8 (n=12) 6908.9 (n=25)
Cumulative number of new active lesions at Week 24: • ADA negative • ADA positive • NAb negative • NAb positive	 1.8 (n=27) 1.3 (n=99) 1.1 (n=13) 1.3 (n=86)	 1.9 (n=33) 1.9 (n=94) 2.8 (n=10) 1.8 (n=84)
Annualized Relapse Rate at Week 24: • ADA negative • ADA positive • NAb negative • NAb positive	 0.24 (n=27) 0.20 (n=104) 0.17 (n=14) 0.20 (n=90)	 0.06 (n=35) 0.18 (n=98) 0.00 (n=10) 0.21 (n=88)

Includes n=30 subjects who switched to PB006 at week 24

Table 60: Summary of ADA & NAb response parameters vs. clinical impact in Week 0 to Week 48 of study PB006-03-01 (Safety Analysis Population)

Parameter	**PB006 (N=131)**	**EU-Tysabri (N=103)**
%ADA positive (Week 0 to 48): • Total treatment-emergent • Transient • Persistent	 79% (n=104) 22% (n=29) 57% (n=75)	 74% (n=76) 22% (n=23) 51% (n=53)
Geometric mean maximal (Week 0 to 48) ADA titer for total treatment-emergent ADA positive	229.1 (n=104)	131.5 (n=76)
%NAb positive (Week 0 to 48)	69% (n=90)	67% (n=69)
Geometric mean maximal (Week 0 to 48) NAb titer for NAb positive	39.8 (n=90)	26.5 (n=69)
Geometric mean drug trough concentration at Week 48: • ADA negative • ADA positive • NAb negative • NAb positive	 30005.2 (n=102) 7373.3 (n=13) 32246.3 (n=5) 2931.9 (n=8)	 34273.1 (n=84) 9493.9 (n=39) 21462.5 (n=5) 3424.9 (n=4)
Cumulative number of new active lesions at Week 48: • ADA negative • ADA positive • NAb negative	 1.9 (n=26) 1.4 (n=96) 1.1 (n=13)	 1.4 (n=25) 2.7 (n=71) 6.4 (n=7)

• NAb positive	1.4 (n=83)	2.3 (n=64)
Annualized Relapse Rate at Week 48:		
• ADA negative	0.28 (n=27)	0.12 (n=27)
• ADA positive	0.14 (n=104)	0.14 (n=76)
• NAb negative	0.16 (n=14)	0.15 (n=7)
• NAb positive	0.14 (n=90)	0.13 (n=69)

Excludes n=30 subjects who switched to PB006 at week 24

Finally, switching of 30 subjects who were treated for 24 weeks with EU-Tysabri to treatment with PB006 for 24 weeks was not associated with any impact on either the treatment-related humoral immune response to natalizumab or its clinical impact.

2.6.8.6. Discontinuation due to adverse events

Discontinuation due to adverse events occurred only in study PB006-03-01.

Overall, 12 patients experienced TEAEs leading to study drug discontinuation (8 [5.0%] patients in the PB006 group and 4 [3.0%] patients in the Tysabri group). The most common TEAEs leading to discontinuation in the PB006 group were pruritus (2 [1.5%] patients) and urticaria (2 [1.5%] patients), which are known common adverse drug reactions of natalizumab. One (0.8%) patient discontinued due to a TEAE of COVID-19. One (0.8%) patient experienced multiple TEAEs of asthenia, hyperhidrosis, blood pressure fluctuation, and dizziness after study drug infusion at Week 0. No treatment was given and all TEAEs resolved, but the patient withdrew from the study following the events. One (0.8%) patient experienced multiple TEAEs of ear infection and herpes simplex (an AESI) approximately 1 month after study drug infusion at Visit 5; both events resolved. The patient later experienced a TEAE of trigeminal neuralgia and was withdrawn from the study following these events.

In the Tysabri group, 1 (0.8%) patient experienced multiple TEAEs of urticaria and angioedema (both AESIs) approximately 20 minutes after the start of the patient's second Tysabri IV infusion. The infusion was stopped and the patient was treated for both events. The events of angioedema and urticaria resolved and the patient withdrew from the study.

2.6.9. Discussion on clinical safety

The assessment of safety in the PB006 clinical program was focused on the comparison of PB006 to Tysabri. Safety data with PB006 were collected in studies PB006-01-02, PB006-01-03 and PB006-03-01. The principal safety data for PB006 and comparative safety data with Tysabri is derived from the pivotal, Phase 1, single-dose study in 450 healthy subjects (PB006-01-03) and the pivotal Phase 3, multiple-dose study in 264 adult male and female RRMS patients (PB006-03-01) treated for up to 48 weeks (131 patients in the PB006 group and 133 in the Tysabri group up to week 24; afterwards a subset of 30 patients (22.6%) from the Tysabri group switched to PB006). Comparability of safety, tolerability and immunogenicity between the biosimilar candidate PB006 and the reference product, Tysabri EU, were secondary objectives of these studies. In both studies, safety analyses were based on the SAF, defined as all subjects who received at least one dose of study drug. In the Phase 3 study PB006-03-01, AESIs were defined in line with the SmPC of the reference product. Additional safety analyses were performed for the SSW population, defined as patients who received at least one infusion of study drug after the time point of re-randomization.

The overall concept of the safety evaluation is considered adequate to conclude on similarity between PB006 and Tysabri EU and the safety database is considered sufficient for establishment of safety for

PB006 taking into account the well-known safety profile of the active substance and the fact that it is a biosimilar candidate.

Non-conformity with GCP was described for one of the study centres participating in the safety and efficacy study PB006-03-01. However, it was confirmed that all patients received the correct study drug and dose at all attended visits and there was no relevant impact on the safety data.

The safety profile of the switch population is difficult to interpret, since it cannot always be determined, if events are due to treatment with the test or reference product. AEs are therefore not assessed in detail in this population.

Overall, PB006 was administered to 159 healthy subjects as single dose (for 149 of these subjects at 3 mg/kg, and for 10 of these subjects at 300 mg), and to 161patients with RRMS as multiple doses of 300 mg in 4-weekly intervals for up to 12 doses. Tysabri (US-Tysabri or EU-Tysabri) was administered to 337 healthy subjects as single dose (1 to 6 mg/kg), and EU-Tysabri was administered to 133 patients with RRMS as multiple doses of 300 mg in 4-weekly intervals.

The overall number of subjects (healthy volunteers and patients) exposed to the study drugs as well as the dose levels administered and the duration of exposure are considered adequate.

In study PB006-01-03, approximately two-thirds of subjects in each group reported any TEAE, and approximately one third of subjects across groups had study drug-related TEAEs. No fatal TEAEs occurred. SAEs were reported for two subjects (1.3%; 6 SAEs overall) treated with US-Tysabri, while no SAEs occurred in the other two groups. No subject reported TEAEs leading to study drug discontinuation.

Most frequently reported were TEAEs in the SOCs nervous system disorders (30-33% across groups) and general disorders and administration site conditions (29-34% across groups).

On the PT level, most frequently reported was headache (26-30% across groups), followed by AEs related to the injection/infusion, with PTs of injection site reaction (18-21% across groups), infusion site reaction (1-4% across groups), vessel puncture site reaction (1-5% across groups), and catheter site related reaction (2-3% across groups).

In the majority of subjects (65.5%), TEAEs were mild and were reported with similar frequencies across the three study groups. Slightly more moderate TEAEs were reported in PB006 group (15 TEAEs in 11 subjects (7.4%)) and in Tysabri US (19 TEAEs in 12 subjects (8.0%)) than in Tysabri EU (5 TEAEs in 4 subjects (2.6%)).

Slight differences were noted in the PTs Infusion site reaction and Pyrexia, which required follow-up as they could be indicative of immunogenic reactions to PB006. Of the 4 subjects with pyrexia events in study PB006-01-03, pyrexia did not occur in close temporal relationship to study drug infusion in the 3 subjects who received PB006 and noneof these 3 subjects reported concomitant AEs indicative of a systemic immunogenic reaction. Thus, it is unlikely that pyrexia was indicative of an immunological event in these subjects. Further it was assessed that the PT "infusion site reaction" was used only temporarily at one study site, and corresponds to PT "injection site reaction". Overall, the frequency of injection site reaction was comparable across the 3 groups in study PB006-01-03.

In study PB006-03-01, the overall percentages of patients with TEAEs and with TEAEs related to study drug were similar across groups. Approximately two-thirds of patients in each group reported any TEAE, while in approximately 25% of patients in each group, study drug-related TEAEs occurred. However, the frequencies of TEAEs of Grade 3 and of TEAEs leading to discontinuations were numerically higher in the PB006 group than in the other groups. Despite minor imbalances observed between treatments, the nature and frequency of these AEs reported in study PB006-03-01 correspond to the safety profile of the reference product Tysabri and thus do not give reason to concern.

The percentage of patients with study drug-related TEAEs was similar in the PB006, switch and the EU-Tysabri group (23.7, 26.7 and 21.4%), with event rates per 100 PY of 46.13, 54.90 and 56.40, respectively.

Most frequently reported in both groups were study drug-related TEAEs in the SOCs infections and infestations, nervous system disorders and general disorders and administration site conditions. On the PT level, most frequently reported was headache, followed by dizziness.

Though, on the level of individual PTs the frequencies slightly differed between groups. Most strikingly, distinctly more patients in the PB006 group (10 patients) had nervous system disorders compared to the Tysabri group (2 patients), mostly in the PT headache (7 versus 2 patients). However, these events were only graded as mild or moderate; they are known adverse drug reactions of natalizumab and do not give rise to concern.

There were no fatal AEs in any of the studies conducted for this MAA.

There were 6 SAEs reported for 2 subjects in the PK/PD study. All of these SAEs were unlikely related to study drug (based on investigator assessment) and were resolved within 1 or 2 days.

There were 5 SAEs reported for 5 patients in the phase 3 study, 3 and 2 in the PB006 and Tysabri group, respectively. Three SAEs were not considered study drug related, 1 event of hypotension in the PB006 group was considered possibly related and led to study drug discontinuation; one event of tremor in the control group was considered unlikely related. All events in the PB006 group resolved without sequelae, whereas the two SAEs in the Tysabri group resolved with sequelae.

Adverse events of special interest were defined in the study protocol based on the Tysabri SmPC and included PML, JCV granule cell neuropathy, opportunistic infection, liver injury, hypersensitivity, encephalitis, meningitis, and ARN. The overall number of treatment emergent AESI was low (approx. 6%) and similar between groups. The most frequently reported AESIs were events of urticaria and herpes.

Discontinuation due to adverse events accounted for 4.5% overall and was twice as abundant in the PB006 group compared to the Tysabri group. Most common TEAEs leading to discontinuation were pruritus and urticaria, which are known and common adverse drug reactions of natalizumab. All of the events resolved.

In both pivotal studies, increases from baseline in mean numbers of circulating leukocytes, lymphocytes, eosinophils and monocytes were reported, which were similar between treatment groups. The increases are consistent with expression of α4β1 on these white-cell subpopulations and are a known pharmacodynamic effect of natalizumab (Tysabri SmPC). Results from study PB006-01-03 (which assessed laboratory parameters up to and including 85 days post-dose) suggested that the increases were transient, with values returning to or below baseline levels at Day 85.

In study PB006-03-01, AEs of anaemia were reported for 4 patients in the PB006 group versus none in the Tysabri group, whereas one event each of iron deficient anaemia and normocytic anaemia was observed in the Tysabri group. All of the anaemia events were judged as not related or unlikely related to study drug and none of the events was serious. Of note, anaemia has been reported as common AE for Tysabri. In this context, the findings of AEs pertaining to anaemia in study PB006-03-01 are not considered to be unusual.

The use of natalizumab has been associated with an increased risk of PML, an opportunistic infection caused by JCV, which may be fatal or result in severe disability. This virus also causes JCV granule cell neuronopathy which has been reported in patients treated with natalizumab. Symptoms of JCV GCN are similar to symptoms of PML (i.e. cerebellar syndrome).

The presence of anti-JCV antibodies is considered a risk factor for PML and it has been shown that the level of anti-JCV antibody response (index) is associated with the level of risk for PML in anti-JCV antibody positive natalizumab treated patients who have not used prior immunosuppressants (Tysabri SmPC).

Thus, patients with anti-JCV antibodies were excluded from the phase 1 studies with PB006. Due to the relatively high prevalence of anti-JCV antibodies, patients presenting such antibodies could be enrolled into study PB006-03-01 but the index had to be below 1.5. Further, JCV status was a stratification factor at randomization. Further, all studies included a PML follow-up visit 6 months after (last) dosing with study drug, to assess neurological symptoms which could be suggestive of PML.

Although an increase in JCV index was observed throughout the phase 3 study with approx. 6% of patients having an index >1.5 at week 48, no cases of PML occurred in this study. No subjects had any signs suggestive of PML at the follow-up visit, either.

Throughout clinical development of PB006, STRATIFY JCV DxSelect assay was used for screening of study subjects and as risk minimisation during the biosimilar clinical studies. In parallel, a new anti-JCV antibody assay was developed (ImmunoWELL JCV IgG test) for use in clinical practice.

In response to concerns on test development and test validation, the applicant explained that development and validation were performed on independent data sets and this approach is endorsed.

The applicant further informed that there is no international reference standard for antibodies directed against JCV available. For this reason, the applicant developed the ImmunoWELL JCV IgG test with the intention to provide the same readout as the widely used and established STRATIFY JCV DXSelect, providing estimated values for the ImmunoWELL assay based on a regression fit with values from the STRATIFY JCV DxSelect test, to prove their agreement for healthy individuals and MS patients.

In order to rely on the established PML risk estimate algorithm, it is essential that the ImmunoWELL assay produces comparable test results as the STRATIFY JCV DxSelect assay and reports index values on a comparable scale as the STRATIFY JCV DxSelect assay. The applicant provided further information on the ImmunoWELL JCV IgG test and its comparability with the current standard STRATIFY JCV DxSelect assay, including the instructions for use, the analytical and the clinical validation report of the ImmunoWELL JCV IgG test and the EC declaration of conformity.

Although both tests (STRATIFY JCV DxSelect and ImmunoWell JCV IgG test) should reveal results on a comparable scale, the difference in index cut-off values between the two tests (0.2 – 0.4 for the STRATIFY JCV DxSelect and 0.25 – 0.5 for the ImmunoWELL JCV IgG test) shows that the two tests are not fully identical. For justification of the cut-off values 0.25 and 0.5 for Ab negativity/positivity, the applicant referred to the conducted "cut-off study" and stated that the selection of 0.25 and 0.5 was based on high sensitivity (>99%) and specificity (>90%), respectively. Details from the cut-off study were missing on the performance measures (sensitivity, specificity, NPV, PPV) for different cut-offs to understand the proceeding of cut-off selection. Although the actual question about justification of the cut-off of 0.25 and 0.5 was not answered in detail, it is acknowledged that CE-marking is available based on these.

However, the applicant was asked to elaborate on their proceeding for defining the chosen range for positivity/negativity (0.25-0.5). During the procedure, the applicant explained that the two JCV Ab tests are correlated and that decisions based on positivity cut-off are conservative based on the findings in the cohort of MS patients. While indeed, good correlation is observed in the cohort of MS patients, at first only subjects with a value <1.5 based on the STRATIFY JCV DxSelect test were included, which could bias the regression fit. On request, the applicant presented further comparative test data from the healthy subject population across the whole index range, and based on a regression analysis it is implied that the ImmunoWELL JCV IgG test might slightly underestimate the index estimated from the STRATIFY JCV DxSelect test.

To address this potential difference in PML risk stratification, the applicant proposed to subtract an offset of 0.1 index values from the PML risk threshold values (0.9, 1.5) defined for the STRATIFY JCV DxSelect assay. The proposed offset is based on an analysis that includes only data from the healthy subject population; ideally, data across the entire patient population should have served as the basis for determining the proposed offset. The scatter plot of index values from ImmunoWELL JCV IgG test vs. STRATIFY JCV DxSelect test using data from MS patients presented in Section *Clinical Safety*, shows that both tests agree quite well except in the upper range, where the ImmunoWELL JCV IgG test appears to underestimate the anti-JCV antibody index from the STRATIFY JCV DxSelect test. This trend was also observed for healthy subjects, which the offset was established from. It is questionable if a linear regression actually delivers the best fit for such a dataset over the full range of values. However, in the index range up to 2.5, that clearly goes beyond the index range for clinical decision making, a linear regression appears appropriate, and estimated ImmunoWELL JCV IgG test values from this regression analysis at the cut-offs 0.9 and 1.5 appear similar to those in healthy subjects. Thus, the offset (for deduction from the PML risk threshold values) based on healthy subjects is also considered appropriate in the cohort of MS patients and can be acceptable.

Importantly, lowering the index levels by deducting an offset value for PML risk stratification based on the ImmunoWELL JCV IgG test as compared to the STRATIFY JCV DxSelect test results in a more conservative approach (compared to using the same thresholds) that reduces the number of false negatives tests, i.e. the number of patients that would be eligible for treatment despite being at increased PML risk.

The applicant reported the binary agreement between the two tests in terms of sensitivity, specificity, NPV, and PPV at the newly proposed threshold values of 0.8 and 1.4 for the ImmunoWELL JCV IgG test and at the threshold values of 0.9 and 1.5 for the STRATFIY JCV DxSelect test, for healthy individuals and MS patients, respectively. Both analyses revealed performance measures (sensitivity, specificity, PPV and NPV) around 90% in healthy individuals as well as MS patients, which indicate that treatment decisions would be similar for most patients regardless of the applied JCV test.

Eventually relevant specific information on risk stratification testing including applicable tests is provided as part of the educational material for healthcare professionals.

In order to account for potential differences between the results of different anti-JCV antibody assays, the applicant provided a general wording for SmPC section 4.4 without the use of specific antibody index values.

The applicant provided a comprehensive Integrated Summary of Immunogenicity presenting data from all three clinical studies where PB006 was administered. The analytical portfolio included a screening, confirmation and neutralisation assay, and respective methods were considered suitable for their intended use.

The overall data showed up to 79% ADA positive patients in the phase 3 study. The peak value was observed at week 8 for PB006 (65% ADA positive) and EU-Tysabri (61% ADA positive), declining progressively thereafter to 11% for PB006 and 10% for EU-Tysabri at week 48. Through to Week 24, in total, 79% of subjects in the PB006 treatment group were confirmed positive for treatment-emergent ADA compared to 74% for EU-Tysabri. A relatively high proportion (approx. 87%) of ADA positive subjects were also NAb positive.

The relative numbers of ADA and NAb positive subjects are much higher than described in the Tysabri SmPC. The higher detected ADA and NAb incidence compared to the originator's studies is most plausibly explained by superior drug tolerance of the methods applied in the PB006 program.

Although ADA and NAb frequency declined progressively after reaching a peak at week 8, subjects with persistent ADA and NAb showed increasing titer levels up to and including week 48.

The applicant conducted a comprehensive evaluation of the impact of ADA or NAb on PK, efficacy and safety parameters in the two pivotal clinical studies.

Overall, no apparent differences in the treatment-emergent ADA or NAb profiles for PB006 compared to EU-Tysabri (or US-Tysabri), or in impact of ADA on PK or PD parameters were revealed in the single dose comparative PK/PD study PB006-01-03 in healthy volunteers.

Further, despite slightly higher ADA and NAb levels in the PB006 compared to the Tysabri group, the immunogenicity profile of PB006, including ADA/NAb responses and the impact on relevant clinical parameters, was similar to that of EU-Tysabri during chronic administration for 48 weeks to RRMS patients in the pivotal safety and efficacy study, PB006-03-01.

Overall, no clinically relevant differences in immunogenicity were detected between PB006 and Tysabri.

2.6.10. Conclusions on the clinical safety

Considering the provided safety data from the clinical development programme, PB006 and Tysabri can be concluded to be biosimilar in terms of safety and immunogenicity.

2.7. Risk Management Plan

2.7.1. Safety concerns

Table 61: Summary of safety concerns

Summary of safety concerns	
Important identified risks	Progressive multifocal leukoencephalopathy (PML)
	Serious herpes infections
Important potential risks	Malignancies
Missing information	PML risk following switch from disease modifying therapies with immunosuppressant effect

2.7.2. Pharmacovigilance plan

No additional pharmacovigilance activities.

2.7.3. Risk minimisation measures

Table 62: Summary of pharmacovigilance activities and risk minimization activities by safety concerns

Safety concern	Risk minimization measures	Pharmacovigilance activities
Progressive multifocal leukoencephalopathy (PML)	Routine risk communication: Information in SmPC Sections 4.2, 4.3, 4.4, 4.8, and 5.1; and PL Sections 2 and 4 Legal status: Restricted medical prescription Additional risk minimization measures: Educational tools for HCPs (Physician Information and Management Guideline) Educational tools for patients/carers (patient alert card, treatment initiation form, treatment continuation form, and treatment discontinuation form)	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: Specific adverse reaction follow-up questionnaire Additional pharmacovigilance activities: None
Serious herpes infections	Routine risk communication: Information in SmPC Sections 4.3, 4.4, 4.8; and PL Sections 2 and 4 Legal status: Restricted medical prescription Additional risk minimization measures: None	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: Specific adverse reaction follow-up questionnaire Additional pharmacovigilance activities: None
Malignancies	Routine risk communication: Information in SmPC Sections 4.3 and 4.8; and PL Section 2 Legal status: Restricted medical prescription Additional risk minimization measures: None	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: Specific adverse reaction follow-up questionnaire Additional pharmacovigilance activities: None
PML risk in patients switching from DMTs with immuno-suppressant effect	Routine risk communication: Information in SmPC Section 4.4 and PL Section 2. Legal status: Restricted medical prescription Additional risk minimization measures: None	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activities: None

2.7.4. Conclusion

The CHMP considers that the risk management plan version 1.2 is acceptable.

2.8. Pharmacovigilance

2.8.1. Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

2.8.2. Periodic Safety Update Reports submission requirements

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

2.9. Product information

2.9.1. User consultation

No full user consultation with target patient groups on the package leaflet has been performed on the basis of a bridging report making reference to Tysabri (text) and Ziextenzo (layout/design). The bridging report submitted by the applicant has been found acceptable.

2.9.2. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Tyruko (natalizumab) is included in the additional monitoring list as it is a biological product that is not covered by the previous category and authorised after 1 January 2011

Therefore the summary of product characteristics and the package leaflet includes a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

3. Biosimilarity assessment

3.1. Comparability exercise and indications claimed

Natalizumab, a monoclonal IgG4 antibody, is developed to have the same intravenous dosage form, route of administration, dosing regimen and presentation as the reference product EU-Tysabri. The concentration of natalizumab is the same as for Tysabri (20 mg/mL in 15 mL) but the formulation of PB006 differs from the formulation of the reference medicinal product.

The marketing authorization is claimed for treatment of multiple sclerosis for the following patient groups:

• Patients with highly active disease despite a full and adequate course of treatment with at least one disease modifying therapy (DMT).

• Patients with rapidly evolving severe RRMS defined by 2 or more disabling relapses in one year, and with 1 or more Gadolinium enhancing (GdE) lesions on brain magnetic resonance imaging (MRI) or a significant increase in T2 lesion load as compared to a previous recent MRI.

Quality

The applicant presented detailed information about its comprehensive analytical assessment to demonstrate similarity between PB006 and the reference medicinal product EU-Tysabri (INN: natalizumab). The analytical similarity study includes PB006 (natalizumab) data from batches manufactured at full commercial scale. Biosimilarity was evaluated against an appropriate number of batches of the reference medicinal product, EU-approved Tysabri.

The relevant quality attributes of the natalizumab molecule were assessed using a broad panel of orthogonal standard methods that are state-of-the-art and are suitable to characterise and compare relevant structural and functional features of the natalizumab PB006 in comparison to the RMP. Analytical methods cover primary and higher order structure, potency/binding and Fab arm exchange kinetics as well as purity and product related variants. For each parameter under investigation, the methodology and performance of the analyses was well described, batches used and experimental data derived were well presented including raw data or chromatograms/spectra where applicable. Based on the provided information it is concluded that the analytical methods are suitable and sensitive to detect minor differences.

The quality attributes were either evaluated against a min-max range (primary criterion) or assessed qualitatively. Generally, the critical quality attributes assessment was well described and the criticality ranking can be followed. Assigning a different overall risk for each QA considering abundance, methodology and type of QA, is acceptable.

Summary of clinical data

Two pivotal clinical studies were conducted to establish clinical similarity between PB006 and the reference product, Tysabri.

A clinical phase 1 study was conducted to demonstrate comparability in PK and PD of the biosimilar candidate PB006 and the reference medicinal product EU-Tysabri and US-Tysabri. Study PB006-01-03 was a randomized, double-blind study with 3 parallel arms in 453 healthy male and female subjects. Subjects received a single dose of 3 mg/kg PB006, EU-approved Tysabri, or US-licensed Tysabri as an IV infusion over a 60-minute period. Dosing was followed by PK and PD sampling for 85 days and a final follow-up visit 6 months (24 weeks) after dosing to assess new neurological symptoms that could be suggestive for PML. Safety was monitored throughout the study by repeated clinical and laboratory evaluations. Samples were collected for immunogenicity assessments for 85 days.

The primary objective of the study was to demonstrate PK and PD similarity of PB006 to both US-licensed Tysabri and EU-approved Tysabri.

The primary PK endpoint was $AUC_{0\text{-}inf}$ of total natalizumab, as secondary PK endpoints, $AUC_{0\text{-}t}$, C_{max}, and t_{max} of total natalizumab were selected to support the PK comparability of PB006 to EU-Tysabri and US-Tysabri. Further secondary PK endpoints were $AUC_{0\text{-}inf}$, $AUC_{0\text{-}t}$, C_{max}, and t_{max} of unexchanged natalizumab. The primary and secondary PK endpoints are in line with guidance ("*Guideline on similar biological medicinal products containing monoclonal antibodies – non-clinical and clinical issues*" (EMA/CHMP/BMWP/403543/2010)).

For PD, two co-primary endpoints were selected: $AUEC_{0\text{-}12w}$ of baseline-adjusted CD19+, and $AUEC_{0\text{-}12w}$ of alpha-integrin % receptor saturation. As an additional analysis, $AUEC_{4\text{-}12w}$ for α4-integrin %RS was specified. As secondary PD endpoints, E_{max} and t_{max} of baseline-adjusted CD19+, $AUEC_{base\_neg}$, E_{min}, t_{min} of sVCAM and sMAdCAM, and $AUEC_{0\text{-}t}$, E_{max}, t_{max} of CD34+ were used.

The pivotal efficacy and safety study PB006-03-01 was conducted to compare PB006 to the EU reference product Tysabri in terms of efficacy, safety and immunogenicity.

This was a randomized, double-blind, active-controlled, parallel-group study to compare the efficacy, safety and immunogenicity of PB006 versus EU-Tysabri, and to demonstrate similarity between PB006 and EU-Tysabri in patients with RRMS.

A total of 265 patients were randomized and 264 patients overall were treated with study drug (131 and 133 patients in the PB006 and Tysabri groups, respectively).

The dose of 300 mg, dosing frequency (once every 4 weeks), route and method of administration are in line with the SmPC of the reference product. The study was conducted over 44 weeks and 12 doses were administered to the PP population.

The primary endpoint, cumulative number of new active lesions over 24 weeks, and secondary endpoints, i.e., cumulative number of new active lesions over 48 weeks, cumulative number of new GdE T1-weighted lesions over 24 and 48 weeks, number of patients without new GdE T1-weighted lesions over 24 and 48 weeks, cumulative number of new/enlarging T2-weighted lesions over 24 and 48 weeks, number of patients without new/enlarging T2-weighted lesions over 24 and 48 weeks, number of persistent lesions after 24 and 48 weeks, annualized relapse rate after 24 and 48 weeks and change from baseline in EDSS after 24 and 48 weeks are considered adequate to assess the clinical similarity between the biosimilar candidate and the reference product because they examine the effect on brain lesion activity and measure the delay of the disability, respectively. Moreover, the efficacy endpoints are in line with the "Guideline on clinical investigation of medicinal products for the treatment of Multiple Sclerosis" (EMA/CHMP/771815/2011, Rev. 2), the efficacy endpoints studied in the clinical trials which supported the MA of the reference product, Tysabri EU and the "Guideline on similar biological medicinal products containing interferon beta" (EMA/CHMP/BMWP/652000/2010).

3.2. Results supporting biosimilarity

Quality

Analytical similarity assessment was based on the analysis of PB006 batches and an appropriate number of EU-approved Tysabri batches, based on a broad panel of orthogonal, sensitive and state of the art analytical methods, including supportive data from US-licensed Tysabri batches. The comparison included the assessment of strength, composition, physicochemical, biophysical and *in vitro* functional properties, as well as structural characterisation and in-depth assessment of isolated product-related variants. Stability and degradation pathways were compared by a degradation study and by long-term, accelerated and stress stability studies. Acceptance ranges were pre-defined as min-max ranges (primary criteria) and quality ranges (secondary criteria) for the EU-approved RMP, depending on their criticality, which was elaborated based on a comprehensive criticality assessment covering all relevant quality attributes. For all quality attributes including multiple attributes covering the mechanism of action high degree of analytical similarity of PB006 to the reference product EU-Tysabri has been demonstrated: Primary structure was demonstrated to be similar by 100% confirmation of the sequence (peptide mapping), molecular mass analyses (intact and deglycosylated), and the same locations of posttranslational modifications (N-terminal pyroglutamic acid, oxidation, deamidation, N-glycosylation). Highly similar higher order structure was shown. The main mechanism of action, binding to the α4-subunit of α4β1 and α4β7 integrins expressed on the surface of all leukocytes except neutrophils inhibiting the α4-mediated adhesion of leukocytes to their counter-receptor(s) VCAM-1, and MAdCAM-1, was evaluated by ELISA method and SPR. Potency values of analysed PB006 batches were comparable to EU-Tysabri and US batches. It was also shown that PB006 lacks for immunogenic Galili-motifs present in EU-licensed Tysabri. Minor differences with regard to charge and size heterogeneity are not considered impactful. Differences were observed for oxidation, free thiols, open disulfide bonds, C-terminal amidation, N-glycan profiles, and basic variants. The stability and degradation profiles of PB006 and its RMP were comparable. The observed analytical differences have been adequately justified and it is agreed that their impact on safety and efficacy seems minor. In addition, analytical comparability of US-Tysabri to EU-Tysabri has been sufficiently demonstrated as presented in a separate bridging report.

Clinical

Pharmacology/PK

The primary PK endpoint $AUC_{0\text{-}inf}$ of total natalizumab was met. The ratio of the pairwise comparison PB006 vs EU-Tysabri was 0.9864, 90% CI: 0.9410, 1.0340.

The secondary PK endpoints were supportive of similarity between PB006 and EU-Tysabri.

Pharmacology/PD

Both co-primary PD endpoints were met.

$AUEC_{0\text{-}12wk}$ α4-integrin %RS: The ratio of the pairwise comparison PB006 vs EU-Tysabri was 1.0142, 95% CI: 0.9667, 1.0641. The ratio of the comparison of PB006 vs pooled Tysabri was 0.9933, 95% CI: 0.9523, 1.0362.

$AUEC_{0\text{-}12wk}$ of baseline-adjusted CD19+: The ratio of the pairwise comparison PB006 vs EU-Tysabri was 1.0163, 95% CI: 0.8787, 1.1754. The ratio of the comparison of PB006 vs pooled Tysabri was 1.0159, 95% CI: 0.8955, 1.1525.

The secondary PD endpoints were supportive of similarity between PB006 and EU-Tysabri.

Efficacy

The primary efficacy endpoint cumulative number of new active lesions over 24 weeks was met. The point estimate for the exponentiated difference between Tysabri and PB006 was 0.17 and the 95% CI (-0.613; 0.944) for the difference Tysabri minus PB006 was narrow and well within the specified margins (-2.1; 2.1).

The secondary efficacy endpoints were supportive of similarity between PB006 and EU-Tysabri.

Safety and immunogenicity

A comprehensive safety and immunogenicity evaluation was conducted in the two pivotal clinical trials. Overall, no clinically relevant differences in safety or immunogenicity were detected between PB006 and Tysabri.

3.3. Uncertainties and limitations about biosimilarity

Clinical

Pharmacology

The study protocol was amended at several time points, with extensive changes to its design, including the primary endpoints, sample size, study population, conduct of the study (introduction of a pooling criterion to allow pooling of results of EU- and US-Tysabri for PD evaluation), and evaluation of study results. However, no severe impact on study integrity was identified.

Pharmacology/PD

The relevance of the increase in CD19 positivity used as a co-primary PD endpoint is difficult to interpret.

The used equivalence margins for assessment of PD endpoints were already criticised during EMA scientific advice provided prior to study initiation as being too wide. Also, no sound scientific justification for the acceptance ranges was provided.

No PD parameter was evaluated in the multiple-dose safety and efficacy study in patients.

Efficacy

The equivalence margin for the primary efficacy evaluation was based on a single reference study with small sample size and the predefined acceptance range was considered too large to rule out a clinically

relevant difference. However, the actual results accounted for a difference of less than one lesion and the 95% CI for the difference between the treatments displayed a narrow range of [-0.613; 0.944]. These results are considered sufficient to demonstrate similarity in clinical efficacy.

Safety and immunogenicity

The switch of 30 patients from Tysabri EU to PB006 at week 24 is considered a study limitation as a comparative safety and immunogenicity assessment up to 48 weeks of the initial treatment groups PB006 and Tysabri EU are considered more useful for the biosimilarity assessment.

3.4. Discussion on biosimilarity

Overall, at the quality level similarity between PB006 and EU-sourced Tysabri, which was used as clinical comparator, could be demonstrated in a comprehensive analytical similarity exercise. In the PK/PD study EU-and US-sourced Tysabri were applied, and comparability between both products could be demonstrated in the analytical similarity exercise.

Overall, the clinical comparison between PB006 and EU-sourced Tysabri demonstrated biosimilarity in terms of the pre-defined primary parameters for PK, PD and efficacy. Additionally, the secondary PK and efficacy parameters support the conclusion of biosimilarity, as does the assessment of safety and immunogenicity.

3.5. Extrapolation of safety and efficacy

Not applicable

3.6. Additional considerations

Not applicable

3.7. Conclusions on biosimilarity and benefit risk balance

Based on the review of the submitted data, Tyruko is considered biosimilar to Tysabri. Therefore, a benefit/risk balance comparable to the reference product can be concluded.

4. Recommendations

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the benefit-risk balance of Tyruko is favourable in the following indication(s):

Tyruko is indicated as single disease modifying therapy in adults with highly active relapsing remitting multiple sclerosis (RRMS) for the following patient groups:

- Patients with highly active disease despite a full and adequate course of treatment with at least one disease modifying therapy (DMT) (for exceptions and information about washout periods see sections 4.4 and 5.1)

or

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

The CHMP therefore recommends the granting of the marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to restricted medical prescription (see Annex I: Summary of Product Characteristics, section 4.2).

Other conditions and requirements of the marketing authorisation

- **Periodic Safety Update Reports**

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

- **Risk Management Plan (RMP)**

The marketing authorisation holder (MAH) shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

- **Additional risk minimisation measures**

Based on how patients treated with Tyruko are currently monitored at national level, the MAH shall discuss and agree with the National Competent Authorities measures to enhance further this monitoring (e.g. registries, post-marketing surveillance studies) as appropriate. The MAH shall implement agreed measures for monitoring within a time frame agreed with the National Competent Authorities.

The educational programme is aimed at educating healthcare professionals and patients/carers of the potential and risk factors for the development of PML, its diagnosis and treatment, and the identification and management of possible sequelae.

The MAH shall ensure that in each Member State where Tyruko is marketed, all healthcare professionals and patients/carers who are expected to prescribe/use Tyruko have access to/are provided with the following educational materials:

- Physician educational materials:
  - Summary of Product Characteristics
  - Physician Information and Management Guidelines
- Patient information pack:

- Package leaflet
- Patient alert card
- Treatment initiation and treatment continuation forms
- Treatment discontinuation form

These educational materials shall contain the following key elements:

Physician Information and Management Guidelines:

- Background information on the increased risk of atypical/opportunistic infections, in particular PML, which may occur with Tyruko therapy, including a detailed discussion of data (including **epidemiology, aetiology, and pathology**) pertaining to the development of PML in Tyruko-treated patients.
- Information relating to the **identification of risk factors** for Tyruko-associated PML, including details of the PML risk estimates algorithm summarising PML risk by risk factor (anti-John Cunningham virus [JCV] antibody status, prior IS use, and duration of treatment [by year of treatment]), and stratification of this risk by index value when applicable.
- **Information on extending the dosing interval for PML risk mitigation**, including a reminder of the approved dosing schedule.
- Inclusion of **monitoring guidance** for MRI and anti-JCV antibody based on PML risk, including recommended timing, protocols, and interpretation of results.
- Detail regarding the **diagnosis of PML**, including principals, clinical assessment (including MRI and laboratory testing), and differentiation between PML and MS.
- **Management** recommendations in the event of cases of suspected PML, including considerations on the effectiveness of PLEX treatment and the management of associated IRIS (immune reconstitution inflammatory syndrome).
- Detail on the **prognosis** on PML, including information on improved outcomes observed in asymptomatic PML cases.
- A reminder that irrespective of the presence or absence of PML risk factors, heightened clinical vigilance for PML should be maintained in all patients treated with Tyruko and for 6 months following **discontinuation of therapy**.
- A reminder on the need to discuss the benefit/risk profile of Tyruko treatment with the patient, and the requirement to provide the patient information pack.

Patient alert card:

- Reminder to patients to show the card to any doctor and/or caregiver involved with their treatment, and to keep the card with them for 6 months after the last dose of Tyruko treatment.
- Reminder to patients to read the package leaflet carefully before starting Tyruko, and not to start Tyruko if there is a serious problem with their immune system.
- Reminder to patients no to take any other long-term medicines for MS while receiving Tyruko.
- A description of PML, potential symptoms and management of PML.
- A reminder of where to report side effects.

- Details of the patient, treating doctor and date Tyruko was started.

Treatment initiation and treatment continuation forms:

- Information on PML and IRIS, including the risk of developing PML during Tyruko treatment stratified by prior treatment with immunosuppressants and JCV infection.
- Confirmation that the doctor has discussed the risks of PML and the risk of IRIS if treatment is discontinued following suspicion of PML, and confirmation of patient understanding of the risks of PML and that they have received a copy of the treatment initiation form and a patient alert card.
- Patient details and prescriber name.

The treatment continuation form should contain the elements of the treatment initiation form and, in addition, a statement that the risks of PML increase with duration of treatment and that treatment beyond 24 months carries additional risk.

Treatment discontinuation form

- Information for the patient that PML has been reported up to 6 months after stopping Tyruko,and to therefore keep the patient alert card with them after treatment discontinuation.
- Reminder of PML symptoms, and when MRI imaging may be warranted.
- Reporting of side effects.

Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States

Not applicable.