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ΠΤΥΧΙΑΚΗ ΕΡΓΑΣΙΑ

**A protocol for RCT for an assessing the relative effectiveness of
Tysabri vs. Gilenya in the treatment of RRMS**

(Κλινικό Πρωτόκολλο τυχαιοποιημένης κλινικής μελέτης για την αξιολόγηση της
αποτελεσματικότητας του Tysabri έναντι του Gilenya στη θεραπεία της
Υποτροπιάζουσας Διαλείπουσας Πολλαπλής Σκλήρυνσης)

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ΓΕΩΡΓΙΑ ΔΕΛΗΓΙΑΝΝΗ

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A.ABSTRACT

Natalizumab (Tysabri) and Fingolimod (Gilenya) are approved for relapsing-remitting MS patients. They're the most effective second line treatments for MS that have been approved in recent years, but few head-to-head comparisons and no randomized controlled comparisons of the safety and efficacy of these drugs have been done.

Objective: The aim of this study is to compare natalizumab and fingolimod on both clinical and MRI outcomes in patients with relapsing-remitting multiple sclerosis (RRMS). Primary endpoint is the Annualised Relapse Rate (year 1) and the cumulative probability of sustained progression of disability at 12 and 24 weeks (year2).

Methods: We designed a Phase III, multicenter, double blind, randomized trial with duration of 96 weeks (2 years).

Patients with RRMS included in the study were aged from 18 to 55 years with an Expanded Disability Status Scale score of 0–5.5 and an available brain MRI performed within the year before treatment initiation.

LIST OF ABBREVIATIONS AND TERMS

ABBREVIATION	TERM
ADR	Adverse Drug Reaction
AE	Adverse Event
AESI	Adverse Event of Special Interest
ARR	Annualized Relapse Rate
CI	Confidence Intervals
CIS	Clinically Isolated Syndrome
CRO	Contract Research Organization
CSF	Cerebrospinal Fluid
CSR	Clinical Study Report
CTCAE	Common Terminology Criteria for Adverse Events
DMT	Disease Modifying Treatment
eCRF	Electronic Case Report Form
EDSS	Expanded Disability Status Scale
GPP	Good Pharmacovigilance Practices
ICF	Informed Consent Form
MRI	Magnetic Resonance Imaging
MS	Multiple Sclerosis
PPS	Per Protocol Set
PRO	Patient Reported Outcome
RRMS	Relapsing Remitting Multiple Sclerosis
SADR	Serious Adverse Drug Reaction
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SDV	Source Data Verification
SmPC	Summary of Product's Characteristics

SYNOPSIS

Title of study:	A randomized double blind multicenter study to compare the efficacy of natalizumab versus fingolimod in patients with relapsing remitting multiple sclerosis
Planned study period:	2 years (96 weeks)
Clinical phase:	III
Objectives:	<p><u>Primary</u> To compare the Annualized Relapse Rate (year 1) and the cumulative probability of sustained progression of disability at 12 and 24 weeks (year2).</p> <p><u>Secondary</u> The secondary objectives of this study is to evaluate safety of both fingolimod and Tysabri in RRMS patients after swithing from other first line DMTs due to efficacy reasons.</p> <p>○</p>
Methodology:	<p><u>Study Design:</u> The study will take place in Greece in multiple centers. Investigators will participate from a broad geographical distribution in order to ensure a proper representation from all areas of Greece.</p> <p>The total duration of the study is 96 weeks. The recruitment period will be 12 months. Visits are scheduled every 3 months while the recruitment period is 12 months. Patients visits will be documented every 3 months(baseline, month 3, month 6, month 9, month12, month15, month18, month21, month24).</p> <p><u>Data collection:</u> The study follows closely the methodology used in several earlier studies of multiple sclerosis in Europe and United States. All data will be entered at the study sites into a central database using an electronic case report form (eCRF), specifically designed for the purpose of the study, with a delegated Contract Research Organization (CRO) as an administrator. This eCRF will contain information such as demographic characteristics, medical history, clinical data, resource utilization, intangible costs and utility, obtained during the patient's participation in the current study.</p>

	<p>Collected data are divided into two main categories: baseline data and follow up data.</p> <p><u>Baseline data</u> Patients' baseline data will be collected during the baseline visit:</p> <ol style="list-style-type: none"> 1) <u>Demographic characteristics:</u> age, gender, marital status, educational status, place of residence, etc. 2) <u>Medical history:</u> the age of the first onset of MS symptoms, the date of MS diagnosis, the number of relapses two years prior to study participation, the severity level of the disease, co-morbidities, and medication history (including previous Disease Modifying Treatments [DMT] and non-DMT for MS, as well as non-MS therapies) if any. 3) <u>Laboratory and examination data</u> <p><u>Follow up data</u> Patients' follow up data will be collected 3, 6,9,12,15,18,21 and 24 months post recruitment.</p> <ol style="list-style-type: none"> 1) <u>Adverse events occurring during study period, including SAE and AESI.</u> 2) <u>Number of disease relapses at each visit since the previous one, for the whole duration of the follow-up period.</u>
Total number of subjects planned:	612 patients is the enrolment target.
Diagnosis and main criteria for inclusion:	<p><u>Inclusion:</u></p> <ol style="list-style-type: none"> 1. Persons aged 18–55 years. 2. Patients with a confirmed diagnosis of RRMS. 3. Baseline EDSS score between 0.0 and 5.5, inclusive. 4. Have experienced at least one relapse within the 12 months prior to randomization. 5. Cranial MRI scan demonstrating lesion(s) consistent with MS.

	<p>6. Patients that have been previously treated with a first line DMT for RRMS but are eligible to therapy switch due to efficacy reasons.</p> <p>7. Patients must have signed an informed consent document.</p> <p><u>Exclusion</u></p> <p>8. Primary progressive, secondary progressive, or progressive relapsing MS.</p> <p>9. MS relapse has occurred, in the opinion of the investigator, within 50 days prior to randomization and/or the subject has not stabilized from a previous relapse.</p> <p>10. A clinically significant infectious illness within 30 days prior to randomization.</p> <p>11. History of, or abnormal laboratory results indicative of any significant cardiac, endocrinologic, hematologic, hepatic, immunologic, metabolic, urologic, pulmonary, gastrointestinal, dermatologic, psychiatric, renal and/or other major disease, that in the opinion of the investigator, would preclude the administration of a recombinant humanized antibody immunomodulating agent for 116 weeks.</p> <p>12. History of severe allergic or anaphylactic reactions or known drug hypersensitivity.</p> <p>13. Unable to perform the Timed 25-foot Walk, 9HPT, and PASAT 3.</p> <p>14. Abnormal blood tests performed at the Screening Visit.</p>
Treatment of interest	<p>Patients will be randomized in two groups and will receive the First Group: capsules(oral) fingolimod 0.5 mg once daily and Placebo, IV infusion, every 4 weeks, for up to 96 weeks and the Second Group: Natalizumab 300 mg IV infusion, every 4 weeks, for up to 96 week plus Placebo capsules once daily.</p>
Duration of observation:	<p>24 months follow up.</p>
Safety:	<p>At baseline and at weeks 12, 24, 36, 48, 60, 72, 84 and 96 post recruitment adverse events, serious adverse events, and adverse events of special interest will be collected.</p>

Statistical methods:**Sample size**

The estimate of sample size is based on data from previous trials of natalizumab and fingolimod with the power approach test. The annualized rate of relapse at one year is predicted to be 0.23 natalizumab and 0.18 with fingolimod. For an annualized relapse rate, the sample size required for 90 percent power (n=489). With an assumed dropout rate of 25%, the number of patients needed was estimated to be **612**.

Primary and secondary endpoints

Descriptive statistics will be used to evaluate demographic data, clinical characteristics, laboratory data, medical history, and resource utilization data. The annualized rate of relapse (the primary end point year 1) will be calculated by Poisson regression. The predefined statistical models include baseline scores on the EDSS for sustained progression of disability and the number of relapses in the previous year for the relapse rate. Additional baseline factors will be tested for inclusion in each of the models, including the EDSS score (≤ 3.5 or > 3.5), the presence or absence of lesions as detected by gadolinium-enhanced MRI, the number of hyperintense lesions as detected by T2-weighted MRI (< 9 or ≥ 9), and age (< 40 or ≥ 40 years). For the progression of disability (the primary end point year 2), a sensitivity analysis will be conducted on the change in EDSS scores that is sustained for 12 and 24 weeks. For the annualized relapse rate, sensitivity analyses will be performed with and without censoring, as well as with and without adjustment for significant covariates. The unadjusted relapse rate will be calculated as the total number of relapses divided by the total number of patient-years followed for each treatment group.

Secondary efficacy end points will be analyzed by logistic regression that include a term for the treatment group and the respective baseline measure as a covariate. In the analyses of secondary end points, missing values will be imputed using the mean for the respective measures in the study population.

Differences between treatment groups with regard to

	<p>adverse events will be analyzed by the chi-square test, and serious adverse events will be analyzed by Fisher's exact test. Poisson regression will be used to calculate the difference between the rates of infection in each treatment group.</p> <p>All analyses will follow the intention-to-treat principle. All reported P values are two-tailed. The one-year analyses will occur when 612 patient-years of data will be collected.</p> <p><u>Missing data handling</u></p> <p>For any of the post-enrolment time-points for which the drop-out rate pertaining to the questionnaires' completion is high, the specific analysis will also be performed using the last observation carried forward (LOCF) approach in order to account for missing data.</p> <p>If there are missing items, subscale scores can be prorated as long as answers have been provided in more than 50% of the items of the specific subscale.</p>
Date:	January 2017

STUDY RATIONALE

European Medical Agency (EMA) eligibility criteria for both fingolimod and natalizumab define that they are both used when the disease remains active despite appropriate treatment with at least one other disease modifying therapy, or is severe and getting worse rapidly, thus prompting a careful balancing between potential risks and efficacy profiles.

To date, few observational studies compared efficacy between natalizumab and fingolimod in real clinical setting, while there are still no head-to-head trial comparing efficacy of these drugs, which would eventually improve clinical decision making after switching to second line therapies.

The aim of this clinical study is to compare the efficacy of fingolimod and natalizumab in patients with RRMS after switching from a first line DMT due to efficacy reasons in a double blind randomized multicenter study with duration of 2 years.

B. INTRODUCTION

Multiple sclerosis (MS) is a chronic inflammatory, demyelinating, and neurodegenerative disease that affects the central nervous system (CNS). Affecting over 2 million people worldwide, MS usually starts at 20–40 years of age and is the most common cause of neurological disability in young adults. Although not all genetic and environmental risk factors are known, the etiology of MS involves both complex genetic trait and environmental factors. There are two major forms of MS. Relapsing-remitting (RR) MS is the most frequent form (85%–90%) and affects women about twice as often as men. The majority of patients with RRMS later develop secondary progressive disease. About 10%–15% of patients present with insidious disease onset and steady progression, termed primary progressive MS. Although the factors responsible for the different disease courses are not completely understood, the relapsing forms of MS seem to be driven mainly by acute inflammation that is initiated by adaptive immune cells, while neurodegeneration and chronic microglia-sustained inflammation seems to play a more important role in the progressive forms of the disease.

Treatment options for RRMS have broadened remarkably in recent years. Glatiramer acetate, interferon (IFN)- β preparations, mitoxantrone, natalizumab, fingolimod, alemtuzumab, dimethyl fumarate, and teriflunomide are drugs approved to treat RRMS in several countries and have different efficacy and safety profiles. While glatiramer acetate and IFN- β preparations have been used to treat MS for more than 20 years and have overall good safety profiles, the more recently approved treatments are more effective but also come with more safety complications.

NATALIZUMAB

Natalizumab, a monoclonal antibody against the $\alpha 4$ subunit (CD49d) of $\alpha 4$ integrins ($\alpha 4\beta 1$ and $\alpha 4\beta 7$), is one of these new treatments, and is very effective but has a complicated safety profile. $\alpha 4\beta 1$ integrin is expressed by all leukocytes except neutrophils and serves as an adhesion molecule mediating attachment to endothelial cells. Natalizumab prevents transmigration of leukocytes across the blood-brain barrier and the vascular endothelium of the gut by blocking $\alpha 4\beta 1$ integrin/vascular cell adhesion molecule-1 and mucosal vascular addressin cell adhesion molecule-1 interaction.

Natalizumab was approved by the US Food and Drug Administration (FDA) in 2004 for RRMS based on its high efficacy and good safety profile even before completion of two placebo-controlled, multicenter Phase III clinical trials, ie, AFFIRM (Natalizumab Safety and Efficacy in RRMS) and SENTINEL (Safety and Efficacy of Natalizumab in Combination with Interferon Beta-1a in Patients with RRMS). Only a few months after approval, a completely unexpected severe complication of natalizumab, ie, three cases of progressive multifocal leukoencephalopathy (PML), were identified in patients with MS and Crohn's disease. The manufacturers voluntarily suspended natalizumab in 2005 pending a safety review. After the review, it was considered that the estimated risk of PML was acceptable given the outstanding demonstrable therapeutic benefits of natalizumab, and the drug was reintroduced in the market in 2006 and approved by the FDA and European Medicines Agency for highly active RRMS, although with a pharmacovigilance plan to minimize the risk of PML. Eight years after licensing of natalizumab, considerable progress has been made in identification of risk factors for PML and approaches to risk minimization, and long-term studies to define better when to start or stop treatment and to optimize treatment strategies after cessation of natalizumab are ongoing. However, the PML complication still jeopardizes one of the most valuable treatments available for MS.

For patients with multiple sclerosis (MS) who are treated with injectable disease-modifying therapies (DMTs) for more than 2 years, the risk of relapse is 55 to 80%, and the risk of disability progression is 33 to 46%. Patients who demonstrate suboptimal response to these therapies are often switched to natalizumab (Tysabri) or fingolimod (Gilenya) for treatment escalation. Though these agents are considered to have higher efficacy than the injectable DMTs, there is limited data regarding their impact in breakthrough disease.

Clinical trials

AFFIRM and SENTINEL are two large, randomized, double-blind, placebo-controlled, multicenter Phase III clinical trials that provided the initial data regarding the short-term efficacy and safety of natalizumab in patients with RRMS. AFFIRM compared the efficacy of natalizumab monotherapy (300 mg intravenously every 4 weeks) with placebo, while SENTINEL compared the efficacy of natalizumab when added to IFN- $\beta 1a$ with that of IFN- $\beta 1a$ monotherapy. GLANCE (Glatiramer Acetate and Natalizumab Combination Evaluation) is also a published randomized, 24-week, double-blind, placebo-controlled Phase II clinical trial that evaluated the efficacy, safety, and tolerability of natalizumab

when added to glatiramer acetate in patients with RRMS. The short-term efficacy and impact on quality of life of natalizumab in these clinical trials have been extensively reviewed previously, so are not covered in this review.

Clinical practice and real-life settings

The efficacy of natalizumab and its impact on quality of life have also been studied in different clinical practice and real-life settings. Natalizumab was approved as a second-line treatment for patients in whom first-line treatments have failed and for patients with rapidly evolving MS. Consequently, patients treated with natalizumab in clinical practice had in general more severe disease than patients in clinical trials, and the majority had received disease-modifying treatments. For this reason, it was assumed that the efficacy of natalizumab in clinical trials might differ from the experience in clinical practice. Several studies have examined the clinical efficacy of natalizumab in open-label cohorts and shown that these patient populations also benefit from treatment with natalizumab. The effect of natalizumab on Expanded Disability Status Scale (EDSS) scores was evaluated in a retrospective study of patients treated for 44 weeks. In this study, treatment with natalizumab improved EDSS scores mainly in patients who had relapse-mediated worsening of EDSS. In addition, other studies also demonstrated an effect of treatment, with natalizumab improving visual acuity, visual evoked potential sum scores, and cognitive performance. In a small, prospective, magnetic resonance imaging (MRI) study, it was found that treatment with natalizumab over 2 years reduced cortical lesions and cortical atrophy when compared with untreated patients and patients treated with first-line therapies. This observation suggests that natalizumab might, by reducing inflammation, generate a more favorable environment for remyelination. Supporting a role for natalizumab in reducing axonal damage, it was observed in a longitudinal cerebrospinal fluid study of 92 MS patients treated with natalizumab that neurofilament light chain, a marker of neurodegeneration, decreased to the levels found in normal donors.

Long-term follow-up

There are two large, ongoing observational studies of note for which some results are partially available. These studies are STRATA (Safety of Tysabri Re-dosing and Treatment) and TOP (Tysabri Observational Program).

STRATA is a long-term (up to 10 years), open-label, multinational, follow-up study of natalizumab monotherapy (300 mg intravenously every 4 weeks) in patients with RRMS who completed the AFFIRM, SENTINEL, and GLANCE trials and their open-label extensions, and is evaluating the long-term safety and efficacy of natalizumab. In March 2013, after 4,135 patient-years of natalizumab exposure in STRATA (median of 64 natalizumab infusions), the annualized relapse rate remained low at 0.15 relapses per patient per year. Mean EDSS scores increased in the time between completing the feeder studies and enrollment in STRATA. Mean EDSS scores for patients who originally received natalizumab or placebo, respectively, were 2.9 and 3.1 at STRATA baseline, 2.7

and 3.1 at week 48, 2.7 and 3.2 at week 96, 2.8 and 3.2 at week 144, 2.9 and 3.2 at week 192, 2.8 and 3.2 at week 240, and 2.9 and 3.2 at week 288. Overall, patients on natalizumab generally experienced stable EDSS scores and a low annualized relapse rate over time. Patients initially randomized to 2 years of treatment with natalizumab in the Phase III clinical trials retained their lower EDSS scores compared with those initially randomized to placebo. This suggests important advantages of early treatment with natalizumab.

TOP is a 10-year prospective, multinational, open-label, post-marketing, observational study of the long-term clinical safety and efficacy of natalizumab in the European, Australian, and Canadian settings. Patients must have active RRMS and be natalizumab-naïve (≤ 3 doses prior to enrollment), but may have received other disease-modifying therapies prior to natalizumab. A preliminary report on the TOP study was presented in June 2011. At that time, 3,484 patients from 15 countries were enrolled. Overall, the mean EDSS score was 3.5 at baseline and 3.4 after 3 years. The overall annualized relapse rate was significantly decreased from baseline (1.98) to post-baseline (0.28, $P < 0.0001$). Annualized relapse rates at baseline were similar (1.91–2.03) regardless of the patient's previous therapy, but after 3 years of treatment with natalizumab, the annualized relapse rates differed significantly according to pre-enrollment therapy ($P < 0.0001$), ie, therapy-naïve (0.16), prior IFN (0.20), prior glatiramer acetate (0.23), prior IFN/glatiramer acetate or glatiramer acetate/IFN (0.25), and prior immunosuppression (0.34).

After this general update, two subsequent updates have been presented. One update reported TOP results obtained in the UK until June 2012. At that time, 117 patients were enrolled in the UK and received a median of 15 (range 1–27) natalizumab infusions. The median number of relapses in the year prior to treatment with natalizumab was 2.0, 24.8% of the patients had one relapse, and 75.2% had more than one relapse. The overall annualized relapse rate decreased from 2.26 at baseline to 0.38 on treatment ($P < 0.0001$). The annualized relapse rate decreased significantly from baseline regardless of baseline treatment history and relapse history. In treatment-naïve patients, the relapse rate was 2.54 at baseline versus 0.48 on treatment ($P < 0.0001$). In patients previously treated with one disease-modifying therapy, the relapse rate was 2.14 at baseline versus 0.36 on treatment ($P < 0.0001$). In patients with more than one prior disease-modifying treatment, the relapse rate was 2.00 at baseline versus 0.26 on treatment ($P = 0.0003$). Regarding history of relapse, in patients with one relapse in the prior year, the relapse rate was 1.00 at baseline versus 0.27 on treatment ($P < 0.001$), and in patients with more than one relapse in the prior year, the relapse rate was 2.67 at baseline versus 0.43 on treatment ($P < 0.0001$). EDSS scores were stable, with a mean of 4.2 at baseline, 4.0 at month 6, 4.4 at year 1, and 4.3 at year 2. This interim analysis of TOP results in the UK indicated that treatment with natalizumab improves the annualized relapse rate, regardless of baseline treatment or relapse history, and stabilizes EDSS scores.

A second observational study reported TOP results obtained in Germany until February 2012. In this study, the annualized relapse rate in the year prior to treatment with natalizumab was 2.2, the EDSS was 3.5, and the Demtect cognitive function score at baseline was 14.9. After treatment with natalizumab, the relapse rate decreased to 0.14, the EDSS stabilized at 3.5, and the Demtect score increased to 16.0. This interim study confirms the sustained efficacy of natalizumab in reducing clinical disease activity.

Safety of natalizumab and impact on quality of life

Progressive multifocal leukoencephalopathy

PML is a demyelinating disorder of the CNS caused by John Cunningham virus (JCV) infection. JCV is a ubiquitous virus, which infects 60%–80% of the population worldwide without clinical consequences, but can cause PML under circumstances of immunocompromise and especially impaired CD4+ T cell function, such as in late-stage human immunodeficiency virus infection, hematological malignancies, and organ transplantation, and also in clinically inconspicuous idiopathic CD4+ lymphopenia. PML has recently emerged as a serious adverse event during monoclonal antibody treatment of autoimmune diseases, especially in MS patients treated with natalizumab. The first two cases of PML in natalizumab-treated patients developed during premarketing studies, with one case occurring in the SENTINEL trial after 28 doses of natalizumab and the other in the open-label extension of SENTINEL after 37 doses of natalizumab. One patient died from PML and the other patient with PML progressed rapidly. These cases were followed by a case of PML after eight infusions of natalizumab in a patient with Crohn's disease.

As mentioned above, after these PML cases, the manufacturer voluntarily suspended natalizumab in 2005 pending a safety review. In March 2006, an extensive safety study of 3,116 patients with MS, Crohn's disease, or rheumatoid arthritis who received treatment with natalizumab were assessed for PML. Of these, 44 were referred to the expert panel because of clinical findings of possible PML, abnormalities on MRI, or a high plasma load of JCV. No patient had detectable JCV DNA in cerebrospinal fluid. PML was ruled out in 43 of the 44 patients, but could not be ruled out in one patient because cerebrospinal fluid and follow-up MRI were not available. No new PML cases were identified in this study, suggesting a risk of PML of roughly one in 1,000 patients treated with natalizumab for a mean of 17.9 months.

Based on this study, it was considered that the estimated risk of PML was acceptable given the demonstrably outstanding therapeutic benefits of natalizumab, and the drug was reintroduced in the market in June 2006 and approved by the FDA and European Medicines Agency for highly active RRMS. Since then, the number of cases of PML has continued to increase. As of February 2012, 212 confirmed PML cases among 99,571 patients treated with natalizumab were reported, giving an incidence of 2.1 cases per 1,000 patients. Twenty-two percent of these PML patients died and 40% of the evaluable survivors had severe disability. As of March 2013, the number of patients with confirmed PML was 343 among more than 112,000 treated patients, giving a risk of PML associated with natalizumab of 2.96 cases per 1,000 patients. The mean duration of treatment in these patients was 39 months. The most recent media release, in December 2013, indicates an overall incidence of PML in natalizumab-treated patients of 3.41 cases per 1,000 patients.

In general, the outcome of natalizumab-associated PML is better than in PML associated with human immunodeficiency virus. Two case series studies demonstrated that around 70% of natalizumab-associated PML cases survived and that survival is correlated with younger age, lower EDSS prior to diagnosis, lower JCV load at diagnosis, and more

localized brain involvement on MRI at the time of diagnosis. The most recent media release from December 2013 indicates that 77% of the PML patients are alive, with varying levels of disability. However, despite this better outcome of PML in MS patients, cessation of natalizumab therapy in these patients could have serious negative consequences. Unlike general immunosuppression, natalizumab therapy selectively compromises immune surveillance only in the CNS and gut, and only during treatment. Cessation of natalizumab therapy in MS patients who develop PML re-establishes immune surveillance in the CNS and leads to an inflammatory response known as PML immune reconstitution inflammatory syndrome, which allows efficient destruction of JCV-infected cells and eradication of the virus, but can also lead to rapid deterioration of the patient's clinical state and death in about 30%–50% of cases. The cellular and molecular pathogenesis of PML immune reconstitution inflammatory syndrome is not completely understood, but histological analysis of biopsy samples demonstrated that both CD8 and CD4 inflammation dominated the T cell infiltrate.

During 10 years of use of natalizumab as a treatment for MS, considerable progress has been made in identification of risk factors for PML and approaches to risk minimization. Positive status with respect to anti-JCV antibodies, prior use of immunosuppressants, and increased duration of treatment with natalizumab (more than 2 years) are the main PML risk factors identified. The estimated incidence of PML in patients with all three risk factors was 11.1 cases per 1,000 patients, compared with an estimated incidence of 0.09 cases per 1,000 in the group of patients negative for JCV antibodies. Based on this risk stratification, treatment recommendations have been proposed that include testing for JCV antibodies prior to starting treatment with natalizumab and every 6 months thereafter. As of December 2013, results of the anti-JCV antibody assay have been reliable in stratifying PML risk; 99% of PML patients with available pre-PML samples tested positive for anti-JCV antibodies.

The incidence of PML in natalizumab-treated patients is higher in Europe than in the USA, and the treatment duration beyond which the risk for PML increases is also longer in the USA. Although the reason for this difference is unknown, it has been proposed that it might be related to body mass index. It has been hypothesized that a low body mass might translate into higher concentrations of natalizumab in plasma, higher or more complete VLA-4 saturation rates in immune cells, and a consequently decreased immunosurveillance of the CNS and a higher incidence of PML. Reducing the natalizumab dose or increasing the interval between infusions may reduce this risk.

Regarding biomarkers for risk of PML, it was recently reported that the percentage of L-selectin-expressing CD4+ T cells was significantly lower in patients on long-term treatment with natalizumab (40.2%) when compared with patients not receiving natalizumab (47.2%, $P=0.016$) and healthy controls (61.0%, $P<0.0001$). This unusually low percentage correlated with the risk of developing PML in the patient group with available pre-PML samples when compared with natalizumab-treated patients without PML ($P\leq 0.0001$). These results suggest that a low percentage of L-selectin-expressing CD4+ T cells might be useful as a biomarker for individual PML risk.

It has also been suggested that natalizumab might reduce T cell responses against JCV in peripheral blood, facilitating reactivation of JCV. It has been reported that subclinical

reactivation of JCV occurs frequently in natalizumab-treated patients and that viral shedding is associated with a transient drop in JCV-specific cellular immune responses, suggesting that monitoring JCV-specific T cell responses might be an interesting biomarker for risk of PML. However, neither the reduced T cell response nor viral reactivation in peripheral blood during treatment with natalizumab has been reproduced in other studies.

Treatment with natalizumab not only compromises immune surveillance of the CNS, but also mobilizes lymphoid hematopoietic precursor cells from the bone marrow and induces significant sustained phenotypic changes in the immune cell composition of peripheral blood, such as an increase in T cells, natural killer cells, and especially B cells, and a decrease in monocytes, suggesting that there is no desensitization effect after prolonged exposure to natalizumab. Mobilization of lymphoid precursors and B cells might contribute to development of PML, given that this might result in a significant increase in cells that are in principle able to produce and spread progeny virus. Monitoring circulating JCV-infected hematopoietic cells and B cells might be an interesting biomarker for risk of PML and deserves further investigation.

Other issues with natalizumab

Short-term side effects

Natalizumab is generally well tolerated, and few short-term side effects were reported in the Phase III AFFIRM and SENTINEL clinical trials. The more common reported side effects were nonserious, and included fatigue, anxiety, pharyngitis, sinus congestion, peripheral edema, and allergic reactions. Allergic reactions are often associated with the presence of antibodies against natalizumab. Data from the Phase III clinical trials indicated a higher percentage of allergic reactions in treated individuals than in those on placebo, and around 10% of treated patients developed anti-natalizumab antibodies. Persistently high anti-natalizumab antibody titers are associated with a lack of drug efficacy, a reduction in the monoclonal antibody concentration in serum, and infusion-related hypersensitivity reactions. The short-term side effects of natalizumab in these clinical trials have been previously reviewed in detail.

Natalizumab, like other T cell immunomodulatory treatments, has been associated with liver injury. Although this is not a common side effect, some cases have been reported of MS patients who developed serious liver injury after their first natalizumab infusion. Patients developed increased serum aminotransferases and hyperbilirubinemia and sometimes signs of autoimmune hepatitis, such as autoantibodies and liver inflammation. These adverse events normally appeared after the first natalizumab infusion and remitted after discontinuation of treatment. Although the mechanism by which natalizumab might induce liver damage is unknown, it is recommended to monitor for signs of autoimmune hepatitis or atypical viral infections before and during treatment with natalizumab.

Long-term side effects

Long-term impaired immunosurveillance in MS patients treated with natalizumab has been associated with several opportunistic infections other than JCV, although a higher risk has not been proven. Human herpesvirus-6 (HHV-6) is a pleiotropic β -herpesvirus that infects cells in the CNS. HHV-6 is commonly reactivated in situations of immunosuppression and has been associated with MS. Several lines of evidence, including an increase in the level of HHV-6-specific antibodies in serum and an increase in HHV-6 DNA in the cerebrospinal fluid of natalizumab-treated MS patients, suggest that HHV-6 might reactivate on treatment with natalizumab. Herpes simplex and varicella zoster CNS infections have recently been reported in 20 natalizumab-treated MS patients. Finally, a single case of ocular toxoplasmosis has been reported after 11 months of treatment with natalizumab. *Toxoplasma gondii* infects 30% of the population without causing symptoms, but in immunocompromised individuals can reactivate and lead to organ damage.

The relationship between long-term treatment with natalizumab and a higher susceptibility to developing malignancy is still under debate. Several cases of malignant melanoma while on treatment with natalizumab have been reported. However, the prevalence of melanoma was not different when comparing natalizumab-treated and untreated MS patients. Single cases of primary CNS lymphoma and peripheral T cell lymphoma have been reported in natalizumab-treated MS patients, although a direct cause-effect relationship has not been demonstrated. Other hematologic changes, such as hypereosinophilia, immune-mediated acute hemolytic anemia, and immune thrombocytopenic purpura, have occasionally been reported in natalizumab-treated MS patients.

Impact on quality of life

Not only the risk of PML, but also other adverse events, such as hypersensitivity reactions, recurrent infections, or malignancies, or other conditions, such as pregnancy, might render it advisable to discontinue natalizumab. Given that natalizumab is a treatment with high benefits along with very severe risks, experts have advocated an informed re-consent for patients after 2 years of treatment with natalizumab. Although a good understanding of the benefits and risks is important regarding the decision whether to continue or stop treatment, the perception of risk differs between patients and neurologists. In a study published in 2010, when the estimated risk for natalizumab-induced PML in MS patients treated for more than 2 years was about one in 1,000, patients perceived MS as a malignant disease, so were willing to accept a considerably higher PML risk than neurologists. Identification in recent years of JCV seropositivity, duration of treatment with natalizumab, and prior immunosuppression as risk factors, has modified this acceptance of risk. Although the large majority of JCV-seropositive patients are still willing to continue treatment, seropositive patients have elevated anxiety levels, are less secure about their decision to continue treatment, feel less safe, and are more afraid of PML. One important complication of cessation of natalizumab with crucial consequences for patients is that it is presently not known which treatment strategy is the more appropriate to follow after stopping treatment with natalizumab. It is known that

disease activity returns to pretreatment levels or even above within 4–7 months from the last natalizumab infusion, so patients stopping natalizumab are at considerable risk of rebound relapse/inflammatory activity and worsening MS-related disability. Several strategies have been used to avoid disease activity after discontinuation of natalizumab. One of these strategies, which unfortunately has not been very effective, was use of methylprednisolone as a bridge before switching to glatiramer acetate or IFN- β . Better results have been obtained in some case series using fingolimod as subsequent therapy, although long-term data on the efficacy and safety of fingolimod after cessation of natalizumab are still missing. Other therapies available, such as fumarate, might represent a good alternative to optimize escalation treatment for patients who stop natalizumab, but further long-term studies are needed.

The clinical experience with natalizumab has provided important lessons. While the drug continues to be perceived as highly effective and well tolerated overall, the risk of PML remains an important safety concern that is not solved at present despite risk stratification algorithms. Accordingly, last December, the FDA approved a label update, announcing that natalizumab is no longer generally recommended for those who have responded inadequately to or are unable to tolerate alternative MS therapy. Further important questions that merit more detailed investigation are whether certain markers can allow us to identify patients who will show severe rebound disease activity after cessation of natalizumab, how to switch over from natalizumab to other drugs, and over what periods of time.

FINGOLIMOD

In addition to efficacy issues for current MS therapies, side effects are a major concern for all currently used agents. Interferons are often associated with injection-site reactions and flu-like symptoms, with other less commonly reported events including liver dysfunction and cytopenias. Of greater concern with the efficacious agent natalizumab is the increasing incidence of progressive multifocal leukoencephalopathy (PML), a rare but serious infection associated with immunosuppression that has resulted in multiple fatalities in MS patients receiving this therapy. Another example is the cytostatic agent mitoxantrone that has a cumulative dose-dependent cardiac toxicity with additional risk of leukemia, both of which limit its long-term use. The latter two agents underscore immunosuppressive liabilities of these and other current and future MS therapies that primarily inhibit immune function as their primary mechanism of action. Efforts to develop new MS treatments, as evidenced by those in current development, underscore both limitations and a need for therapies with more convenient, effective, and safe treatment profiles.

Despite their introduction nearly 20 years ago, interferon therapies still represent the most commonly prescribed disease modifying agents for MS. This situation epitomizes deficiencies in the current state of therapeutics using agents that lack ease of delivery, such as those requiring injection, as well as efficacy characteristics, particularly for preventing neurodegeneration. Drugs under development for MS include the monoclonal antibodies named rituximab, ocrelizumab, and ofatumumab (which target CD20 to deplete B cells); alemtuzumab/Campath-1H (which targets CD52 to deplete T and B cells and some monocyte-derived dendritic cells); and small molecules including the oral

agents. These oral agents include cladribine (a cytotoxic adenosine deaminase-resistant purine nucleoside, recently withdrawn from commercial development), dimethylfumarate BG-12 [an activator of the nuclear factor-E2-related factor 2 (Nrf2) transcriptional pathway that alters glutathione levels , but is also associated with tumorigenesis and immunosuppression], laquinimod (unknown cellular target), and teriflunomide (an inhibitor of dihydroorotate dehydrogenase, which catalyzes the rate-limiting step in the de novo synthesis of pyrimidines). All of these agents target lymphocytes as well as other immune-related cells. As of this writing, they also all remain subject to additional clinical and regulatory evaluation before possible entry into the therapeutic armamentarium.

A major step towards achieving the goal of a mechanistically novel, orally bioavailable agent has recently been taken through FTY720/fingolimod (commercial name Gilenya; Novartis) that represents the first oral MS therapy approved by the United States Food and Drug Administration (FDA), the European Union, and several other countries. This compound interacts with a new, until now clinically unassessed molecular target: receptors for the signaling lysophospholipid known as sphingosine 1-phosphate (S1P).

Origins of Fingolimod: A Folk Medicine from Fungi

Fingolimod emerged from the study of the fungal phylum Ascomycota, historically, commonly, and taxonomically referred to as class Ascomycetes within the kingdom Fungi. Within this group of “sac fungi” is the species *Cordyceps* of family Cordycipitaceae, which constitutes over 400 members characterized prominently by their entomopathogenic activity wherein the fungus infects a range of insect host species at different points in their development, parasitize them to grow out of the corpse to form the stalk and fruiting body of the fungus . Efforts to produce chemically modified compounds derived from myriocin led, in 1994, to the identification of a novel compound, fingolimod , whose original name, FTY720 , reflects the discoverers: Fujita and colleagues at Kyoto University, Taito Company , and Yoshitomi Pharmaceutical Industries . The compound was of interest based upon preclinical data that supported activities that might be relevant to improved organ transplantation , which merged with Ciba-Geigy in 1996 to form Novartis. Fingolimod showed activity in models of organ transplantation when combined with Cyclosporin A, which led to in-licensing of fingolimod by Novartis for evaluation as a therapeutic agent in renal transplantation.

In particular, chemical modification efforts of the parental myriocin compound to produce fingolimod might have been expected to maintain some of the myriocin activities; however, fingolimod had no activity against SPT . Consistent with an altered activity profile and contrasting with myriocin, fingolimod did not inhibit activation, proliferation, or memory formation of T cells; moreover, it did not affect the production of antibodies by B cells or cytokines by T cells and, as a consequence, did not impair immune against systemic viral infection . Thus, fingolimod appeared to be acting in a way distinct from classically defined immunosuppressants through an unclear molecular target(s) and mechanism of action.

Parallel Discovery of Lysophospholipid S1P Receptors, a Molecular Target for Fingolimod

Around the same period of fingolimod's discovery, a completely independent line of research was investigating genes involved in CNS development, which led to the identification of the first member for what is now known as the lysophospholipid family of receptors. These G protein-coupled receptors (GPCRs) mediate the actions of at least two major classes of signaling lysophospholipids that are known as lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P), the latter of which comprises the major receptor target for fingolimod. The first family member recognized was for LPA and this identity aided deorphanization of other LPA receptors, as well as the S1P receptors that at one point were hypothesized to interact with both LPA and S1P reflecting their marked amino acid sequence similarities. There are currently 11 receptors, six for LPA and five for S1P. Lysophospholipid receptors were first identified by exploiting knowledge of their expression and activity in the developing CNS, and their other activities and the cell types on which they express have now been identified within the CNS. These receptors also have prominent expression in the immune system as well as other tissues, and have been implicated in a broad range of biological and pathophysiological processes.

The structural similarity of fingolimod to sphingosine led to the key identification of S1P receptors as the target for phosphorylated fingolimod. Sphingosine is a metabolite of the cell-membrane-derived sphingolipids such as sphingomyelin and ceramide. Phosphorylation is produced by the action of endogenous sphingosine kinases (SPHKs) to produce fingolimod-phosphate (fingolimod-P), primarily involving the enzyme SPHK2. Receptor studies revealed that fingolimod-P (but not parent fingolimod) is an agonist at S1P1, S1P4, S1P5 (EC50 values of ~ 0.3–0.6 nM), and S1P3 (EC50 values of ~ 3 nM), with essentially no activity at S1P2 at these concentrations. Stereochemical analyses identified (S)-fingolimod-P as the biologically active form *in vivo*, whereas (R)-enantiomer was not detected. Thus, the discoveries of fingolimod and of their targeted lysophospholipid receptors provided a basis for understanding fingolimod's actions. However, the therapeutic indications for fingolimod evolved in unanticipated ways.

FINGOLIMOD to Multiple Sclerosis

Initial human studies with fingolimod led to two pivotal Phase III clinical trials focused on a possible reduction in the annualized relapse rate (ARR) as the primary endpoint using fingolimod in relapsing remitting MS (RRMS): a placebo-controlled trial of 1,272 patients (the FREEDOMS trial) and an active comparator trial involving 1,292 patients (the TRANSFORMS trial). In FREEDOMS, fingolimod was investigated in a double blind, 2-year study that involved patients randomized to a reduced dose arm of 0.5 mg or 1.25 mg as compared to a placebo. Once again, the ARR was significantly reduced in both experimental arms (0.18 and 0.16, respectively) compared to the placebo (0.40), as was risk of disability progression (17.7% and 16.6%, respectively) compared to placebo (24.1%) and MRI-detected lesions, which included the previously assessed gadolinium-enhancing lesions, and interestingly, a reduction in brain-volume loss (atrophy) (–0.7% in both fingolimod arms compared to –1.0% with placebo). In TRANSFORMS, a 1-year,

double blind, double-dummy trial, fingolimod was compared to IFN2-1a (Avonex, Biogen Idec), a major, first-line therapy for MS. The same fingolimod doses were compared to standard administration of IFN β -1a. Both fingolimod arms showed an ARR that was significantly lower (0.16 and 0.20, respectively), compared to the IFN β -1a arm (0.33). Disability progression was similar, albeit difficult to assess during the short, 1-year trial; however, both fingolimod doses showed superior MRI endpoints of fewer new or enlarged lesions on T2-weighted images, gadolinium-enhancing lesions, and reduced brain atrophy at 12 months.

Serious adverse event rates in the 2-year FREEDOMS trial were similar amongst all groups including placebo (10.1–13.4%), whereas in the 1-year TRANSFORMS study, the incidence of adverse events was higher in the high dose arm (1.25 mg) but comparable in the 0.5 mg fingolimod vs. IFN β -1a arms. The incidence of adverse events that resulted in drug discontinuation was similar between the 0.5 mg fingolimod and all control groups, but was higher in the 1.25 mg fingolimod groups. The combined data from both Phase III trials did not suggest an increased incidence of either infections or malignancies associated with fingolimod treatment. The two lethal herpes infections (one primary varicella zoster infection and one case of herpes simplex encephalitis) that occurred in patients receiving 1.25 mg fingolimod may have involved confounding factors related to the use of high-dose steroids. In view of the comparable efficacy of the lower 0.5-mg fingolimod dose, along with its improved safety profile, the 0.5-mg fingolimod dose was nominated for regulatory approval in providing the best risk-to-benefit profile, and during 2010–2011, fingolimod (commercial name Gilenya) received approvals in the United States, the European Union, and several other countries.

Fingolimod Immunological Activities

In part reflecting its origins in transplantation research, fingolimod has been extensively studied for its effects on immune system. Early studies raised the possibility that fingolimod might interfere with T cell trafficking rather than function, and that its mode of action may involve G α i protein-coupled receptors . It was then found that conversion of fingolimod to its phosphorylated metabolite, fingolimod-P, and the interaction with cognate S1P receptors result in trafficking effects as demonstrated by the sequestration of lymphocytes in secondary lymphoid organs .

These studies provided a link to the biological effects of fingolimod, shown in transfected cell lines, to produce, paradoxically, S1P receptor internalization, removing them from the cell surface to inhibit S1P signaling, despite the initial characterization of fingolimod as a receptor agonist (after phosphorylation) . Immunological studies of knockout mice for S1P1 — whereby this receptor was removed from lymphocytes — resulted in lymphocyte trafficking defects that were similar to the effects of fingolimod exposure, suggesting that fingolimod was acting as a “functional antagonist” to inhibit S1P receptors . Interestingly, mutations in the S1P1 receptor that abrogated internalization, but not signaling of the receptor, were sufficient to blunt lymphocyte trafficking effects of fingolimod, confirming that receptor internalization rather than signaling was required, presumably to prevent S1P-directed migration of cells . In other words, although fingolimod-P initially acts as an

S1P receptor agonist, chronic exposure to it results in S1P receptor loss from the surface of the cell and abrogation of receptor-mediated S1P signaling.

Modulation of S1P1 receptors by S1P and fingolimod. To ensure that extracellular stimuli are translated into intracellular signals of appropriate magnitude and specificity, most signalling cascades are tightly regulated.

The orchestrated role for S1P signaling that allows lymphocytes to egress from the lymph nodes predominates over another signaling system mediated by chemokines and the receptor known as CCR7, which promotes retention of lymphocytes in secondary lymphoid organs with preferential effects on “naïve” and early “central” memory T-cells rather than late, terminally differentiated “effector” memory T-cells . Thus, S1P1 signaling appears to predominate over CCR7-mediated retention by promoting lymphocyte egress from lymph nodes, whereas functional antagonism of S1P1 by fingolimod inhibits egress from lymph nodes. By contrast, egress of CCR7-negative effector memory T cells appears to occur independently of S1P1 receptor signaling and these cells are refractory to the trafficking effects of fingolimod. This latter point is supported by studies in mice and humans .

Two corollaries with particular relevance to MS through this differential effect of fingolimod on CCR7-positive vs. CCR7-negative T cells may contribute to efficacy and safety, respectively. First, fingolimod may produce efficacy by sequestering the CCR7-positive cells, which include naïve and central memory T cells, the latter of which have a key role in immunological memory. Following antigen exposure, central memory T lymphocytes can undergo clonal expansion and differentiation to generate effectors/effector memory T cells which provide adaptive immunity against recognized antigens . Central memory T cell retention by fingolimod could function as a therapy in MS since more than 90% of T cells that are found in the cerebrospinal fluid (CSF) appear to be of the central memory subset . The contained autoreactive, pathological T cells could therefore be prevented from entering the CNS by fingolimod sequestration, thereby abrogating their differentiation into pathological effectors and effector memory T cells upon interaction with CNS-resident antigen-presenting dendritic cells. In animal models, fingolimod prevented accumulation of pathological Th17 cells in the nervous system , supporting Th17 cell- or Th17 cell precursor sequestration as an efficacy mechanism. Accordingly, phenotypic Th17 cells were reduced in the circulation in fingolimod-treated MS patients . In addition to efficacy by CCR7-positive cell sequestration of pathological T cells, fingolimod could provide safety through maintained immunosurveillance. Such functionality would be produced by preferentially not affecting CCR7-negative effector memory T cells of any functional phenotypes , which may leave lymph nodes, independent of S1P1 signaling .

In support of the above, another study proposed that Gai2 null T cells egress independent of S1P-mediated chemotaxis , and these cells were also not retained by fingolimod. Intravital imaging of lymph nodes revealed that T cells approach and engage cortical sinusoids in lymph nodes similarly in the presence or absence of fingolimod. However, after engagement of the sinus, most T cells retract and migrate back into the parenchyma in fingolimod-treated animals, due to a failure of the cells to establish adhesion on the

sinus, whereas Gai2-deficient T cells adhere firmly on the sinus, which prevents their retraction, facilitating their transmigration of the lymphatic endothelial barrier. Interestingly, Gai2-deficient T lymphocytes are hyper-responsive for T cell receptor signaling and cytokine production, with a relaxed costimulatory requirement— a phenotype matching effector memory T cells — again supporting sparing of this subset by fingolimod.

Collectively, the data show that activation and proliferation of naïve and central memory T-cells, as well as differentiation and trafficking of effector memory T-cells, may not be significantly affected by fingolimod, thereby preserving this arm of the adaptive immune system that can reduce the risk of infection and cancers common with immunosuppressive agents. Consistent with this mechanism, the combined data from both aforementioned Phase III trials did not suggest an increased incidence of either infections or malignancies associated with fingolimod treatment.

In addition to the immunomodulatory effects, fingolimod also simultaneously accesses a completely different biology: direct CNS actions on cells relevant to MS, which if present, would be an efficacy mechanism independent of immunomodulation. While the exact role that fingolimod's CNS actions might have in MS are not known, a growing body of basic and clinical literature supports direct CNS influences that are discussed next.

Fingolimod CNS Activities

Based upon its relationship to sphingosine, and its interactions with lysophospholipid S1P receptors, fingolimod was likely to have direct CNS effects. As an analog of the lipid sphingosine, fingolimod could have a range of possible roles within the CNS, since that is where sphingosine and related sphingolipids (e.g., phospholipids that contain sphingosine, like sphingomyelin) were first identified from early studies of the brain during the 1800s. The functions of sphingosine were then as enigmatic as the Sphinx, from which its name was coined. Consistent with CNS actions, fingolimod and fingolimod-P localize to the CNS as revealed by radiolabeling studies. Independent support for CNS activities came from studies of lysophospholipid receptors that were first identified from the brain, with most, including S1P receptors, expressed in CNS lineages where they have a rich neurobiology.

Data supporting possible direct CNS effects included the aforementioned fingolimod localization within the CNS, the rapid onset of therapeutic effects with a “rescue” therapy started 40 days after disease onset, and a discordance between clinical scores and peripheral lymphocyte levels seen in some EAE animal models. Receptor-mediated S1P signaling has been documented in CNS cell lineages that have relevance to MS, consistent with broad expression of lysophospholipid receptors in general within the brain. Astrocytes in particular express S1P1 and S1P3, and these two receptors have been reported to be upregulated in MS astrocytes. Oligodendrocytes and their precursor cells also express S1P receptors particularly S1P5 in mature oligodendrocytes. Neural progenitor cells, and likely some neurons, can also express S1P1, along with other S1P receptor subtypes. In addition, resident non-neural cells like microglia can also express S1P receptors. The diversity of both cell types and S1P receptor subtypes underscore potential effector activities of fingolimod within the CNS in MS. The receptor mechanisms

could involve some degree of transient, initial agonism; however, continuous exposure to fingolimod would be expected to produce functional antagonism — a net loss of S1P receptor signaling — at least for S1P1 that has been best characterized in non-neural cells. Pharmacological S1P1 loss through functional antagonism can be rigorously modeled by use of genetics via the production of null mutation knockouts.

This knockout strategy was used to address functional consequences of removing S1P1 from specific cell lineages in the CNS while leaving the immune system intact, combined with challenge by EAE . The resulting mutants were then assessed for 1) effects on fingolimod activity, and 2) effects on clinical disease, independent of fingolimod exposure, combined with other analyses . S1P1 was conditionally deleted (using loxP technologies) from various CNS cell lineages while still maintaining immunological competence as evidenced by normal responses of peripheral blood lymphocytes to fingolimod exposure, and an ability of mutant lymphocytes to produce disease following adoptive transfer from mutant into normal animals. Of the lineages assessed, S1P1 deletion from astrocytes but not neurons produced a dramatic effect, eliminating fingolimod activity in EAE, compared to vehicle controls, and also attenuating MS-like disease. Consistent with these clinical assessments of disease, S1P1 deletion on its own protected against histologically detected damage as compared to control animals challenged by EAE, including a marked reduction in astrogliosis — a reactive state of astrocytes that increases their number and alters their morphology —along with preservation of axons and myelin that would usually be damaged by EAE. Fingolimod exposure during EAE produced a similar histological picture when assayed in normal (non-mutant) animals, and competitive receptor binding assays using membrane preparations from brains of these animals confirmed down-modulation of S1P receptors by the drug, supporting the functional antagonism model of S1P1 loss that had been previously observed in the immune system, a receptor mechanism that was further shown to occur in astrocytes as well.

Overall, these data identify S1P1 signaling in astrocytes as a major influence on models of MS, as well as a necessary component of fingolimod efficacy . The combined effects of the drug on lymphocyte trafficking and astrogliosis may reduce neurodegeneration and favor remyelination after damage, as observed in models of EAE and cuprizone-induced demyelination . Other S1P receptor subtypes and/or involved cell lineages may also have related influences, and these remain to be addressed in MS models. One or more of these processes might explain the reduction in brain atrophy observed with fingolimod treatment in MS Phase III trials contrasting with distinct and at times increasing atrophy signals, observed with immunologically targeted therapeutics like natalizumab

Immunomodulatory Approaches to MS Therapy

A notable corollary of the dual immunological and CNS fingolimod mechanisms is that fingolimod does not fit the profile of an immunosuppressive agent like those in common use in the transplantation field - e.g., “classical” immunosuppressive agents like calcineurin inhibitors [Cyclosporine, Tacrolimus], high dose corticosteroids [e.g., Prednisone], and cytotoxic and/or antimitotic agents [azathioprine, mycophenolate, or

cladribine] or biologicals, including a growing number of humanized antibodies raised against immune cell targets [CD3, IL-2 receptor, integrins, CD52]. Early approaches to the treatment of MS utilized classical immunosuppressive strategies, some of which continue to be used today . However, risk of serious neoplastic and/or infectious adverse events limits their use. This issue has been underscored by the rare occurrence of progressive multifocal leukoencephalopathy (PML) associated with the use of natalizumab or rituximab . T cell immunosuppression may be involved in both cases; in addition to its effects on T cell trafficking, natalizumab may interfere with the VLA4-VCAM1 costimulatory pathway that is critical to human CD4 T cell proliferation , and therapeutic B cell depletion by rituximab was shown to impair B cell antigen-presentation and, as a consequence, CD4 T cell activation and clonal expansion in response to pathogen challenge . Therefore, the sparing of effector memory T cells in both CD4 and CD8 populations by fingolimod could be critical to immunosurveillance; in the meninges of mice, fingolimod preferentially reduced naive and central memory T cells, whereas anti-VLA4 treatment primarily depleted the effector memory population(see also above for the key role of circulating central memory T cells in pathology of MS).

Compared to human CD8 T cells, circulating CD4 T cells contain larger numbers of CCR7-positive naive and central memory T cells, and this could explain the more profound retention of CD4 T cells in lymph nodes by fingolimod . Importantly, infection-relevant effector memory T cells could still be generated in lymph nodes and would recirculate independent of S1P1 ; thus, the reduced total CD4 T cell count in blood may not prove useful as an indicator of immunosuppression in fingolimod-treated patients.

The above data support the notion that fingolimod at its approved dose may not act as a potent immunosuppressant: 1) CNS effects are unrelated to immunosuppression; 2) suboptimal prevention of graft rejection was achieved in renal transplantation studies in combination with cyclosporine, despite being at 10X the approved MS dose; 3) immunological constituents are maintained (cellular and humoral), with reversible effects on cell location of some (but not all) lymphocyte subsets —without inhibition of proliferation, differentiation, and cytotoxicity; 4) immunological surveillance is maintained through relatively unaffected effector memory T-cells; and 5) clinically, the overall incidence of infections as well as of serious and severe infections was not increased over placebo control in the FREEDOMS trial in phase III studies. Overall, the emerging picture identifies S1P receptor pathways in MS that can provide efficacy through mechanisms different from classical immunosuppressants.

Future Prospects

Fingolimod is the first compound targeting lysophospholipid receptors to receive regulatory approval as a human medicine. Its direct effects on both the immune and nervous systems via a defined class of molecular targets, lysophospholipid S1P receptors, make it unique amongst approved MS therapies. Fingolimod also provides human validation for the efficacy and safety of S1P receptor modulation in MS. Evidence

for direct CNS activity raises the possibility that fingolimod could access mechanisms relevant to non-relapsing forms of MS, particularly PPMS or SPMS. There are currently no approved therapies for PPMS, and a Phase III trial has been started to assess the ability of fingolimod to improve disability progression over a 3–5 year period towards assessing its efficacy in this form of MS.

Beyond MS, fingolimod and other S1P receptor modulating compounds could have relevance to both immunological diseases as well as those of the CNS. Autoimmune disorders that may be susceptible to S1P receptor modulators include lupus, psoriasis, arthritis, and diabetes. In particular, the effects of fingolimod on IL-17 cytokine-secreting Th17 lymphocytes that reduce IL-17 mediated inflammatory sequelae may be of special relevance. Additionally, fingolimod's direct action on neural cells, especially astrocytes, portend the use of fingolimod or related lysophospholipid receptor-modulatory compounds in a range of other neurological diseases. This in part reflects the important roles for astrocytes in most major neurological disorders that include stroke and neurodegenerative disease (Alzheimer's and Parkinson's disease), which gives rise to the possibility of therapeutically treating disease through the pharmacological modulation of S1P receptors. More broadly, the documented effects of lysophospholipid signaling in the CNS, which include cell survival for myelinating and neural progenitor cells as well as modulation of synaptic activity, suggest new approaches to treat major human diseases through lysophospholipid receptor modulation: a first step along this path has now been taken with the introduction of fingolimod into clinical practice for MS.

Fingolimod 0.5 mg once daily is approved for the treatment of relapsing multiple sclerosis (MS) in many countries¹ and in the European Union (EU) for the treatment of patients with high disease activity despite treatment with interferon beta or those with rapidly evolving severe relapsing-remitting MS (RRMS).² Fingolimod has shown superior efficacy on clinical and MRI outcomes compared with both an approved firstline MS therapy—intramuscular interferon beta-1a—and placebo.^{1–6} In TRANSFORMS (Trial Assessing Injectable Interferon versus FTY720 Oral in RRMS),⁴ fingolimod 0.5 mg reduced the annualised relapse rate (ARR) by 52% compared with intramuscular interferon beta-1a over 1 year. In the 2-year FREEDOMS (FTY720 Research Evaluating Effects of Daily Oral therapy in MS) study,³ fingolimod 0.5 mg significantly reduced the ARR by 54%, from 0.40 (95% CI 0.34–0.47) in patients receiving placebo to 0.18 (0.15–0.22); the risk of disability progression after 3 and 6 months was reduced by 30% and 37%, respectively, over 24 months compared with placebo. Kaplan-Meier estimates for the proportions of patients free from 3-month confirmed disability progression were 75.9% (95% CI 71.7–80.2%) in patients receiving placebo and 82.3% (78.6–86.1%) in patients treated with fingolimod 0.5 mg.³ As well as overall efficacy, efficacy in subgroups of patients who have high disease activity, and are therefore in urgent need of therapy, is of specific interest to clinicians. In MS, for which several treatment options are available, subgroup analyses can inform patients and physicians as they make treatment choices. A substantial proportion (41%) of patients included in the FREEDOMS study³ had received previous disease-modifying therapy (DMT) and the study thus provided an opportunity to assess the efficacy of fingolimod treatment in patients who responded suboptimally to other available treatments. This assessment is important because up to

36% of patients who receive first-line treatments for MS discontinue owing to perceived limited efficacy.^{7,8}

C.METHODS

Standard protocol approvals, registrations, and patient consents

This study is being conducted in compliance with the Declaration of Helsinki and the International Conference on Harmonisation Good Clinical Practice guidelines and local regulatory requirements. Approval for the study protocol and all amendments is obtained from local ethics committees. A written informed consent will be obtained from each patient before any evaluations are conducted for eligibility. The trial is registered on ClinicalTrials.gov.

Study Objectives

Primary objective

The primary objective of this study is to assess and compare the efficacy (Annualized Relapse Rate (year 1) and the cumulative probability of sustained progression of disability at 12 and 24 weeks (year2) of natalizumab and fingolimod in RRMS patients after switching from other first line DMTs due to efficacy reasons.

Secondary objectives

The secondary objectives of this study is to evaluate safety of both fingolimod and Tysabri in RRMS patients after switching from other first line DMTs due to efficacy reasons.

End Points and Study Procedures

End points

- **Primary endpoints**

Annualized Relapse Rate (year 1) and the cumulative probability of sustained progression of disability at 12 and 24 weeks (year2) of natalizumab and fingolimod in RRMS patients after switching from other first line DMTs due to efficacy reasons

- **Secondary endpoints**

The number of new or enlarging hyperintense lesions as detected by T2-weighted MRI, the number of lesions as detected by gadolinium-enhanced MRI, and the proportion of relapse-free patients (year 1)

the rate of clinical relapse, the volume of lesions as detected by T2-weighted MRI, the number of new hypointense lesions as detected by unenhanced T1-weighted MRI, and the progression of disability as measured by EDSS.

STUDY PROCEDURES

At each study site, primary and backup examining neurologists and primary and backup treating neurologists are designated. Treating neurologists are responsible for all aspects of patient care, including the management of adverse events and the treatment of relapsing disease. Examining neurologists will perform objective evaluation with use of the EDSS and neurologic examination during all study visits; they will not be in contact with patients in any other capacity, so as to reduce the possibility of being unblinded by side effects or laboratory assessments.

EDSS is an ordinal scale in half-point increments that quantifies disability in participants with MS. It assesses 7 functional systems (visual, brainstem, pyramidal, cerebellar, sensory, bowel/bladder and cerebral) as well as ambulation. EDSS total score: 0 (normal neurological examination) to 10 (death due to MS). Sustained progression of disability is defined as an increase of 1.0 or more on the EDSS from a baseline score of 1.0 or more or an increase of 1.5 or more from a baseline score of 0 that is sustained for 12 and 24 weeks.

Patients will visit the clinic every 12 weeks for scoring on the EDSS, blood chemical and hematologic analyses, evaluation of adverse events, and testing for anti-natalizumab antibodies. Patients will also be seen by the treating neurologist at unscheduled visits within 72 hours after the onset of new neurologic symptoms. If a relapse is suspected, the patient will be referred to the examining neurologist, who will evaluate the patient within five days after the event. Relapses are defined as new or recurrent neurologic symptoms not associated with fever or infection that last for at least 24 hours and are accompanied by new neurologic signs found by the examining neurologist. At the discretion of the treating neurologist, relapses will be treated with intravenous methylprednisolone at a dose of 1000 mg per day for three or five days. Patients are strongly encouraged to remain in the study for follow-up assessments even if they have discontinued the study drugs.

Proton-density-weighted or T2-weighted and gadolinium-enhanced T1-weighted MRI scans of the brain will be obtained at baseline, at week 48, and at week 96. Contiguous, 3-mm-thick axial slices through the whole brain were acquired. MRI analysis will be

performed at the same Institute with the same protocol, by experienced raters who will be unaware of treatment assignment.

The study has primary and secondary end points. An assessment of the inflammatory characteristics of the disease will be performed at one year and of the progression of the irreversible destructive process at two years. At one year, the primary end point is the annualized relapse rate, and secondary efficacy end points are the number of new or enlarging hyperintense lesions as detected by T2-weighted MRI, the number of lesions as detected by gadolinium-enhanced MRI, and the proportion of relapse-free patients. At 2 years, the primary end point is the cumulative probability of sustained progression of disability at 12 and 24 weeks, which is defined as an increase of 1.0 or more on the EDSS from a baseline score of 1.0 or more or an increase of 1.5 or more from a baseline score of 0 that was sustained for 12 and 24 weeks (progression could not be confirmed during a relapse). Secondary efficacy end points at two years are the rate of clinical relapse, the volume of lesions as detected by T2-weighted MRI, the number of new hypointense lesions as detected by unenhanced T1-weighted MRI, and the progression of disability as measured by the Multiple Sclerosis Functional Composite.

Binding antibodies against natalizumab will be assessed with the use of an enzyme-linked immunosorbent assay. Samples that are positive for binding antibodies (0.5 µg per milliliter) will be further tested by flow cytometry to assess the ability of the antibodies to interfere with the binding of natalizumab to $\alpha 4$ integrin.

As prespecified, MRI analyses will be primarily performed in a population of patients who will have undergone randomization and who will have completed 6 months of treatment, have no major protocol violations, and for whom MRI scans are available at baseline and on three or more visits. Use of a per-protocol-like population for MRI analyses is appropriate for a proof-of-concept study. The intention-to-treat population comprises all patients who are randomly assigned to receive at least one dose of study medication and have at least one post-baseline MRI. MRI analyses will be repeated for the intention-to-treat population to assess the sensitivity of the results with the population with data that could be evaluated. Clinical outcomes will be evaluated in the intention-to-treat population. Safety analyses will be undertaken for patients who are randomly assigned to receive at least one dose of study drug and completed at least one safety assessment.

Visits

Visits are scheduled every 12 weeks while the recruitment period is 12 months.

Patients visits will be documented every 12 weeks (baseline, weeks 12, 24, 36, 48, 60, 72, 84, 96).

All data will be entered at the study sites into a central database using an electronic case report form (eCRF), specifically designed for the purpose of the study, with a delegated Contract Research Organization (CRO) as an administrator. This eCRF will contain information such as demographic characteristics, medical history and clinical data obtained during the patient's participation in the current study.

Data Collection

Collected data are divided into two main categories: baseline data and follow up data.

Baseline data

Patients' baseline data will be collected during the baseline visit:

1. Demographic characteristics: age, gender, marital status, educational status, place of residence, etc.
2. Medical history: the age of the first onset of MS symptoms, the date of MS diagnosis, the number of relapses two years prior to study participation, the severity level of the disease, co-morbidities, and medication history (including previous Disease Modifying Treatments [DMT] and non-DMT for MS, as well as non-MS therapies) if any.
3. Laboratory and examination data

Randomization & Treatment Of Interest

- Using a computer-generated sequence, we will randomly allocate eligible patients—those aged 18–55 years with relapsing-remitting multiple sclerosis—to receive the First Group: fingolimod 0.5 mg once daily and Placebo, IV infusion, every 4 weeks, for up to 96 weeks and the Second Group: Natalizumab 300 mg IV infusion, every 4 weeks, for up to 96 week plus Placebo capsules once daily.

. Randomization will be performed centrally, with the use of a validated system and stratification according to site, with a block size of six within each site.

Enrollment is limited to men and women who are between the ages of 18 and 55 years and have a diagnosis of relapsing multiple sclerosis, who have a score of 0 to 5.5 on the Expanded Disability Status Scale (EDSS), a rating that ranges from 0 to 10, with higher

scores indicating more severe disease; who have undergone magnetic resonance imaging (MRI) showing lesions consistent with multiple sclerosis; and who had have at least one medically documented relapse within the 12 months before the study began. Patients with disease that is categorized as primary progressive, secondary progressive, or progressive relapsing are excluded. Additional exclusion criteria include the following: a relapse within 50 days before the administration of the first dose of the study drug or treatment with cyclophosphamide or mitoxantrone within the previous year.

NUMBER AND TYPE OF PATIENTS

Inclusion Criteria:

- Diagnosis of MS, as defined by McDonald et al
- Between the ages of 18 and 55, inclusive.
- Baseline EDSS score between 0.0 and 5.5, inclusive.
- Have experienced at least one relapse within the 12 months prior to randomization.
- Cranial MRI scan demonstrating lesion(s) consistent with MS.
- Have given written informed consent to participate in the study.

Exclusion Criteria:

- Primary progressive, secondary progressive, or progressive relapsing MS.
- MS relapse has occurred, in the opinion of the investigator, within 50 days prior to randomization and/or the subject has not stabilized from a previous relapse.
- A clinically significant infectious illness within 30 days prior to randomization.
- History of, or abnormal laboratory results indicative of any significant cardiac, endocrinologic, hematologic, hepatic, immunologic, metabolic, urologic, pulmonary, gastrointestinal, dermatologic, psychiatric, renal and/or other major disease, that in the opinion of the investigator, would preclude the administration of a recombinant humanized antibody immunomodulating agent for 96 weeks.
- History of severe allergic or anaphylactic reactions or known drug hypersensitivity.
- Abnormal blood tests performed at the Screening Visit.

All study personnel, patients, sponsor personnel involved in the conduct of the study, and the investigator advisory committee will be unaware of treatment assignments throughout the study.

DEFINITION OF ENROLLED PATIENTS

A patient will be considered enrolled after reading and signing the ICF.

EARLY WITHDRAWAL OR STUDY DISCONTINUATION

As the decision for treatment lies with the treating physician and is not bound to the participation of a patient in the study, the Investigator has the right to withdraw a patient from the study at any time. In addition, patients have the right to voluntarily withdraw from the study at any time and for any reason. Reasons for discontinuation of treatment with the medicinal product or withdrawal from the study may include, but are not limited to, the following:

- Patient withdrawal of consent at any time;
- The patient meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation;
- Enrolment or planned participation in any clinical trial, at any time during the study observation period, in which the patient has been or will be exposed to an investigational product (pharmaceutical agent or device) or intervention;
- Investigator's/physician's decision;
- Significant protocol deviation/violation.
- Patient is lost to follow-up.

The last visit for these patients will constitute the end-of-study/early termination data collection point for this patient.

For patients who are lost to follow-up (i.e., those patients whose status is unclear because they fail to appear for study visits without stating an intention to withdraw), the Investigator should contact the patient in accordance with their own routine clinical practice.

WITHDRAWAL PROCEDURE

Patients, who voluntarily discontinue study treatment or withdraw from the study, should be asked about the reason(s) for discontinuation and the occurrence of any adverse events, and, if applicable, they shall be examined and evaluated by the investigator as per the procedures defined in the End of Treatment or Early Discontinuation Visit. Adverse events must be monitored until their resolution or stabilization.

In addition, if a patient is prematurely withdrawn from the study, he/she will be contacted by telephone or other methods to assess his/her status at the end of study unless the patient has actively withdrawn consent for all forms of contact.

If the patient specifically withdraws his/her consent to be contacted for additional information, no study-related contacts can be conducted.

STATISTICAL ANALYSIS

Sample size Estimation

The estimate of sample size is based on data from previous trials of natalizumab and fingolimod with the power approach test. The annualized rate of relapse at one year is predicted to be 0.23 natalizumab and 0.18 with fingolimod. For an annualized relapse rate, the sample size required for 90 percent power (n=489). With an assumed dropout rate of 25%, the number of patients needed was estimated to be **612**.

Statistical Analysis

P values that are reported for most baseline demographic and disease characteristics will be calculated with the use of a t-test to compare differences in means. The exceptions are sex, race, and diagnosis of multiple sclerosis by the McDonald criteria,¹⁸ for which a chi-square statistic will be used to compare treatment groups.

The annualized rate of relapse (the primary end point year 1) will be calculated by Poisson regression.. The predefined statistical models include baseline scores on the EDSS for sustained progression of disability and the number of relapses in the previous year for the relapse rate. Additional baseline factors will be tested for inclusion in each of the models, including the EDSS score (≤ 3.5 or > 3.5), the presence or absence of lesions as detected by gadolinium-enhanced MRI, the number of hyperintense lesions as detected by T2-weighted MRI (< 9 or ≥ 9), and age (< 40 or ≥ 40 years).²¹⁻²³ .

For the progression of disability (the primary end point year 2), a sensitivity analysis will be conducted on the change in EDSS scores that is sustained for 12 and 24 weeks. For the annualized relapse rate, sensitivity analyses will be performed with and without censoring, as well as with and without adjustment for significant covariates. The unadjusted relapse rate will be calculated as the total number of relapses divided by the total number of patient-years followed for each treatment group.

Secondary efficacy end points will be analyzed by logistic regression that include a term for the treatment group and the respective baseline measure as a covariate. In the analyses of secondary end points, missing values will be imputed using the mean for the respective measures in the study population.

Differences between treatment groups with regard to adverse events will be analyzed by the chi-square test, and serious adverse events will be analyzed by Fisher's exact test. Poisson regression will be used to calculate the difference between the rates of infection in each treatment group.

All analyses will follow the intention-to-treat principle. All reported P values are two-tailed. The one-year analyses will occur when **612** patient-years of data will be collected.

Replacements of Patients

Sample size calculation has taken into account possible drop-outs or withdrawals, and no replacements will be made during the study follow-up period. However, replacement of patients will be permitted during the study recruitment period in order to ensure sufficient study population in case that drop-out rate is larger than the predicted one.

ADVERSE EVENTS AND OTHER SAFETY ASPECTS

DEFINITION OF AN ADVERSE EVENT

An AE is defined as the appearance or worsening of any undesirable sign, symptom, or medical condition occurring after signing the informed consent form (ICF), even if the event is not considered to be related to the use of Tysabri (natalizumab) or Gilenya (fingolimod). Medical conditions/diseases present at study entry are only considered AEs if they worsen after signing the ICF. Medical judgment should be exercised in deciding whether examination findings should be classified as AEs.

An AE can therefore be, but is not limited to, any of the following:

- Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.
- An exacerbation of an existing disease.
- Recurrence of an intermittent medical condition not present at baseline.
- Any deterioration in a laboratory value or other clinical test (if judged by the investigator to have unexpectedly worsened in severity or frequency or changed in nature at any time during the study) that is associated with symptoms or leads to a change in study treatment or concomitant treatment or discontinuation from study medicine.
- Overdose, abuse, off-label use, misuse, and medication error associated with a medicinal product.
- Lack of therapeutic efficacy of a medicinal product.

A preexisting medical condition should be recorded as an AE only if the frequency, severity, or character of the condition worsen during the study. When recording such events in the AE section of the eCRF, it is important to convey the concept that the preexisting condition has changed by including applicable descriptors (e.g., “more frequent headaches”). Events that are clearly consistent with the expected pattern of progression of the underlying disease should not be recorded as AEs. This includes relapses of MS that cannot be attributed to the expected course of disease progression under treatment, taking into account the initial clinical and laboratory findings and the individual patient’s medical history. These data will be captured as effectiveness assessment data only. In most cases, the expected pattern of progression will be based

on clinical outcomes. In rare cases, the determination of clinical progression will be based on symptomatic deterioration. However, every effort should be made to document progression through use of objective criteria. If there is any uncertainty as to whether an event is due to disease progression, it should be reported as an AE. This exception from reporting includes events of disease progression with a fatal outcome which are clearly attributable to disease progression.

The occurrence of AEs/ADRs should be sought by non-directive questioning of the patient at each visit during the study. AEs may, also, be detected when they are volunteered by the patient during or between visits or through physical examination, laboratory test, or other assessments. All AEs must be recorded on the AE eCRF with the following information:

1. The severity grade (mild, moderate, severe).
2. Its relationship to natalizumab or fingolimod (related/not related/possibly related).
3. Its duration (start and end dates or if continuing at final exam).
4. Whether it constitutes a serious adverse event (SAE) or an AESI.

During completion of the respective field in the AE eCRF of relation of the non-serious AE to Tysabri Or Gilenya, if the Investigator selects “yes”, “unknown” or does not select anything, then the respective non-serious AE is considered to be an ADR.

Investigators will seek safety information as defined per this protocol at each patient contact. All events meeting the criteria for an AE or SAE should be reported on the relative AE field in the eCRF, whether reported by the patient or noted by study personnel. If an AE has been marked as serious in the AE eCRF by the Investigator, the Investigator will be required to fill in the SAE form in the eCRF and submit the case (with an electronic signature). Information about ADRs observed in relation to Tyasabri or Gilenya can be found in the local product labeling.

DEFINITION OF AN ADVERSE DRUG REACTION

An ADR is a response to the administration of a medicinal product which is noxious and unintended, with a causal relationship between the drug and the reported reaction being a reasonable possibility.

AWARENESS DAY (DAY 0)

Date when information, containing the 4 minimum elements relating to a SAE or a pregnancy report is notified to the distributor of each drug in Greece.

Awareness Date for follow-up information would be the date that the new information is notified to the Sponsor.

The 4 minimum elements that must be provided for a case to be considered as valid are:

- ✓ An identifiable reporter.
- ✓ An identifiable patient (i.e. either eCRF number, patient/subject number, initials, gender, date of birth, age, or age group).
- ✓ A drug.
- ✓ An adverse event (or pregnancy).

The term “awareness” is also used in the document for the Investigator to specify the time point when the Investigator receives information relevant to an AE (or a pregnancy).

REPORTING REQUIREMENTS FOR SERIOUSNESS AND SEVERITY

A serious AE/ADR is any AE/ADR which:

- results in death,
- is life-threatening,
- requires in-patient hospitalization or prolongation of existing hospitalization,
- results in persistent or significant disability or incapacity,
- is a congenital anomaly or birth defect

Medical and scientific judgement should be exercised in deciding whether other situations should be considered serious reactions, such as important medical events that might not be immediately life threatening or result in death or hospitalisation but might jeopardise the patient or might require intervention to prevent one of the other outcomes listed above. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalisation or development of dependency or abuse.

Any suspected transmission of an infectious agent via a medicinal product is also considered a SAE.

Life threatening in this context refers to a reaction in which the patient was at risk of death at the time of the reaction; it does not refer to a reaction that hypothetically might have caused death if more severe.

The terms “severe” and “serious” are not synonymous. Severity refers to the intensity of an AE (e.g., rated as mild, moderate, or severe, or according to NCI CTCAE criteria); the event itself may be of relatively minor medical significance (such as severe headache without any further findings).

Severity and seriousness need to be independently assessed for each AE recorded on the AE eCRF.

The AE severity grading scale for the NCI CTCAE (v4.0) will be used for assessing AE severity. The table below will be used for assessing severity for AEs that are not specifically listed in the NCI CTCAE.

TABLE 1: ADVERSE EVENT SEVERITY GRADING SCALE

Grade	Severity
1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; or intervention not indicated
2	Moderate; minimal, local, or non-invasive intervention indicated; or limiting age-appropriate instrumental activities of daily living ^a
3	Severe or medically significant, but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; or limiting self-care activities of daily living ^{b, c}
4	Life-threatening consequences or urgent intervention indicated ^d
5	Death related to AE ^d

ADVERSE EVENTS OF SPECIAL INTEREST

The adverse events found in the following list constitute AESI, and regardless of their seriousness, must be handled and reported to the Sponsor using the same expedited processes as SAEs (within 24 hours of awareness):

- Anaphylactic Shock
- Angioedema
- Aplastic Anaemia
- Cancer (all types)
- Cardiomyopathy
- Cardiomegaly
- Cirrhosis
- Congestive Heart Failure
- Convulsion/Seizure (all types)
- Hepatitis
- Heart Attack/Myocardial Infarction
- Ischemic Colitis
- Pancytopenia
- Primary Biliary Cirrhosis
- Primary Pulmonary Hypertension
- Primary Sclerosing Cholangitis
- Progressive Multifocal Leukoencephalopathy.

CAUSALITY ASSESSMENT

Investigators should use their knowledge of the patient, the circumstances surrounding the event, and an evaluation of any potential alternative causes to determine whether or not an adverse event is considered to be related to the study drug, indicating "related", "not related" or "possibly related" accordingly. The following guidance should be taken

into consideration:

- Temporal relationship of event onset to the initiation of study drug.
- Course of the event, considering especially the effects of dose reduction, discontinuation of study drug, or reintroduction of study drug (where applicable).
- Known association of the event with the study drug or with similar treatments.
- Known association of the event with the disease under study.
- Presence of risk factors in the patient or use of concomitant medications known to increase the occurrence of the event.
- Presence of non-treatment-related factors that are known to be associated with the occurrence of the event.

ADVERSE EVENT REPORTING

Immediate reporting requirements from Investigator to Sponsor

Certain events require immediate reporting to allow appropriate measures to be taken to address potential new risks associated with the use of the medicine. The Investigator must report such events to the Sponsor immediately; under no circumstances should reporting take place more than 24 hours after the Investigator becomes aware of the event. The following is a list of events that the Investigator must report to the Sponsor within 24 hours from awareness:

SAEs

AESI related to Tysabri Or Gilenya

Pregnancies.

Investigators should record all case details that can be gathered immediately (i.e., within 24 hours) in the AE page of the eCRF and submit the SAE or pregnancy form via the EDC system. A report will be generated and sent to the Sponsor by the EDC system. The Investigator must report new significant follow-up information for these events to the Sponsor immediately (i.e., no more than 24 hours after becoming aware of the information). New significant information includes the following:

New signs or symptoms

Significant new diagnostic results

Change in the AEs outcome, including recovery

Additional information on the clinical course of the AE.

Other reporting requirements

AEs that are suspected to be related to medicinal products other than the studied medicine should be reported by the Sponsor to the Marketing Authorization Holder of the suspected medicinal product, or to the concerned competent authorities via the national spontaneous reporting system as per local requirements.

Pregnancy reporting

Female patients of childbearing potential will be instructed to immediately inform the Investigator if they become pregnant during the study or within 7 days after the last dose of medicine. A Pregnancy form in the eCRF should be completed by the Investigator immediately (i.e., no more than 24 hours after awareness of the pregnancy) and submitted via the EDC system. A pregnancy report will automatically be generated and sent to the Sponsor.

The Investigator should discontinue the medicine, counsel the patient, discussing the risks of the pregnancy and the possible effects on the fetus. Monitoring of the patient should continue until conclusion of the pregnancy. Any AE/SAE associated with the pregnancy (e.g., an event in the fetus, an event in the mother during or after the pregnancy, or a congenital anomaly/birth defect in the child) should be reported on the AE section of the eCRF. Any abortion should be classified as a SAE (medically significant), recorded in the AE section of the eCRF, and reported to the Sponsor immediately (i.e., no more than 24 hours after awareness).

Congenital Anomalies / Birth Defects

Any congenital anomaly/birth defect in a child born to a female patient exposed to the medicine or the female partner of a male patient exposed to natalizumab or fingolimod should be classified as a SAE, recorded in the AE section of the eCRF, and reported to the Sponsor immediately (i.e., no more than 24 hours after awareness).

ADVERSE EVENT REPORTING PERIOD

Investigators will seek information on AEs at Baseline, at month 6, 12, 18 and month 24 post-recruitment, as well as in any early termination instances. All AEs subject to the collecting and reporting requirements outlined in this protocol, whether reported by the patient or noted by study personnel, will be recorded in the patient's medical record and on the AE eCRF.

During the study period, resolution of AE, SAE, and AESI (with dates) should be documented in the AE section of the eCRF and in the patient's medical record to facilitate SDV. All pregnancies reported during the study should be followed until pregnancy outcome.

FOLLOW-UP

The Investigator should make every effort to follow all AEs until a final outcome can be reported, e.g. until the events have resolved to Baseline grade or better, assessed as stable by the Investigator, the patient is lost to follow-up, or the patient withdraws consent.

Follow-up may be performed by telephone, fax, electronic mail, and/or a monitoring visit to obtain additional case details and outcome information (e.g., from hospital discharge summaries, consultant reports, autopsy reports) in order to perform an independent medical assessment of the reported case.

Follow-up information is sent using a new SAE report form stating that this is a follow-up to the previously reported SAE and giving the date of the original report. The follow-up information should describe whether the event has resolved or continues, if and how it was treated, and whether the patient continued or withdrew from study participation.

At the study completion/early termination visit, the Investigator should instruct each patient to report to the Investigator any subsequent AEs that could be related or not to study drugs. The Investigator should notify the Sponsor of any death and AE occurring at any time after a patient has discontinued study participation. The Sponsor should also be notified if the Investigator becomes aware of the development of congenital anomaly/birth defect in a subsequently conceived offspring and fertility-related problems of a patient that participated in this study.

The Investigator should report these events directly to the Sponsor or its designee, either by faxing or by scanning and emailing the relative SAE reporting form using the fax number or email address provided to Investigators.

Critical Documentation

Before study initiation the following documents must be collected and archived by the study Sponsor:

- Curricula Vitae of the principal investigator and co-investigators (current, signed and dated);
- Signed and dated investigator's agreement of the final protocol;
- Signed and dated investigator's agreement of any study documents' amendments, if applicable;
- Copy of the IRB approval letter for study sites that are planned to participate in the study;
- Financial Agreement/Investigator's Contract.

Source Documents and Records Storage and Retention

The investigator should ensure that only appropriately qualified persons are delegated with duties associated with this study.

During the study and after termination of the study -including study's early termination- the investigator must maintain copies of all documents and records relating to the conduct of the study.

This documentation includes, but is not limited to, protocols, eCRF archivals, AE reports, patient source data, correspondence with regulatory authorities and IRBs, ICFs, investigator's curricula vitae and monitor visit logs.

Source documents are original documents, data, and records from which the patient's case report form data are obtained. These include but are not limited to hospital records, clinical and office charts, laboratory and pharmacy records, diaries, microfiches and radiographs. All information entered in the eCRF must be traceable to these source documents in the patient's file. Before study initiation, the types of source documents that contain study-relevant information will be clearly defined in the Trial Monitoring Plan. This includes any protocol data to be entered directly into the eCRF (i.e., no prior written or electronic record of the data) and considered source data.

The physician must also keep the original informed consent form signed by the patient (a signed copy is given to the patient). The physician must provide the study Sponsor (or designee) access to all relevant source documents to confirm their consistency with the eCRF entries. No information in source documents about the identity of the patients will be disclosed.

Patient files and other source documents must be kept for the maximum period of time permitted by the hospital/clinic, or as specified below. The study monitor must be consulted if the investigator wishes to assign the files to someone else, remove them to another location, or if he/she is unable to retain them for the specified period.

The investigator must retain study records for the amount of time specified by applicable laws and regulations. At a minimum, study records must be retained for the amount of time specified by the standing legislation, i.e., study records must be retained for at least 15 years after study completion. These documents may be retained for a longer period if required by the study Sponsor and this period and method of retention will be agreed to separately between the study Sponsor and the study Principal Investigator. The investigator should consult with the study monitor prior to discarding study's and/or patient's files.

Electronic Case Reports Forms (eCRF)

Data will be entered electronically on the eCRF by the investigator or authorized personnel in the participating sites via an electronic data capture (EDC) platform using a web-based secure server.

All patient data collected during the course of this study will be recorded while maintaining anonymity and the patient will be recognized based on the subject identification code.

Patient Identification Code

For the identification of the patients during the conduct of the study and following its completion, the investigator is responsible for maintaining a file with Patient Identification Codes for patients of his/her site.

Audits and Inspections

The Sponsor may perform audit(s) of the study files at the study sites in order to ensure compliance with the study requirements, the GPP guidelines and the standing regulatory requirements.

In addition, the competent authorities may also inspect the sites at any time during the conduct or following the completion of the study. In case of an audit or inspection, the investigator (and the institution) shall agree to permit the auditor(s) and inspector(s) to have direct access to all relevant documents and to provide adequate time, both he/she and his/her staff, for the discussion regarding any findings/major issues.

Patients shall be informed that properly authorized staff of the Sponsor or the regulatory authority may inspect their medical files.

Patient data will remain confidential during the study and following its completion, and they will not be disclosed to any non-authorized third parties.

ETHICS AND REGULATORY REQUIREMENTS

Scientific Committee

This protocol, the Informed Consent Forms, any information to be given to the patient, and relevant supporting information must be submitted to the Scientific Committee of the healthcare institution that the site belongs to, by the Primary Investigator or equivalent board for review and approval prior to initiation of the study at that site.

Prior to study start, the Investigator is, also, required to sign a protocol signature page confirming her/his agreement to conduct the study in accordance with these documents and all of the instructions and procedures found in this protocol.

The Sponsor (unless this activity is delegated to CRO) is responsible for promptly informing the Scientific Committee of any protocol amendments. The Primary Investigator of each site (if applicable) is responsible for promptly informing the Scientific Committee of any protocol amendments.

Regulatory/Ethical Conduct Of The Study

Compliance with regulatory standards provides assurance that the rights, safety, and wellbeing of patients participating in non-interventional studies are protected; consistent with the principles that have their origin in the Declaration of Helsinki; and that the study data are credible and responsibly reported.

This study was designed and shall be implemented and reported in accordance with the ethical principles laid down in the Declaration of Helsinki, the Guidelines for GPP of the International Society for Pharmacoepidemiology (ISPE 2008), the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines (Vandenbroucke, et al 2008) where applicable, and the local rules and regulations.

The Declaration of Helsinki is included in the protocol .

Patient Information And Consent

The study sample Informed Consent Form (ICF) will be provided to each site in a certified translation of the local language.

The ICF must be signed and dated by the patient (or the patient's legally authorized representative, if applicable) before his or her participation in the study. In case the patient or his legally acceptable representative is unable to read, then an impartial witness must be present during the discussion regarding the informed consent, and the witness must also sign and date the ICF. Following the obtainment of the written informed consent, the person who conducted the informed consent procedure shall sign and date the ICF. The case history or clinical records for each patient shall document the informed consent process and that written informed consent was obtained prior to participation in the study.

By signing the ICF, the patient confirms that she/he has been informed about the study and agrees to pseudonymous data collection, pooling of data with similar scientific data (if applicable), and the possibility of monitoring activities. It is the responsibility of the Investigator to obtain written informed consent from each patient participating in the study, after adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study. The Investigator must also explain that the patient is completely free to refuse to enter the study or to withdraw from it at any time, for any reason and without losing the benefit of any medical care to which the patient is entitled or is presently receiving.

The ICF should be revised whenever there are changes to study procedures or when new information becomes available that may affect the willingness of the patient to participate.

Patients must be re-consented to the most current version of the ICF (or to a significant new information/findings addendum in accordance with applicable laws) during their participation in the study. For any updated or revised ICF, the case history or clinical records for each patient shall document the informed consent process and that written informed consent was obtained using the updated/revised ICF for continued participation in the study.

A copy of each signed ICF must be provided to the patient or the patient's legally authorized representative and witness, where and if applicable. All signed and dated ICFs must remain in each patient's study file or in the site file and must be available for verification by study monitors at any time.

Regulatory Approval

According to the local regulatory requirements, the clinical study protocol and the Informed Consent Form (ICF) must be submitted to the IRBs (Scientific Committee/Board of Directors) of the participating hospitals as part of the approval process.

The Scientific Council/Board of Directors of the participating hospitals must also approve any amendment to the protocol or Patient ICF, prior to the implementation of the amendment or the use of the ICF, according to local regulations. The conduct of this non interventional study will adhere to the applicable national regulatory requirements governing the conduct of such type of clinical studies.

This study will be conducted in compliance with Good Pharmacovigilance practice Guidelines (GVP) released by the European Medicines Agency, and is aligned with recommendations for non-interventional studies.

Patient Confidentiality

The Sponsor maintains confidentiality standards by coding each patient enrolled in the study through assignment of a unique patient identification number. This means that patient names are not included in datasets that are transmitted to any Sponsor location.

Patient medical information obtained by this study is confidential and may be disclosed to third parties only as permitted by the Informed Consent Form (or separate authorization for use and disclosure of personal health information) signed by the patient, unless permitted or required by law.

By signing this protocol, the investigator agrees to treat all patient data used and disclosed in connection with this study in accordance with all applicable privacy laws, rules and regulations.

The confidentiality of patient data will be maintained at all times and no documents containing the patient's name or other identifying information will be collected by the Sponsor. It may be necessary for the Sponsor's representatives, the IRBs and regulatory authority representatives to have direct access to the patient's medical records. If study documents need to be photocopied during the process of verifying eCRF data, the patient will be identified by a unique code only; full names/initials and other identifying information will be masked.

By signing this protocol, the investigator also affirms to the Sponsor that information provided to the investigator by the Sponsor will be maintained in confidence and will be divulged only as necessary to the IRBs and institution employees directly involved in the study. IRB members and employees also understand the confidentiality requirements for any information divulged to them. The data generated by this study will be considered confidential by the investigator, except to the extent that it is included in a publication as agreed in the publication policy of this protocol.

By signing the ICF, the patient accepts being informed of the following:

- The kind of personal information (data) that will be collected from participants in this study;
- The persons that have access to the study information;
- The persons that may use or disclose that information;
- The rights of the participant pertaining to the potential revoke of his/her authorization for use of their personal data.

In case of patient authorization revoking, the investigator maintains the right to use the information collected prior to the revoke and attempts should be made to obtain permission to collect all available information at the end of their scheduled study period.

Completion of the Study

The IRB (Scientific Council/Board of Directors) of the participating hospital sites will be notified about the end of the study (last patient out) or early termination of the study, unless otherwise mandated by the national regulations governing the conduct of such type of studies which may have been altered by the time of study completion.

Insurance Of Patients

As this is an interventional Phase III study in which non standard clinical practice will be chosen for each participant, therefore insurance of participants will be involved throughout the duration of the study.

APPENDIX 4: DECLARATION OF HELSINKI

World Medical Association Declaration of Helsinki. Ethical Principles for Medical Research Involving Human Subjects

Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the:

29th	WMA	General	Assembly,	Tokyo,	Japan,	October	1975
35th	WMA	General	Assembly,	Venice,	Italy,	October	1983
41st	WMA	General	Assembly,	Hong	Kong,	September	1989
48th	WMA	General	Assembly,	Somerset	West, Republic of South	October	1996
52nd	WMA	General	Assembly,	Edinburgh,	Scotland,	October	2000
53th	WMA	General	Assembly,	Washington	2002 (Note of Clarification on paragraph 29 added)		
55th	WMA	General	Assembly,	Tokyo	2004 (Note of Clarification on Paragraph 30 added)		
59th	WMA	General	Assembly,	Seoul,	October 2008		
64th	WMA	General	Assembly,	Fortaleza,	Brazil,	October 2013	

Preamble

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.

The Declaration is intended to be read as a whole and each of its constituent paragraphs should be applied with consideration of all other relevant paragraphs.

2. Consistent with the mandate of the WMA, the Declaration is addressed primarily to physicians. The WMA encourages others who are involved in medical research involving human subjects to adopt these principles.

General Principles

3. The Declaration of Geneva of the WMA binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act in the patient's best interest when providing medical care."
4. It is the duty of the physician to promote and safeguard the health, well-being and rights of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.
5. Medical progress is based on research that ultimately must include studies involving human subjects.
6. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best proven interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.
7. Medical research is subject to ethical standards that promote and ensure respect for all human subjects and protect their health and rights.
8. While the primary purpose of medical research is to generate new knowledge, this goal can never take precedence over the rights and interests of individual research subjects.

9. It is the duty of physicians who are involved in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects. The responsibility for the protection of research subjects must always rest with the physician or other health care professionals and never with the research subjects, even though they have given consent.
10. Physicians must consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.
11. Medical research should be conducted in a manner that minimises possible harm to the environment.
12. Medical research involving human subjects must be conducted only by individuals with the appropriate ethics and scientific education, training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional.
13. Groups that are underrepresented in medical research should be provided appropriate access to participation in research.
14. Physicians who combine medical research with medical care should involve their patients in research only to the extent that this is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.
15. Appropriate compensation and treatment for subjects who are harmed as a result of participating in research must be ensured.

Risks, Burdens and Benefits

16. In medical practice and in medical research, most interventions involve risks and burdens.

Medical research involving human subjects may only be conducted if the importance of the objective outweighs the risks and burdens to the research subjects.

17. All medical research involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and groups involved in the research in comparison with foreseeable benefits to them and to other individuals or groups affected by the condition under investigation.

Measures to minimise the risks must be implemented. The risks must be continuously monitored, assessed and documented by the researcher.

18. Physicians may not be involved in a research study involving human subjects unless they are confident that the risks have been adequately assessed and can be satisfactorily managed.

When the risks are found to outweigh the potential benefits or when there is conclusive proof of definitive outcomes, physicians must assess whether to continue, modify or immediately stop the study.

Vulnerable Groups and Individuals

19. Some groups and individuals are particularly vulnerable and may have an increased likelihood of being wronged or of incurring additional harm.

All vulnerable groups and individuals should receive specifically considered protection.

20. Medical research with a vulnerable group is only justified if the research is responsive to the health needs or priorities of this group and the research cannot be carried out in a non-vulnerable group. In addition, this group should stand to benefit from the knowledge, practices or interventions that result from the research.

Scientific Requirements and Research Protocols

21. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.
22. The design and performance of each research study involving human subjects must be clearly described and justified in a research protocol.

The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, potential conflicts of interest, incentives for subjects and information regarding provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study.

In clinical trials, the protocol must also describe appropriate arrangements for post-trial provisions.

Research Ethics Committees

23. The research protocol must be submitted for consideration, comment, guidance and approval to the concerned research ethics committee before the study begins. This committee must be transparent in its functioning, must be independent of the researcher, the sponsor and any other undue influence and must be duly qualified. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration.

The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No amendment to the protocol may be made without consideration and approval by the committee. After the end of the study, the researchers must submit a final report to the committee containing a summary of the study's findings and conclusions.

Privacy and Confidentiality

24. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information.

Informed Consent

25. Participation by individuals capable of giving informed consent as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no individual capable of giving informed consent may be enrolled in a research study unless he or she freely agrees.
26. In medical research involving human subjects capable of giving informed consent, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, post-study provisions and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information.

After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

All medical research subjects should be given the option of being informed about the general outcome and results of the study.

27. When seeking informed consent for participation in a research study the physician must be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent must be sought by an appropriately qualified individual who is completely independent of this relationship.
28. For a potential research subject who is incapable of giving informed consent, the physician must seek informed consent from the legally authorised representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the group represented by the potential subject, the research cannot instead be performed with persons capable of providing informed consent, and the research entails only minimal risk and minimal burden.
29. When a potential research subject who is deemed incapable of giving informed consent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorised representative. The potential subject's dissent should be respected.
30. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research group. In such circumstances the physician must seek informed consent from the legally authorised representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research must be obtained as soon as possible from the subject or a legally authorised representative.
31. The physician must fully inform the patient which aspects of their care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never adversely affect the patient-physician relationship.
32. For medical research using identifiable human material or data, such as research on material or data contained in biobanks or similar repositories, physicians must seek informed consent for its collection, storage and/or reuse. There may be exceptional situations where consent would be impossible or impracticable to obtain for

such research. In such situations the research may be done only after consideration and approval of a research ethics committee.

Use of Placebo

33. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best proven intervention(s), except in the following circumstances:

Where no proven intervention exists, the use of placebo, or no intervention, is acceptable; or

Where for compelling and scientifically sound methodological reasons the use of any intervention less effective than the best proven one, the use of placebo, or no intervention is necessary to determine the efficacy or safety of an intervention and the patients who receive any intervention less effective than the best proven one, placebo, or no intervention will not be subject to additional risks of serious or irreversible harm as a result of not receiving the best proven intervention.

Extreme care must be taken to avoid abuse of this option.

Post-Trial Provisions

34. In advance of a clinical trial, sponsors, researchers and host country governments should make provisions for post-trial access for all participants who still need an intervention identified as beneficial in the trial. This information must also be disclosed to participants during the informed consent process.

Research Registration and Publication and Dissemination of Results

35. Every research study involving human subjects must be registered in a publicly accessible database before recruitment of the first subject.
36. Researchers, authors, sponsors, editors and publishers all have ethical obligations with regard to the publication and dissemination of the results of research. Researchers have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. All parties should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results must be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest must be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

Unproven Interventions in Clinical Practice

37. In the treatment of an individual patient, where proven interventions do not exist or other known interventions have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorised representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. This intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information must be recorded and, where appropriate, made publicly available

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