ΠΡΟΓΡΑΜΜΑ ΜΕΤΑΠΤΥΧΙΑΚΩΝ ΣΠΟΥΔΩΝ (ΠΜΣ)

«ΜΕΘΟΔΟΛΟΓΙΑ ΒΙΟΪΑΤΡΙΚΗΣ ΕΡΕΥΝΑΣ, ΒΙΟΣΤΑΤΙΣΤΙΚΗ ΚΑΙ ΚΛΙΝΙΚΗ ΒΙΟΠΛΗΡΟΦΟΡΙΚΗ»

ΔΙΠΛΩΜΑΤΙΚΗ ΕΡΓΑΣΙΑ

Επιβλέπων καθηγητής: καθηγητής κος Χατζηγεωργίου Γεώργιος

Κουτουκόγλου Πρόδρομος

Long term, prospective, non-interventional, multicenter, double-blinded parallel-cohort study monitoring effectiveness and tolerability of teriflunomide (Aubagio®) in the progress of disability in patients with MS

TABLE OF CONTENTS

Table of contents……...2

Abbreviations and Definition of Terms……8

Abstract……...11

Protocol Synopsis……12

1. Introduction…….....16

1.1 Background……...16

1.2 Relevant Literature and Data……17

1.3 Purpose and Rationale……..21

1.4 Compliance Statement 21

1.5 Potential Risks and Benefits……22

1.5.1 Potential Risks……....22

2. Objectives……...23

2.1 Study Objectives……...23

2.1.1 Primary Objective……...23

2.1.2 Secondary Objectives…….....23

2.1.3 Tertiary Objectives……23

2.2 Study Outcome Measures……23

3. Investigational Plan……24

3.1 General Schema of Study Design……24

3.2 Study Duration, Timeline and other Protocol-Specific Details……24

3.3 Total Number of Study Sites/Total Number of Subjects Projected……24

4. Study Population, Enrollment and Withdrawal……..25

4.1 Study Population……..25

4.1.1 Subject Inclusion Criteria……...25

4.1.2 Subject Exclusion Criteria……..26

4.2 Strategies for Recruitment and Retention……27

4.3 Subject Withdrawal……..27

4.3.1 Reasons for Withdrawal……27

4.3.2 Handling of Subject Withdrawals……...27

4.4 Premature Termination or Suspension of Study……..28

5. Study Schedule……28

5.1 Screening Visit……28

5.2 Observation Period……...29

5.2.1 Enrollment/Baseline Visit……...29

5.2.2 Intermediate Visits……..30

5.2.2.1 Visit 3……30

5.2.2.2 Visit 4……30

5.2.2.3 Visit 5……30

5.2.2.4 Visit 6……30

5.2.2.5 Visit 7……31

5.2.2.6 Visit 8……31

5.2.2.7 Visit 9……...31

5.2.2.8 Visit 10……..32

5.2.2.9 Visit 11……..32

5.2.2.10 Visit 12……32

5.2.2.11 Visit 13……32

5.2.2.12 Visit 14……32

5.2.2.13 Visit 15……33

5.2.2.14 Visit 16……33

5.2.2.15 Visit 17……33
5.2.2.16 Visit 18……34
5.2.2.17 Visit 19……34
5.2.2.18 Visit 20……34
5.2.2.19 Visit 21……35
5.2.2.20 Visit 22……35
5.2.2.21 Visit 23……35
5.2.2.22 Visit 24……35
5.2.2.23 Visit 25……36
5.2.2.24 Visit 26……36
5.2.2.25 Visit 27……36
5.2.2.26 Visit 28……36
5.2.2.27 Visit 29……37
5.2.2.28 Visit 30……37
5.2.2.29 Visit 31……37
5.2.3 Terminal Visit……....37
5.2.4 Withdrawal Visit……38
5.2.5 Unscheduled Visit……...38
6. Study Procedures/ Evaluations……...39
6.1 Medical Record Review/ Medical History……...39
6.2 Physical Evaluation/ Vital Signs/ Paraclinical Tests……40
6.3 Laboratory Procedures/ Evaluations……41
6.4 Radiologic Evaluations……41
6.5 Study Specific Biospecimens……...41
6.5.1 Specimen Collection Procedures……41

6.5.2 Specimen Preparation, Handling, Storage and Shipment……..42

6.5.3 Questionnaire Administration……..42

7. Assessment of Safety……..42

7.1 Specification of Safety Parameters……...42

7.2 Adverse Event Reporting……42

7.3 Definition of an Adverse Event……42

7.4 Definition of a Serious Adverse Event……43

7.5 Reporting Procedures……...43

7.6 Follow-up Report……..44

7.7 Pregnancy Reporting……44

7.8 Medical Emergencies……...45

8. Study Oversight and Monitoring……45

8.1 Principal Investigator……45

8.2 Independent Data and Safety Monitoring Board……..45

9. Clinical Site Monitoring……46

10. Statistical Considerations……..46

10.1 Primary Endpoint……46

10.2 Secondary Endpoints……..46

10.3 Tertiary Endpoints……..47

10.4 Control of Bias and Confounding……...47

10.5 Statistical Methods……47

10.5.1 Patient Demographic and other Baseline Characteristics……48

10.6 Analysis of the Main Variables……..48

10.6.1 Analysis of the Primary Outcome of Interest……...48

10.6.2 Analysis of the Secondary Outcomes of Interest……48

10.6.3 Analysis of the Tertiary Outcomes of Interest……..48
10.7 Sample Size and Power……..49
11. Source Documents and Access to Source Data/ Documents……50
12. Quality Management and Quality Assurance……...50
12.1 Quality Management Activity Schedule and Tools……51
12.2 Quality Management Documentation and Reporting……51
12.3 Ad Hoc Quality Management Activities……52
12.3.1 New Study Personnel……52
12.4 Subject Study Visit Quality Management Activities……..52
12.4.1 Subject Record Review……52
12.4.2 Consent Process Completion and Documentation……52
12.4.3 Source Document Completion……..53
12.4.4 Case Report Form Completion……53
12.5 Quarterly Quality Management Activities……53
12.5.1 Consent Process Completion and Documentation……53
12.5.2 Laboratory Specimens……..53
12.5.3 Equipment Set-up and Calibration……53
12.5.4 Source Document Completion……53
12.5.5 Case Report Form Completion……54
12.5.6 Study Drug……54
12.5.7 Staff Training/ Qualifications……...54
12.6 Annual Quality Management Activities……54
12.6.1 Process Documents……...54
13. Ethics/ Protection of Human Subjects……..54
13.1 Ethical Standard……..54

13.2 Institutional Review Board……55

13.3 Informed Consent Process……55

13.4 Participant Confidentiality……55

14. Data Handling and Record Keeping……56

14.1 Data Management Responsibilities……56

14.2 Data Capture and Document Retention……56

14.3 Protocol Deviations……56

15. Publication Data/ Sharing Policy……57

16. Limitations……57

17. References……58

ABBREVIATIONS AND DEFINITION OF TERMS

AE	Adverse Event
ARR	Annualized Relapse Rate
AV	Atrio-ventricular Block
CI	Confidence Interval
CIS	Clinically Isolated Syndrome
CMP	Clinical Monitoring Plan
CNS	Central Nervous System
CQMP	Clinical Quality Management Plan
CRF	Case Report Form
CROMS	Clinical Research Operations and Management Support
CSF	Cerebrospinal Fluid
DRL	Delegation of Responsibilities Log
DSMB	Data and Safety Monitoring Board
EDSS	Extended Disability Status Scale
FIS	Fatigue Impact Scale
GP	General Practitioner
HFPMS	Hellenic Federation of Persons with Multiple Sclerosis
HNS	Hellenic Neurological Society
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICU	Intensive Care Unit
IM	Intramuscular

INF	Interferone
IRB	Institutional Review Board
IUD	Intrauterine Device
IV	Intravenous
JCV	John Cunningham Virus
MMOR	Medical Monitor Oversight Report
MOP	Manual of Procedures
MRI	Magnetic Resonance Imaging
MS	Multiple Sclerosis
MSFC	Multiple Sclerosis Functional Composite
MSQOL-54	Multiple Sclerosis Quality Of Life-54
NNT	Number Needed to Treat
OCT	Optical Coherence Tomography
OCTOM	Office of Clinical Trials and Operations Management
OHRP	Office for Human Research Protection
PBVC	Percentage Brain Volume Change
PI	Principal Investigator
PML	Progressive Multifocal Leucoencelopathy
POP	Progestin-only Pill
PPMS	Primary Progressive Multiple Sclerosis
PRMS	Progressive relapsing Multiple Sclerosis
QM	Quality Management
RCT	Randomized Controlled Trial
RIS	Radiologically Isolated Syndrome

RRMS	Relapsing/Remitting Multiple Sclerosis
SAE	Serious Adverse Event
SC	Study Coordinator
SPMS	Secondary Progressive Multiple Sclerosis
TB	Tuberculosis
TIA	Transient Ischemic Attack
TSQM	Treatment Satisfaction Questionnaire for Medication
VZV	Varicella-Zoster Virus

ABSTRACT

Background: Multiple sclerosis is the most common autoimmune disorder affecting the central nervous system. Teriflunomide (Aubagio®), used in the treatment of relapsing forms of MS since its approval (on September 13, 2012 by the FDA and on August 26, 2013 in the European Union), is one of the currently available disease-modifying therapeutic choices and simultaneously a very convenient one because of its oral administration; consequently, every additional clinical evidence about it is deemed welcome.

Objectives: The primary objective of the current study is to evaluate the effectiveness of teriflunomide, as far as the progression of disability is concerned, in patients with relapsing forms of MS. For the secondary objectives, we aimed at assessing the risk of disability progression and improvement in terms of number of relapses while for the tertiary objectives we assessed the tolerability of the drug, the MRI quantitative changes and the quality of life of our patients.

Study Design: Long term, prospective, non-interventional, multicenter, double-blinded parallel-cohort study.

Setting/Participants: The study will be conducted at 9 sites in four cities of Greece (Athens, Thessaloniki, Patra, Larissa), all of them neurologic clinics in tertiary care hospitals. The sites' facilities will serve mainly as referral centers and all participants-outpatients will be hospitalized for two days once every three months for clinical, radiological and biochemical evaluation except in case of AEs. The initial plan is to enroll at least 1150 patients (575 per cohort) to recompensate for potential dropouts, patients lost to follow-up and to compulsory termination of participation due to SAEs. Patients 18 to 55 years old with relapsing forms of MS (RRMS and relapsing form of SPMS) diagnosed with McDonald criteria with a score of 0 to 5 on the EDSS who haven't taken teriflunomide ever in the past and who don't suffer from an immunocompromising condition or receive any concomitant immunosuppressive medication are eligible to enter the study.

Study Interventions and Measures: The first cohort will receive teriflunomide as monotherapy while the parallel cohort will receive one of the other two approved oral disease-modifying therapies available [fingolimod (Gilenya®) and dimethyl fumarate (Tecfidera®)]. In case of a relapse (a definition will be given in the main part) IV or oral methylprednisolone according to routine medical practice will be administered.

The primary objective of the study is to evaluate the improvement or deterioration from former disability state, with disability expressed in terms of the EDSS and the MSFC. Secondary objectives include improvement in terms of ARR and proportion of patients with a relapse as well as estimating the risk of disability progression in the form of the Rio Score. Finally, tertiary objectives include reporting of any AEs, evaluation of MRI images and evaluation of the MSQOL-54 questionnaire depicting the quality of life of our patients from their point of view.

PROTOCOL SYNOPSIS

Study title: Long term, prospective, non-interventional, multicenter, double-blinded parallel-cohort study monitoring effectiveness and tolerability of teriflunomide (Aubagio®) in the progress of disability in patients with MS.

Purpose and Rationale: Purpose is primarily to explore the effectiveness of teriflunomide, as far as the progression of disability is concerned, in patients with relapsing forms of MS, secondarily to assess the risk of disability progression and improvement in terms of number of relapses and tertiarily to assess the tolerability of the drug, MRI quantitative changes and the quality of life of our patients.

Teriflunomide (Aubagio®) was approved for the treatment of relapsing forms of MS on September 13, 2012 by the FDA and on August 26, 2013 in the European Union and is one of the currently available disease-modifying therapeutic. The fact that treatment with teriflunomide consists of a tablet with active substance either 7 or 14 mg administered orally every day as well as its few reported AEs make it rather popular among patients. The rationale of the study is to compare teriflunomide's effectiveness and tolerability with those of the other two approved oral disease-modifying therapies available (fingolimod and dimethyl fumarate).

Study Objectives:

Primary

- To evaluate the improvement or deterioration from former disability state, with disability expressed in terms of the EDSS and the MSFC.

Secondary.

- To assess the risk of disability progression with the help of the Rio score.
- To estimate the severity of the disease and the number of relapses in terms of ARR and proportion of patients with a relapse.

Tertiary

- To assess the tolerability of the drug in terms of AEs.
- Radiologic evaluation of MRI images for quantitative changes.
- To assess the quality of life of our patients based on their answers from their point of view in the MSQOL-54 questionnaire.

Study Design: Long term, prospective, non-interventional, multicenter, double-blinded parallel-cohort study.

Subject Population: This study will include patients with relapsing forms of MS who either have been recently initiated with teriflunomide or are about to start their therapy with it and haven't received it ever in the past; moreover, patients who are being treated with other approved oral disease-modifying therapy or are about to start their therapy with it in accordance with routine clinical practice will be eligible for the study.

Inclusion/Exclusion Criteria:

Inclusion Criteria

- Patients 18 to 55 years old, male or female, with relapsing forms of MS (RRMS and relapsing form of SPMS) diagnosed with McDonald criteria with a score of 0 to 5 on the EDSS who either have been recently initiated with teriflunomide or are about to start their therapy with it and haven't received it ever in the past as well as patients who are being treated with other approved oral disease-modifying therapy or are about to start their therapy with it in accordance with routine clinical practice will be eligible for the study.

Exclusion Criteria

- Patients who have received teriflunomide in the past or started receiving it more than two months ago.
- Patients who suffer from a liver condition-disease that may or may not lead to liver insufficiency
- Patients who suffer from an immunocompromising condition or receive any concomitant immunosuppressive medication.
- Pregnant patients and women of childbearing potential not using reliable contraception should be excluded from the study or discontinue from it should it emerge during its conduct because of the occurrence of teratogenicity and embryotoxicity in the offspring of teriflunomide and fingolimod-treated rats and rabbits *(Chan et al. 2016 [1])*.
- Male patients who plan to father a child should also be excluded for the reasons stated above.
- Patients with recent (<6 months) myocardial infarction, unstable angina, class III/IV heart failure or heart decompensation, Mobitz II second-degree or third-degree AV block, sick-sinus syndrome, prolonged QTc interval, severe

and persistent lymphopenia, symptomatic cerebrovascular disease (stroke or TIA) and those who take certain antiarrhythmics because they are not allowed to take fingolimod.

Treatment of Interest: Patients who fulfill the eligibility criteria will receive teriflunomide 14 mg once daily as monotherapy in accordance with routine clinical practice while patients who receive one of the other two oral disease-modifying therapies (fingolimod 0.5 mg once daily or dimethyl fumarate 120 mg taken twice daily for a week and 240 mg taken twice daily thereafter) available for MS will constitute a parallel cohort. In case of a relapse (a definition will be given in the main part) IV or oral methylprednisolone according to routine medical practice will be administered.

Number of Subjects: The initial plan is to enroll at least 1150 patients (575 per cohort) to recompensate for potential dropouts, patients lost to follow-up and to compulsory termination of participation due to SAEs. The study will be conducted at 9 sites in four cities of Greece (Athens, Thessaloniki, Patra, Larissa), all of them neurologic clinics in tertiary care hospitals.

Study Duration: Each subject's participation will last at least 2 years and the entire study is expected to keep going for at least 4 years.

Study Phases: The study will mainly consist of a *screening phase*, where fulfillment of eligibility criteria will be assessed and the process of informed consent will take place, and an *observation period.*

Effectiveness Evaluations: The effectiveness of teriflunomide, as far as the progress of disability is concerned, will be assessed in terms of the EDSS and the MSFC. ARR and proportion of patients with a relapse will be utilized to estimate the severity of the disease and the Rio score will be utilized for the estimation of the risk of disability progression.

For the tolerability of the drug we will depend on the patients' and our own discoveries of AEs; the quality of life of our patients will be assessed based on their answers in the MSQOL-54 questionnaire while the thorough examination of MRI images for quantitative changes (more detail will be given in the main part) will provide a useful tool.

Data Analysis: For the statistical processing of the data the IBM® SPSS v23.0 will be used. Summary statistics for continuous variables (results from the EDSS, the MSFC, the Rio score, the ARR, proportion of patients with a relapse, number of new or enlarging hypointense lesions on T1–weighted scans, hyperintense lesions on T2–weighted scans and gadolinium-enhancing lesions on T1–weighted scans and the MSQOL-54) will include N, mean, median, standard deviation, minimum, maximum, lower and upper quartile; the t-test will be used for comparisons between the two cohorts and paired t-test for the analysis of the endpoints (change between baseline and last visit). Likewise, for discrete variables (AEs) absolute and relative frequencies will be used.

Data and Safety Monitoring Plan: An independent data and safety monitoring board will be responsible for processing all the data regarding the benefits and potential risks of the treatments examined, subject safety, protocol compliance and data integrity.

1 INTRODUCTION

1.1 Background

Multiple sclerosis, a chronic inflammatory demyelinating disorder of the CNS defined by episodes disseminated in time and neuroanatomical location, is the most common autoimmune disorder affecting the central nervous system. According to the Atlas of MS, there are about 2.3 million people in the world with MS, although the number may be much higher as it is likely that many people with MS remain undiagnosed in certain parts of the world.

MS typically emerges in young adulthood, and its incidence is highest in Northern Europe and North America, where it occurs in up to 1 in 1,000 individuals. At diagnosis, approximately 85% of patients have RRMS, characterized by recurrent, acute episodes (relapses) of neurological symptoms. After 10-15 years, 50-60% of patients with RRMS enter the SPMS where a less inflammatory, and more neurodegenerative, course of disease takes precedence. Patients with SPMS can also be further divided based on whether they still continue to experience relapses (relapsing form of SPMS) or not (purely progressive SPMS). About 10-15% of MS patients present with PPMS defined by a gradual accumulation of disability from symptom onset without superimposed exacerbations or remissions. Moreover, PRMS, which is the least frequent form of MS, constitutes a chronic progressive from onset with infrequent relapses even though this term is encouraged to be dropped due to its overlapping with other disease course subtypes. Last but not least, according to the 2013 revisions, multiple sclerosis is classified into two phenotypes, relapsing and progressive plus RIS, which is the incidental discovery of asymptomatic MRI lesions with location and morphology suggestive of multiple sclerosis, something that may represent preclinical MS in some patients; relapsing MS is further divided into CIS and RRMS (active or not active) while progressive MS is further divided into PPMS and SPMS, both of them active or inactive, with or without progression.*(Lublin et al. 2014 [2])*

There are currently 14 disease-modifying medications approved by the FDA for use in relapsing forms of MS. Specifically, the approved drugs in chronological order of their approval year are: interferon beta-1b (Betaseron®), glatiramer acetate (Copaxone®), interferons beta-1a (Avonex® and Rebif®), mitoxantrone (Novantrone®) available only as a generic drug, natalizumab (Tysabri®), interferon beta-1b (Extavia®), fingolimod (Gilenya®), teriflunomide (Aubagio®), dimethyl fumarate (Tecfidera®), pegylated interferonbeta-1a (Plegridy®), alemtuzumab (Lemtrada®), glatiramer acetate, generic equivalent of Copaxone® (Glatopa™) and the most recently approved daclizumab (Zinbryta™) *[Wingerchuk et al. 2016 [3]]*.

1.2 Relevant Literature and Data

All of the disease-modifying therapies mentioned above have their own different mechanisms of action. Regardless of it, they all improve the clinical course of relapsing/remitting MS for the duration of RCT as assessed by reductions in ARR and changes in MRI imaging such as number of new or enlarging hyperintense lesions on T2-weighted images or hypointense lesions on T1-weighted images. *(Torkildsen et al. 2016 [4])*. On the other hand, only some of them reduced accumulation of disability as measured by the EDSS and brain atrophy in MRI images (*Tsivgoulis et al. 201[5])*. For those diagnosed with CIS, in some RCTs there was evidence of disease-modifying drugs that delayed dissemination in time. Finally, the recommendations for RIS are clinical and MRI surveillance at the beginning *(Okuda et al. 2014 [6])*.

Based on current knowledge of risk−benefit and clinical evidence of the approved MS medications and taking patients' adherence into consideration, it is suggested that oral treatment with dimethyl fumarate or teriflunomide should be preferred as a starting therapy amongst the first-line available medications for *de novo* RRMS which include, apart from the two mentioned, INFs and glatiramer acetate. In the case of disease that can't be controlled with first-line therapy, or rapidly evolving severe RRMS, second-line therapy with natalizumab, fingolimod or alemtuzumab should be chosen based on careful risk−benefit stratification *(Torkildsen et al. 2016 [4])*.

Before focusing on teriflunomide, there will be a brief overview of the mechanism of action and efficacy, effectiveness and safety of the other two approved oral disease-modifying therapies available that will constitute the parallel cohort in our study. To begin with, dimethyl fumarate, a fumaric acid ester approved for the treatment of relapsing forms of MS in 2013 while fumaric esters have been used for several decades against psoriasis, is a 120 mg capsule taken twice daily for a week followed by a 240 mg capsule taken twice daily thereafter. It has an enteric coating in order to improve gastrointestinal tolerability and is metabolized to monomethyl fumarate, which is eliminated by respiration with minimal hepatic or renal excretion. It is an immunomodulatory agent with both anti-inflammatory and neuroprotective properties due to the reduction of oxidative stress *(Dubey et al. 2015 [7])*; however its mechanism of action in MS is only partially understood. Pre-clinical studies indicate that dimethyl fumarate responses are primarily mediated through activation of the nuclear factor (erythroid-derived 2)-like 2 (Nrf2) transcriptional pathway. Dimethyl fumarate has also been shown to upregulate Nrf2-dependent antioxidant genes in patients *(Linker et al. 2013 [8])*.

In two 24 month phase III RCTs, DEFINE and CONFIRM, the investigators assessed the efficacy and safety of oral dimethyl fumarate (proportion of patients who had a relapse by 2 years, annualized relapse rate over the same time interval, time to confirmed progression of disability, and findings on MRI) comparing a 240 mg dose twice or thrice daily versus placebo (in the CONFIRM trial glatiramer acetate was also included as a reference comparator). Results showed that the 240 mg twice daily (and three times a day) dose was significantly superior to placebo, reducing ARR by

53% with a NNT=9 for proportion relapse-free (48%) for DEFINE and 44% with NNT=5 for CONFIRM (51% for the group of 240 mg three times a day and 29% for glatiramer acetate). Risk of disability progression was significantly reduced by 38% with NNT=9 (34% with 240 mg three times a day) compared to placebo in the DEFINE trial, but did not reach significance in the CONFIRM study (21% with 240mg twice a day, 24% with 240 mg thrice a day and 7% with glatiramer acetate vs placebo). MRI signs of disease activity (number of gadolinium-enhancing lesions on T1–weighted scans, number of new/enlarged lesions on T2–weighted scans and number of new hypointense lesions on T1–weighted scans) were reduced in both phase III trials. Finally, a post hoc analysis in the CONFIRM study showed no difference in clinical outcomes for twice daily dimethyl fumarate versus glatiramer acetate *(Fox et al. 2012 [9], Gold et al. 2012 [10])*.

Dimethyl fumarate is associated with self limited gastrointestinal upset and flushing, each occurring in about 30% of patients as well as diarrhea (13%) and nausea (11%) *[Fox et al. 2012 [9], Gold et al. 2012 [10]]*. Lymphocytes typically decline by 20-30% and should be monitored; risk factors included age over 50 years, lower baseline lymphocyte count, and previous natalizumab treatment *(Longbrake et al. 2015 [11])*. PML has been reported with prolonged lymphopenia during use of compounded fumaric acid products for psoriasis, with or without concomitant immunosuppressive drugs *(Ermis et al. 2013 [12], van Oosten et al. 2013 [13]*; additionally PML has been reported in three MS patients taking dimethyl fumarate without previous natalizumab exposure with a lymphopenia of below 0.5×10^9/L *(FDA 2014 [14])*, in a psoriasis patient without severe lymphopenia *(Rosenkranz et al. 2015 [15])* and recently in a dimethyl fumarate treated multiple sclerosis patient with lymphocyte levels not lower than 0.6×10^9/L and previous natalizumab use. Therefore and because dimethyl fumarate elevates liver transaminases as well, the EMA recommends obtaining a complete blood count every three months; both the EMA and FDA recommend considering discontinuation of dimethyl fumarate if the lymphocyte count is below 0.5×10^9/L for more than six months (especially in JCV-positive patients) or if liver transaminase levels increase more than three times above normal. Lymphopenic patients continuing dimethyl fumarate should be under PML surveillance, and JCV serological testing is reasonable *(EMA 2015 [16], FDA 2016 [17])*.

Fingolimod is a sphingosine 1-phosphate receptor (S1PR) modulator that subsequent to its phosphorylation binds with high affinity to S1PR, which in turn leads to an internalization and degradation of the receptor in different tissues and cell types, including lymphocytes *(Cohen et al. 2011 [18])*. As a consequence, fingolimod inhibits the ability of autoreactive T and B lymphocytes to egress from the lymph nodes towards the CNS reducing their peripheral blood counts by 70 % of baseline within 3 months of treatment initiation *(Bridel et al. 2014 [19])*. Fingolimod, approved by the FDA as a first-line treatment whereas in the European Union as a second-line treatment except for patients with highly active RRMS, is administered orally once a day in form of a 0.5 mg capsule.

This time three Phase III trials will be examined; FREEDOMS, FREEDOMS II and TRANSFORMS. The first two compared fingolimod 0.5 or 1.25mg/day with placebo while in the

TRANSFORMS study a dose of 30 mcg IM INF beta-1a was used as a reference treatment. In FREEDOMS fingolimod at a dose of 0.5 mg/day (1.25mg/day) significantly reduced ARR by 54% (60%), risk of disability progression by 30% (32%) and MRI outcomes while reductions in brain volume were smaller with both doses of fingolimod *(Kappos et al. 2010 [20])*. In FREEDOMS II trial a significant 48% reduction in ARR was found compared with placebo; furthermore, PBVC was significantly smaller with fingolimod and likewise MRI outcomes were significantly reduced. However there was no statistically significant difference in confirmed disability *(Calabresi et al. 2014 [21])*. Likewise in the TRANSFORMS trial fingolimod significantly reduced ARR compared with intramuscular interferon beta-1a (0.16 with 0.5mg/day, 0.20 with 1.25mg/day and 0.33 with INF beta-1a) while no significant difference was observed with respect to progression of disability; moreover MRI outcomes were significantly reduced with both doses vs. INF beta-1a (significantly fewer new or enlarged hyperintense lesions on T_2-weighted images and gadolinium-enhancing lesions on T_1-weighted images and significantly smaller PBVC) *[Cohen et al. 2010 [22]]*.

Common adverse events include upper respiratory tract infection, headache, cough, diarrhoea and back pain *(Kappos et al. 2010 [20], Calabresi et al. 2014 [21])* Fingolimod also increases the risk of varicella zoster virus infection – with one case of fatal disseminated varicella zoster virus and one of herpes simplex virus-1 infection occurred in the TRANSFORMS trial – so patients must have documented varicella zoster virus immunity or pre-treatment immunization *(Cohen et al. 2010 [22], Arvin et al. 2015 [23])*. There have also been reports of cryptococcal central nervous system and skin infections as well as three cases of PML so routine assessment of JCV antibody status and vigilance for PML symptoms or MRI changes is mandatory *(Torkildsen et al. 2016 [4])*. Another side effect is first dose bradycardia, almost universal but rarely symptomatic which requires monitoring of vital signs for six hours after the first dose and extended cardiac telemetry monitoring if excessive bradycardia is persistent or symptomatic *(Kappos et al. 2010 [20], Calabresi et al. 2014 [21], Cohen et al. 2010 [22])*. Other organ specific side effects because of widespread sphingosine-1-phosphate receptor expression are transient 1st and 2nd degree AV blocks, mild hypertension, macular edema, elevated liver transaminases, basal-cell carcinoma and other neoplasms *(Kappos et al. 2010 [20], Calabresi et al. 2014 [21], Cohen et al. 2010 [22])*; therefore ophthalmological baseline and monitoring examinations are needed to detect macular edema and dermatological assessment for all skin lesions *(Jain et al. 2012 [24])*. Female patients are, as with other MS treatments, advised not to become pregnant while treated with fingolimod, and a 3 month washout period with normalisation of blood tests before conception is recommended because pre-clinical studies with fingolimod demonstrated embryotoxicity and teratogenicity. Contraindications include cardiac disorders (recent (<6 months) myocardial infarction, unstable angina, class III/IV heart failure or heart decompensation, Mobitz II second-degree or third-degree AV block, sick-sinus syndrome, prolonged QTc interval), certain antiarrhythmics, severe and persistent lymphopenia and symptomatic cerebrovascular disease (stroke or TIA).

Finally our treatment of choice for our study, teriflumomide will be presented briefly.

Teriflunomide, the active metabolite of leflunomide which is an anti-rheumatic drug, selectively and reversibly inhibits the mitochondrial enzyme dihydroorotate dehydrogenase, required for de novo pyrimidine synthesis. In this way it impairs proliferation of dividing cells (activated T- and B-lymphocytes) and therefore reduces their ability to participate in a potentially damaging immune attack on the CNS *(Amit Bar-Or et al. 2014 [25])*. Teriflunomide is administered orally as tablets, 7 or 14 mg once daily.

In TEMSO, a 24-month Phase III RCT both approved doses reduced ARR significantly (7mg by 31.2% and 14 mg by 31.5%) compared to placebo whereas only the 14 mg dose significantly reduced disability progression (30%; NNT=14); additionally the 14 mg dose reduced significantly MRI evidence of disease activity (change in total lesion volume from baseline, volume of hypointense lesions on T1–weighted scans, hyperintense lesions on T2–weighted scans or gadolinium-enhancing lesions on T1–weighted scans) compared to placebo. Brain-volume loss did not differ significantly between teriflunomide and placebo-treated groups *(O'Connor et al. 2011 [26])*. In a second 24-month Phase III RCT named TOWER which also compared both approved doses to placebo, significant reductions in ARR took place (22.3% with 7 mg and 36.3% with 14 mg) relative to placebo. However only the 14 mg dose reduced significantly the risk of sustained accumulation of disability by 32% with NNT=5 while the 7mg dose barely showed an effect *(Confavreux et al. 2014 [27])*. In TOPIC, a third Phase III RCT, teriflunomide showed benefit for treatment of CIS as well; compared with placebo, it significantly reduced the risk of multiple sclerosis defining relapse with both 14 mg (43% reduction) and 7 mg (37% reduction). It also significantly reduced the risk of either relapse or new MRI lesions with both doses (35% with 14 mg and 31% with 7 mg) *[Miller et al. 2014 [28]]*. Finally, in Phase III RCT TENERA of teriflunomide 7 or 14 mg once daily, compared to 44 μg of subcutaneous interferon beta-1a three times weekly, no difference in ARR between teriflunomide 14 mg and IFNβ-1a was spotted (0.26 and 0.22 respectively), but ARR was significantly higher with teriflunomide 7 mg. Furthermore, FIS scores indicated more frequent fatigue with IFNβ-1a, though differences were only significant with teriflunomide 7 mg. TSQM scores were significantly higher with teriflunomide *(Vermersch et al. 2014 [29])*.

Adverse effects of teriflunomide include diarrhea, hair thinning and alopecia, elevated transaminases and potential serious hepatotoxicity, hypertension, peripheral neuropathy, paresthesia, nausea, leucopenia and serious infections (upper respiratory tract, urinary tract) *[O'Connor et al. 2011 [26], Confavreux et al. 2014 [27]]*. Moreover, TB reactivation has been reported in patients treated with leflunomide; consequently TB tests (Mantoux, sputum smear, blood tests and cultivation) and treatment (if positive) prior to teriflunomide initiation are mandatory. Live attenuated vaccines are contraindicated in patients treated with teriflunomide, and immune status should be verified prior to treatment initiation *(Amit Bar-Or et al. 2013 [30])*. It has a prolonged half life owing to enterohepatic recirculation and therefore the recommended washout period is 8 months unless accelerated to 11 days by administration of cholestyramine or activated charcoal

(Wingerchuk et al. 2016 [3]). Teriflunomide additionally activates cytochrome P450 and interaction with other drugs including warfarine, antibiotics and anti-epileptics may occur *(Bridel et al. 2014 [19])* Because of the previously stated hepatotoxicity, a relatively frequent (every second week) transaminase screening during the first 6 months of treatment is recommended and thereafter every second month; discontinuation of therapy should be considered if a serum transaminase increase more than three times the upper normal level is confirmed *(O'Connor et al. 2011 [26], Confavreux et al. 2014 [27])*. Last but not least, teriflunomide is excreted in breast milk and semen; therefore reproductive counseling is necessary and female patients are advised not to get pregnant and male patients not to father a child while treated with teriflunomide because preclinical studies with it in rats, rabbits and mice have demonstrated embryotoxicity and teratogenicity *(Lu et al. 2013 [31])*.

1.3 Purpose and Rationale

The purpose of this prospective, non-interventional, multicenter, double-blinded post-approval parallel-cohort study is primarily to explore the effectiveness of teriflunomide, as far as the progression of disability is concerned, in patients with relapsing forms of MS, secondarily to evaluate, the improvement in terms of number of relapses and risk of disability progression and tertiarily to assess the tolerability of the drug, the radiologic progress of the disease in terms of MRI quantitative changes and the quality of life of our patients under conditions of routine medical practice.

1.4 Compliance Statement

This study will be conducted in full accordance with all applicable federal and state laws and regulations, the Good Clinical Practice: Consolidated Guideline approved by the ICH and the ethical principles laid down in the Declaration of Helsinki. All episodes of noncompliance will be documented.

The investigators will perform the study in accordance with this protocol, will obtain informed consent and will report unanticipated problems involving risks to subjects or others in accordance with the University Hospital of Larissa IRB Policies and Procedures and all federal requirements. The investigators will bear in mind that the outmost goal is to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of the study participants. Collection, recording, and reporting of data will be accurate and will ensure the privacy, health, and welfare of research subjects during and after the study.

1.5 Potential Risks and Benefits

1.5.1 Potential Risks

Patients who are treated with teriflunomide may present with diarrhea, hair thinning and alopecia, elevated transaminases and potential serious hepatotoxicity, hypertension, peripheral neuropathy, paresthesia, nausea, leucopenia and serious infections (upper respiratory tract, urinary tract). Teriflunomide has also a prolonged half-life, is embryotoxic and teratogenic (Pregnancy Category X) and is secreted in breast milk and semen; TB reactivation may take place, interaction with other drugs including warfarine, antibiotics and anti-epileptics may occur. Therefore a relatively frequent (every second week) transaminase screening during the first 6 months of treatment is recommended and thereafter every second month and discontinuation of therapy should be considered if a serum transaminase increase more than three times the upper normal level is confirmed. TB tests and treatment (if positive) prior to teriflunomide initiation are mandatory and immune status should be verified prior to treatment initiation. Female patients are advised not to get pregnant while treated with teriflunomide and in case of need for fast-paced washout (e.g. for discontinuation of therapy in men wishing to father a child) cholestyramine or activated charcoal should be administered.

Dimethyl fumarate is associated with self limited gastrointestinal upset, flushing, diarrhea, nausea, elevation of liver transaminases, decrease in the number of lymphocytes, PML in patients with or without lymphopenia. Therefore it is highly recommended that a complete blood count should be obtained every three months and that discontinuation should be considered if the lymphocyte count is below 0.5×10^{9}/L for more than six months (especially in JCV-positive patients) or if liver transaminase levels increase more than three times above normal. Lymphopenic patients continuing dimethyl fumarate should be under PML surveillance, and JCV serological testing is reasonable.

Treatment with fingolimod renders patients more possible to present with upper respiratory tract infection, headache, cough, diarrhea, back pain, transient bradycardia and 1st and 2nd degree AV blocks, mild hypertension, macular edema, elevated liver transaminases, basal-cell carcinoma and other neoplasms. Fingolimod also increases the risk of varicella zoster virus infection, cryptococcal central nervous system and skin infections and PML so patients must have documented varicella zoster virus immunity or pre-treatment immunization and undergo routine assessment of JCV antibody status while PML symptoms or MRI changes should be discovered as soon as possible after onset. Ophthalmological baseline and monitoring examinations are needed to detect macular edema as well as dermatological assessment for all skin lesions. Female patients are, as with other MS treatments, advised not to become pregnant while treated with fingolimod (Pregnancy Category C), and a 3 month washout period with normalisation of blood tests before conception is recommended because pre-clinical studies with fingolimod demonstrated embryotoxicity and teratogenicity.

2. OBJECTIVES

2.1 Study Objectives

2.1.1 Primary Objective

The primary objective of this study is to evaluate the improvement or deterioration from former disability state, with disability expressed in terms of the EDSS and the MSFC, in patients with MS that are treated with teriflunomide compared to patients treated with some other approved oral disease-modifying therapy.

2.1.2 Secondary Objectives

The secondary objectives in the same patients are:

- To assess the risk of disability progression with the help of the Rio score.
- To estimate the severity of the disease and the number of relapses in terms of ARR and proportion of patients with a relapse.

2.1.3 Tertiary Objectives

The tertiary objectives in the same patients are:

- To assess the tolerability of the drug in terms of AEs.
- Radiologic evaluation of MRI images for quantitative changes.
- To assess the quality of life of our patients based on their answers from their point of view in the MSQOL-54 questionnaire.

2.2 Study Outcome Measures

For the primary endpoint the EDSS and the MSFC – assessed at screening visit, enrollment visit and every 3 months and at the time of suspected relapse – will be used as instruments. For the risk of **disability progression** (secondary endpoint defined as **an increase in the EDSS score of at least 1.0 point in patients with a baseline score of 1.0 or more or an increase of at least 1.5 points in patients with a baseline score of 0, confirmed at least 12 weeks later**) the Rio score will be utilized once during enrollment and once with the end of the study for each participant while ARR and proportion of patients with a relapse will be defined at the end of each year in order to estimate the severity of the disease (secondary endpoint). In our protocol **relapse** is defined as **new or recurrent neurologic symptoms not associated with fever or infection, lasting at least 24**

hours, accompanied by new objective neurologic findings and separated from the onset of other confirmed relapses by at least 30 days. For the tertiary endpoints MRI images will be evaluated at screening and at month 6, 12 and at termination of study for each participant in a blinded manner at a central MRI evaluation center for **quantitative changes** – defined as **number of new or enlarging hypointense lesions on T1–weighted scans, hyperintense lesions on T2–weighted scans and gadolinium-enhancing lesions on T1–weighted scans** – and the MSQOL-54 questionnaire – taken at enrollment and at month 6, 12, 18 and at termination of study – will be used.

3. INVESTIGATIONAL PLAN

3.1 General Schema of Study Design

This is a long term, prospective, non-interventional, multicenter, double-blinded parallel-cohort study to monitor and assess the effectiveness of teriflunomide in terms of disability and progression of patients with relapsing forms of MS. This study will be based on Greek citizens with MS born and raised in Greece without living abroad for an interval greater than a year. Due to the fact that the treatments compared in this study are orally administered, the majority of participants will consist of outpatients who visit the study sites only for the predefined monitoring dates and in case of an emergency (relapses included).

3.2 Study Duration, Timeline and other Protocol-Specific Details

The study is expected to last at least 4 years. The study will be considered complete once the last patient enrolled in it will have been followed for 2 years. Enrollment of participants and screening is estimated to be complete around the end of the 2nd year of the study and each participant is expected to spend 2 years in the study, with up to 10 days screening period, following the protocol's guidelines. Physical examination, blood and urine sample tests (including lymphocyte count, transaminases and pregnancy test as well as CT imaging of the liver) and immunization profile and status evaluation (including JCV serological status) will be conducted only in the study's official facilities by blinded health professionals. Standardized neurologic assessments and the evaluation of the scales and questionnaires for the study will be conducted at the appropriate time by blinded neurologists specialized in MS and the MRI images for each participant will be evaluated in a blinded manner at a central MRI evaluation center.

3.3 Total Number of Study Sites/Total Number of Subjects Projected

The study will be conducted at 9 sites in four cities of Greece (Athens, Thessaloniki, Patra, Larissa), all of them neurologic clinics in tertiary care hospitals. The hospitals which will provide their facilities and equipment for the study will be the University General Hospital of Larissa, Ippokration General Hospital of Athens, Laikon General Hospital of Athens, Evangelismos General

Hospital of Athens, Attikon General Hospital of Athens, AHEPA Hospital in Thessaloniki, Papageorgiou General Hospital of Thessaloniki, 424 General Military Hospital of Thessaloniki and University General Hospital of Patra. Recruitment will stop when approximately 1150 subjects are enrolled to make up for potential dropouts, patients lost to follow-up and to compulsory termination of participation due to SAEs. It is expected that approximately 575 subjects will be enrolled in each cohort in order to extract reproducible and useful information.

4. STUDY POPULATION, ENROLLMENT AND WITHDRAWAL

4.1 Study Population

The ideal sample size for our study is estimated to be around 1150 participants to recompensate for potential dropouts, patients lost to follow-up and to compulsory termination of participation due to SAEs. The anticipated number of potential recruits to be screened in order to reach our target enrollment is estimated to slightly exceed 1500 patients. The participants will be mostly outpatients and will be drawn from the population of Greek natives born and raised in Greece without living abroad for an interval greater than a year.

4.1.1 Subject Inclusion Criteria

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

- Provide signed and dated informed consent form.
- Willing to comply with all study procedures and be available for the duration of the study.
- Male or female, aged 18 to 55.
- Diagnosed with a relapsing form of RRMS (RRMS and relapsing form of SPMS) according to the McDonald criteria.
- Have a score of 0 to 5 on the EDSS which ranges from 0 to 10 with higher scores indicating greater disability.
- Experienced at least one clinically documented relapse in the previous 12 months or had at least one gadolinium-enhancing lesion 0 to 12 weeks before enrollment.
- Has recently initiated treatment with teriflunomide or is about to start his/her therapy with it and hasn’t received it ever in the past.
- Is currently being treated with other approved oral disease-modifying therapy or is about to start his/her therapy with it in accordance with routine clinical practice.

- Women of reproductive potential must use highly effective contraception (licensed hormonal methods such as combined oral contraceptives, vaginal rings and POPs as well as intrauterine methods like copper or hormonal IUD when indicated).
- Men of reproductive potential must use condoms.

4.1.2 Subject Exclusion Criteria

An individual who meets any of the following criteria will be excluded from participation in this study:

- Has received teriflunomide in the past or started receiving it more than two months ago.
- Suffers from a liver condition-disease that may or may not lead to liver insufficiency
- Suffers from an immunocompromising condition or receives any concomitant immunosuppressive medication.
- Pregnant patients, women of childbearing potential not using reliable contraception and women who want to lactate should be excluded from the study or discontinue from it should it emerge during its conduct because of the occurrence of teratogenicity and embryotoxicity in the offspring of teriflunomide and fingolimod-treated rats and rabbits *(Chan et al. 2016 [1])*. The reason for this is that it has been found that teriflunomide and fingolimod are excreted in maternal milk and paternal semen.
- Male patients who plan to father a child should also be excluded for the reasons stated above.
- Patients with recent (<6 months) myocardial infarction, unstable angina, class III/IV heart failure or heart decompensation, Mobitz II second-degree or third-degree AV block, sick-sinus syndrome, prolonged QTc interval, severe and persistent lymphopenia, symptomatic cerebrovascular disease (stroke or TIA) and those who take certain antiarrhythmics because they are not allowed to take fingolimod.
- Has a heart pacemaker, a metallic foreign body in the eye or an aneurysm clip in the brain or aorta and sometimes in the back after surgery and severe claustrophobia since these are contraindications for an MRI scan.
- Participation in a clinical study that may interfere with participation in this study within 6 months before the onset and 6 months after the termination of the study.
- History of current tobacco (more than 10 cigarettes a day or 10 pack-years), recreational drug and alcohol use (scoring 3 or more in the FAST scale).
- Anything that would place the individual at increased risk or preclude the individual's full compliance with or completion of the study.

4.2 Strategies for Recruitment and Retention

Study subjects will be recruited based upon their presentation with MS for the first time or after tracing back to the registers of the neurological clinics of the hospitals participating in the study and those of the HFPMS and various clubs associated with MS and contacting the possibly eligible patients via phone or direct visit or via their personal caring doctor or GP. Because of the need for long-term participation, several specially appointed members of the nursing staff and senior interns of the neurological clinics participating in the study will be charged with the task of keeping a record with results of clinical, laboratory and radiologic examination of each patient after each visit; additionally they will be responsible for the motivation of patients with rewards and social events as well as for reminding them of their predefined visits according to the study schedule.

4.3 Subject Withdrawal

Subjects may withdraw voluntarily from the study or the investigator may terminate a subject's participation.

4.3.1 Reasons for Withdrawal

Subjects are free to withdraw from participation in the study at any time upon request according to the GCP principles. An investigator may terminate a study subject’s participation in the study if:

- Any medical condition, event or situation occurs such that continued participation in the study would not be in the best interest of the subject (e.g. evolution of the current relapsing form of MS into a rapidly evolving SPMS without relapses or critical worsening of condition and rapid disability progression that necessitates a change in treatment).
- The subject meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation.

4.3.2 Handling of Subject Withdrawals

Even though study participants may withdraw or discontinue from the study, there is the possibility of them being followed-up despite their withdrawal in order to collect safety and other data for the subsequent statistical analysis. In case a participant withdraws or discontinues early (before the milestone of 2 months), he can be replaced with another possibly eligible participant who meets the inclusion and exclusion criteria and is of the same gender and age ± 2 years.

4.4 Premature Termination or Suspension of Study

This study may be suspended or prematurely terminated in case of a sufficient reasonable cause. Written notification, documenting the reason for premature suspension or termination, will be provided by the PI who will also is obliged to promptly inform the University Hospital of Larissa IRB and provide the reason(s) for the suspension or termination.

Circumstances that may warrant termination include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to subjects.
- Insufficient adherence to protocol requirements.
- Data that is not sufficiently complete and/or evaluable and reproducible.
- Determination of futility.

5. STUDY SCHEDULE

In our study, according to the initial plan without considering unscheduled visits, each participant is destined to undergo 32 visits. These include the screening visit, the enrollment visit, 29 intermediate visits and the final one (there can be one additional intermediate visit if need be). In each visit routine clinical examination and blood and urine sample tests will take place. The EDSS and MSFC scales will be assessed at screening visit, enrollment visit and every 3 months and at the time of suspected relapse. The Rio score for the assessment of disability progression will be utilized once during enrollment and once with the end of the study for each participant while ARR and proportion of patients with a relapse will be estimated twice (once at the end of the first year and once at the end of the study. MRI images will be evaluated at screening and at month 6, 12 and at termination of study for each participant and the MSQOL-54 questionnaire will be distributed at enrollment and at month 6, 12, 18 and at termination of study.

5.1 Screening Visit (Visit 1, Day -10 to -1)

For the screening visit, the candidates for participation in the study will visit the study's confirmed sites which will act as referral centers and follow the procedure in order to see if they match the predefined eligibility criteria. Because some screening procedures are required for eligibility, they will be performed under a separate screening consent form. At start, the screening consent form will be distributed and after the necessary briefing by the medical staff, it must be signed in order for the screening procedure to start. Finally, after screening procedure is over and the prospective participant has met the eligibility criteria, the research staff proceeds to the briefing

for the study and the distribution of the study consent form which will be signed by the participant itself or by his appointed representative should he be unable to sign or give informed consent. Screening visit will consist of the following:

- Obtain and document consent from prospective participant on screening consent form.
- Review medical history to determine eligibility based on inclusion/exclusion criteria (focusing on heart disease, hypertension, liver disease, autoimmune disease, condition of the skin and the eyes, drug, alcohol and tobacco use, immunization record especially for VZV and TB, previous surgery for placement of a pacemaker or foreign metal bodies in the eye, the aorta, the back and the joints and possibility of pregnancy for women).
- Review medications history to determine eligibility based on inclusion/exclusion criteria.
- Perform medical examinations needed to determine eligibility (focusing on cardiovascular system with ECG, ultrasonography and blood pressure estimation, routine radiologic examination with X-ray of chest and abdomen, CT scan of the abdomen in order to check the condition of the liver and ophthalmologic examination).
- Collect blood samples for full blood and specifically lymphocyte count, transaminases, control for autoimmune diseases and assessment of JCV antibody status and urine samples for the pregnancy test.
- Perform a Mantoux test.
- Perform a full neurologic examination including assessment in terms of EDSS and MSFC, and MRI scan of the CNS.
- Inform the participants that meet the eligibility criteria that they must use contraception and recommend methods of contraception.
- Schedule study visits for individuals who are eligible and available for the duration of the study.
- Provide potential participants with instructions needed to prepare for first study visit.

5.2 Observation Period

5.2.1 Enrollment/Baseline Visit (Visit 2, Day 0)

During the enrollment visit, evaluations/procedures will take place to assess or confirm whether an individual still meets the eligibility criteria and may be enrolled. Enrollment visit in our study should consist of the following:

- Obtain and document consent from the participants who met the eligibility criteria and still want to proceed with the study on study consent form.

- Verify inclusion/exclusion criteria.
- Obtain demographic information.
- Record medical history, medications history and results of physical examinations.
- Perform a full neurologic examination including assessment in terms of EDSS and MSFC, Rio score and MSQOL-54.

5.2.2 Intermediate Visits

5.2.2.1 Visit 3, Day 15±3

- Perform a full neurologic examination and report any AEs either from the nervous system or from other systems.
- Collect blood sample for transaminase levels control. In case of transaminase levels three times above normal and after elimination of all other possible etiologies, termination of study.

5.2.2.2 Visit 4, Day 30±3

- Perform a full neurologic examination and report any AEs either from the nervous system or from other systems.
- Collect blood sample for transaminase levels control. In case of transaminase levels three times above normal and after elimination of all other possible etiologies, termination of study.

5.2.2.3 Visit 5, Day 45±3

- Perform a full neurologic examination and report any AEs either from the nervous system or from other systems.
- Collect blood sample for transaminase levels control. In case of transaminase levels three times above normal and after elimination of all other possible etiologies, termination of study.

5.2.2.4 Visit 6, Day 60±3

- Perform a full neurologic examination and report any AEs either from the nervous system or from other systems.

- Collect blood sample for transaminase levels control. In case of transaminase levels three times above normal and after elimination of all other possible etiologies, termination of study.

5.2.2.5 Visit 7, Day 75±3

- Perform a full neurologic examination and report any AEs either from the nervous system or from other systems.
- Collect blood sample for transaminase levels control. In case of transaminase levels three times above normal and after elimination of all other possible etiologies, termination of study.

5.2.2.6 Visit 8, Day 90±3

- Perform a full neurologic examination and report any AEs either from the nervous system or from other systems.
- Collect blood sample for transaminase levels control, lymphocyte count and routine assessment of JCV antibody status. In case of transaminase levels three times above normal and after elimination of all other possible etiologies, termination of study. In case of lymphopenia, further investigation is required. Should it persist, termination of study should be considered after eliminating other possible etiologies. In case of a recent JCV infection, termination of study should be considered.
- Examination of the cardiovascular system with ECG, ultrasonography and blood pressure estimation
- Ophthalmologic examination for macular edema.
- Assess the EDSS and MSFC scales.

5.2.2.7 Visit 9, Day 105±3

- Perform a full neurologic examination and report any AEs either from the nervous system or from other systems.
- Collect blood sample for transaminase levels control. In case of transaminase levels three times above normal and after elimination of all other possible etiologies, termination of study.

5.2.2.8 Visit 10, Day 120±3

- Perform a full neurologic examination and report any AEs either from the nervous system or from other systems.
- Collect blood sample for transaminase levels control. In case of transaminase levels three times above normal and after elimination of all other possible etiologies, termination of study.

5.2.2.9 Visit 11, Day 135±3

- Perform a full neurologic examination and report any AEs either from the nervous system or from other systems.
- Collect blood sample for transaminase levels control. In case of transaminase levels three times above normal and after elimination of all other possible etiologies, termination of study.

5.2.2.10 Visit 12, Day 150±3

- Perform a full neurologic examination and report any AEs either from the nervous system or from other systems.
- Collect blood sample for transaminase levels control. In case of transaminase levels three times above normal and after elimination of all other possible etiologies, termination of study.

5.2.2.11 Visit 13, Day 165±3

- Perform a full neurologic examination and report any AEs either from the nervous system or from other systems.
- Collect blood sample for transaminase levels control. In case of transaminase levels three times above normal and after elimination of all other possible etiologies, termination of study.

5.2.2.12 Visit 14, Day 180±3

- Perform a full neurologic examination and report any AEs either from the nervous system or from other systems.
- Collect blood sample for transaminase levels control, lymphocyte count and

routine assessment of JCV antibody status. In case of transaminase levels three times above normal and after elimination of all other possible etiologies, termination of study. In case of lymphopenia, further investigation is required. Should it persist, termination of study should be considered after eliminating other possible etiologies. In case of a recent JCV infection, termination of study should be considered.

- Perform a Mantoux test. If positive, continue with blood tests, sputum smear microscopy and cultivation and if need be, proceed to start treatment.
- Examination of the cardiovascular system with ECG, ultrasonography and blood pressure estimation.
- Ophthalmologic examination for macular edema.
- Assess the EDSS and MSFC scales.
- MRI scan of the CNS.
- Distribute the MSQOL-54 questionnaire.

5.2.2.13 Visit 15, Day 210±3

- Perform a full neurologic examination and report any AEs either from the nervous system or from other systems.

5.2.2.14 Visit 16, Day 240±3

- Perform a full neurologic examination and report any AEs either from the nervous system or from other systems.
- Collect blood sample for transaminase levels control. In case of transaminase levels three times above normal and after elimination of all other possible etiologies, termination of study.

5.2.2.15 Visit 17, Day 270±3

- Perform a full neurologic examination and report any AEs either from the nervous system or from other systems.
- Collect blood sample for lymphocyte count and routine assessment of JCV antibody status. In case of lymphopenia, further investigation is required. Should it persist, termination of study should be considered after eliminating other possible etiologies. In case of a recent JCV infection, termination of study

- should be considered.
- Examination of the cardiovascular system with ECG, ultrasonography and blood pressure estimation
- Ophthalmologic examination for macular edema.
- Assess the EDSS and MSFC scales.

5.2.2.16 Visit 18, Day 300±3

- Perform a full neurologic examination and report any AEs either from the nervous system or from other systems.
- Collect blood sample for transaminase levels control. In case of transaminase levels three times above normal and after elimination of all other possible etiologies, termination of study.

5.2.2.17 Visit 19, Day 330±3

- Perform a full neurologic examination and report any AEs either from the nervous system or from other systems.

5.2.2.18 Visit 20, Day 360±3

- Perform a full neurologic examination and report any AEs either from the nervous system or from other systems.
- Collect blood sample for transaminase levels control, lymphocyte count and routine assessment of JCV antibody status. In case of transaminase levels three times above normal and after elimination of all other possible etiologies, termination of study. In case of lymphopenia, further investigation is required. Should it persist, termination of study should be considered after eliminating other possible etiologies. In case of a recent JCV infection, termination of study should be considered.
- Perform a Mantoux test. If positive, continue with blood tests, sputum smear microscopy and cultivation and if need be, proceed to start treatment.
- Examination of the cardiovascular system with ECG, ultrasonography and blood pressure estimation.
- Ophthalmologic examination for macular edema.
- Assess the EDSS and MSFC scales.

- MRI scan of the CNS.
- Distribute the MSQOL-54 questionnaire.
- Estimate the ARR and proportion of patients with a relapse.

5.2.2.19 Visit 21, Day 390±3

- Perform a full neurologic examination and report any AEs either from the nervous system or from other systems.

5.2.2.20 Visit 22, Day 420±3

- Perform a full neurologic examination and report any AEs either from the nervous system or from other systems.
- Collect blood sample for transaminase levels control. In case of transaminase levels three times above normal and after elimination of all other possible etiologies, termination of study.

5.2.2.21 Visit 23, Day 450±3

- Perform a full neurologic examination and report any AEs either from the nervous system or from other systems.
- Collect blood sample for lymphocyte count and routine assessment of JCV antibody status. In case of lymphopenia, further investigation is required. Should it persist, termination of study should be considered after eliminating other possible etiologies. In case of a recent JCV infection, termination of study should be considered.
- Examination of the cardiovascular system with ECG, ultrasonography and blood pressure estimation
- Ophthalmologic examination for macular edema.
- Assess the EDSS and MSFC scales.

5.2.2.22 Visit 24, Day 480±3

- Perform a full neurologic examination and report any AEs either from the nervous system or from other systems.

- Collect blood sample for transaminase levels control. In case of transaminase levels three times above normal and after elimination of all other possible etiologies, termination of study.

5.2.2.23 Visit 25, Day 510±3

- Perform a full neurologic examination and report any AEs either from the nervous system or from other systems.

5.2.2.24 Visit 26, Day 540±3

- Perform a full neurologic examination and report any AEs either from the nervous system or from other systems.
- Collect blood sample for transaminase levels control, lymphocyte count and routine assessment of JCV antibody status. In case of transaminase levels three times above normal and after elimination of all other possible etiologies, termination of study. In case of lymphopenia, further investigation is required. Should it persist, termination of study should be considered after eliminating other possible etiologies. In case of a recent JCV infection, termination of study should be considered.
- Perform a Mantoux test. If positive, continue with blood tests, sputum smear microscopy and cultivation and if need be, proceed to start treatment.
- Examination of the cardiovascular system with ECG, ultrasonography and blood pressure estimation.
- Ophthalmologic examination for macular edema.
- Assess the EDSS and MSFC scales.
- Distribute the MSQOL-54 questionnaire.

5.2.2.25 Visit 27, Day 570±3

- Perform a full neurologic examination and report any AEs either from the nervous system or from other systems.

5.2.2.26 Visit 28, Day 600±3

- Perform a full neurologic examination and report any AEs either from the nervous system or from other systems.

- Collect blood sample for transaminase levels control. In case of transaminase levels three times above normal and after elimination of all other possible etiologies, termination of study.

5.2.2.27 Visit 29, Day 630±3

- Perform a full neurologic examination and report any AEs either from the nervous system or from other systems.
- Collect blood sample for lymphocyte count and routine assessment of JCV antibody status. In case of lymphopenia, further investigation is required. Should it persist, termination of study should be considered after eliminating other possible etiologies. In case of a recent JCV infection, termination of study should be considered.
- Examination of the cardiovascular system with ECG, ultrasonography and blood pressure estimation
- Ophthalmologic examination for macular edema.
- Assess the EDSS and MSFC scales.

5.2.2.28 Visit 30, Day 660±3

- Perform a full neurologic examination and report any AEs either from the nervous system or from other systems.
- Collect blood sample for transaminase levels control. In case of transaminase levels three times above normal and after elimination of all other possible etiologies, termination of study.

5.2.2.29 Visit 31, Day 690±3

- Perform a full neurologic examination and report any AEs either from the nervous system or from other systems.

5.2.3 Terminal Visit (Visit 32, Day 720±3)

- Perform a full neurologic examination and report any AEs either from the nervous system or from other systems.
- Collect blood sample for transaminase levels control, lymphocyte count and routine

assessment of JCV antibody status. In case of transaminase levels three times above normal and after elimination of all other possible etiologies, termination of study. In case of lymphopenia, further investigation is required. Should it persist, termination of study should be considered after eliminating other possible etiologies. In case of a recent JCV infection, termination of study should be considered.

- Perform a Mantoux test. If positive, continue with blood tests, sputum smear microscopy and cultivation and if need be, proceed to start treatment.
- Examination of the cardiovascular system with ECG, ultrasonography and blood pressure estimation.
- Ophthalmologic examination for macular edema.
- Assess the EDSS and MSFC scales.
- MRI scan of the CNS.
- Distribute the MSQOL-54 questionnaire.
- Estimate the ARR and proportion of patients with a relapse.
- Estimate the Rio score.
- Provide the participants with some final instructions and with the results.

5.2.4 Withdrawal Visit

Should a subject withdraw voluntarily from the study or the investigator terminate a subject's participation for any reason, there is the possibility of him/her being followed-up despite his/her withdrawal in order to collect safety and other data for the subsequent statistical analysis. In the withdrawal visit, the investigator (s) should provide the subject who withdraws with instructions for further treatment and follow-up for the record of data that can be deemed useful for the subsequent analysis.

5.2.5 Unscheduled Visit

An unscheduled visit during the conduct of the study may happen should a serious AE take place (or one that worries the subject more than normal to report it in the upcoming programmed visit) or in case of a relapse that lasts more than before and necessitates hospitalization and treatment with IV methylprednisolone. In any case, after the confrontation of the issue that called the unscheduled visit, the procedures that took place during it will be documented in a separate register and will be entailed in the subsequent analysis and potential publication.

6. STUDY PROCEDURES/EVALUATIONS

6.1 Medical Record Review/Medical History

The following information will be abstracted from the medical record review or will be taken for the first time:

- Date of birth
- Address
- Contact numbers
- Height
- Weight
- Sex
- Race
- Ethnicity
- Religion
- Information regarding health insurance
- Profession
- Marital status
- Socioeconomic status
- Parents/Siblings
- Surgical history
- Obstetric history if female
- Medications and medical allergies
- Family history
- Social history (alcohol, tobacco, recreational drugs)
- Psychiatric history

- Habits
- Immunization history
- Growth chart and developmental history
- Any medical encounters (location, chief complaint, history of the present illness, physical examination, assessment and plan, progress and any diagnostic procedures'/evaluations' results)

6.2 Physical Examination/Vital Signs/Paraclinical Tests

All of the systems will be evaluated with extra care given to the skin (because of the hair thinning-alopecia and especially in intermediate visits where every recently emerged skin lesion should be examined by a specialized dermatologist), the cardiovascular system (because of the bradycardia and atrioventricular block induced by fingolimod and the existence of contraindications to its use associated with this system), the gastrointestinal system (because of the diarrhea and other complaints due to the study's treatments), the eyes (because of the possibility of macular edema coming as a result of fingolimod administration) and of course the nervous system.

Evaluation of the cardiovascular system will include inspection, palpation of the pulse at all possible places for changes in the rhythm, percussion and auscultation of the heart, ECG and ultrasonography of the heart by a specialized cardiologist as well as blood pressure estimation with an automated sphygmomanometer. Additionally, especially for patients treated with fingolimod, because first dose bradycardia is almost universal, monitoring of vital signs for six hours after the first dose is mandatory with need for extended cardiac telemetry monitoring if excessive bradycardia is persistent or symptomatic.

Fluorescein angiography and OCT will be used during the ophthalmologic evaluation to discover macular edema.

During the neurologic examination the following must be evaluated: cranial nerves and peripheral nerves, visually evoked potentials, consciousness, motor and sensory system, speech, memory, meningeal signs and reflexes (lumbar puncture during screening is optional, can be executed for examination and cultivation at suspection of CNS infection along with meningeal signs examination). In case of indentification of steady progression of focal neurologic deficits, MRI imaging must be conducted as soon as possible; should the results bring about distinct multifocal, asymmetric, periventricular and subcortical lesions, hypointense on T1 – weighted scans, hyperintense on T2 – weighted scans with typically no gadolinium enhancement on T1 – weighted scans, corresponding to the clinical deficits, lumbar puncture and PCR for JCV DNA must be conducted for confirmation as well as soon as possible.

The predefined scales and questionnaires for the endpoints of the study, which will be available in the references, will be evaluated by specialized neurologists or senior interns in pairs and any discrepancies will be solved by an additional evaluation by a senior consultant or a professor.

6.3 Laboratory Procedures/Evaluations

Blood samples will be collected for blood and specifically lymphocyte count, transaminases, control for autoimmune diseases, assessment of JCV antibody status and possible cultivation in order to confirm TB infection. Urine samples will be collected for the pregnancy test. Sputum will also be collected for cultivation in case of a possible TB infection. Samples will be evaluated in a blinded manner and results will be given by the laboratory of each hospital included in the study. The Mantoux test will be performed and evaluated by a specialist in infectious diseases.

6.4 Radiologic Evaluations

During the screening a reference X-ray of the chest and the abdomen will be executed as well as a CT scan of the abdomen for assessment of the liver's condition and they will be evaluated by the personnel of each hospital (radiologists). MRI images will be taken at the predefined time points or in case of PML suspection and will be sent for evaluation in a blinded manner at a central MRI reading center.

6.5 Study Specific Biospecimens

6.5.1 Specimen Collection Procedures

Blood and urine samples (and sputum samples if there is need as stated before) will be taken by each participant while lumbar puncture for the acquirement of CSF will be optional in case of suspection for a CNS infection or PML. No special preparation is needed for the blood, urine and sputum samples collection while anti-platelet or anticoagulant treatment should be ceased at least 5 days before lumbar puncture. Blood samples will be collected at best in the morning with the traditional way of phlebotomy in the veins of the upper extremity or at any other place if not available while urine samples will also be collected at best in the morning by urinating in a sterilized glass (in patients unable to urinate, a catheter can be used). CSF collection will be executed when needed in the operation room under sterilized conditions by an anesthesiologist. 10ml of blood and 30 ml of urine will be sufficient while only a few ml of CSF will be deemed enough.

6.5.2 Specimen Preparation, Handling, Storage and Shipment

After collection, the specimens will be processed in a blinded manner by the personnel of each of the hospitals of the study. No special instructions are essential for the handling and storage of the specimens except from the fact that new samples every time will be preferred. In case of difficult specimen collection, blood and urine will be stored in special refrigerators with their serial numbers covered and exposed only by the personnel staff. Since specimens will be evaluated at the place of collection there are no further instructions for shipment as no shipment will take place.

6.5.3 Questionnaire Administration

The questionnaire for assessing the quality of life of our patients will be distributed by the nursing staff and answered by the patients themselves or with the help of a representative in case of a disability in writing, vision, illiteracy or communication.

7. ASSESSMENT OF SAFETY

7.1 Specification of Safety Parameters

This section is in harmony with the University Hospital of Larissa's IRB Guidelines. Safety monitoring for this study will focus on unanticipated problems involving risks to participants, including unanticipated problems that meet the definition of a serious adverse event.

7.2 Adverse Event Reporting

The investigators are responsible for recording and reporting unanticipated problems related to the study that occur during its conduct. All on-site SAEs (University Hospital of Larissa and other sites) will be reported to the IRB of the University Hospital of Larissa in accordance with IRB guidelines. AEs that are not serious will be summarized in narrative or other format and will be submitted to the IRB at the time defined in the next paragraphs.

7.3 Definition of an Adverse Event (AE)

An adverse event is any untoward medical occurrence in a subject who has received an intervention (drug, biologic, or other intervention). The occurrence does not necessarily have to have a causal relationship with the treatment. An AE can therefore be any unfavorable or unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the

medicinal product. Medical conditions/diseases present at study entry are only considered adverse events if they worsen after signing the study ICF.

The occurrence of AEs (including serious AEs) that are not on-site – since our study participants are mainly outpatients – should be sought by non-directive questioning of the patient during his/her visits or may be detected when they are volunteered by the patient during or between visits or through physical examination, laboratory tests and other assessments. All AEs must be noted in the study records and recorded on the CRF with a full description including the nature, date and time of onset, whether it constitutes a SAE or not, severity (mild, moderate, severe), duration, causality (if it is related to teriflunomide or the other treatments), and outcome of the event. The relationship of each AE to the study treatments should be characterized using one of the following terms: definitely, probably, possibly, unlikely or unrelated.

7.4 Definition of a Serious Adverse Event (SAE)

A SAE is any adverse drug experience occurring at any dose that results in any of the following outcomes:

- death,
- a life-threatening event (at risk of death at the time of the event),
- requires inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant disability/incapacity, or
- a congenital anomaly/birth defect in the offspring of a subject.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug event when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

A distinction should be drawn between serious and severe AEs. A severe AE is a major event of its type. A severe AE does not necessarily need to be considered serious. For example, nausea which persists for several hours may be considered severe nausea, but would not be an SAE. On the other hand, a stroke that results in only a limited degree of disability may be considered a mild stroke, but would be an SAE.

7.5 Reporting Procedures

Incidents or events that meet the OHRP criteria for unanticipated problems require the creation and completion of an unanticipated problem report form. OHRP recommends that investigators include the following information when reporting an adverse event, or any other

incident, experience, or outcome as an unanticipated problem to the IRB:

- appropriate identifying information for the research protocol, such as the title, investigator's name, and the IRB project number;
- a detailed description of the unanticipated problem (nature, date and time of onset, whether it constitutes a SAE or not, severity (mild, moderate, severe), duration, causality (if it is related to teriflunomide or the other treatments), and outcome of the event;
- an explanation of the basis for determining that the adverse event, incident, experience, or outcome represents an unanticipated problem;
- a description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the unanticipated problem.

To satisfy the requirement for prompt reporting, unanticipated problems will be reported to the IRB using the following timeline:

- For on-site death or life-threatening SAEs until 12 weeks after the patient has stopped study participation (time of last visit), the initial notification to the IRB via phone, e-mail or fax should take place within the first 24 hours and the written report should be delivered within 2 calendar days.
- For all other on-site SAEs and unanticipated problems, the initial notification to the IRB via phone, e-mail or fax should take place within 7 days after occurence and the written report should be delivered within 7 business days.
- For all other AEs, there is no specific time interval for the initial notification while for their written report, a brief summary of important AEs may be reported at time of continuing review.
- All unanticipated problems should be reported to appropriate institutional officials and OHRP within one month of the IRB's receipt of the report of the problem from the investigator.

7.6 Follow-up Report

If an SAE has not resolved at the time of the initial report and new information arises that changes the assessment of the event, a follow-up report including all relevant new or reassessed information (e.g., concomitant medication, medical history) should be submitted to the IRB within 24 hours by the treating physician or member of the medical staff that receives the information. All SAEs must be followed until either resolved or stable.

7.7 Pregnancy Reporting

Since teriflunomide and fingolimod are considered as Pregnancy Category X and Pregnancy

Category C correspondingly, pregnancy is separated from all other unanticipated problems. To ensure patient safety, each pregnancy must be reported to the University Hospital of Larissa IRB within 24 hours of learning of its occurrence (like death or life-threatening SAEs. The pregnancy should be followed up to determine outcome, including spontaneous or voluntary termination, presence or absence of birth anomalies or defects, congenital abnormalities or maternal and or/newborn complications. Pregnancy follow-up should be recorded on the same form and include an assessment of the possible relationship of any pregnancy outcome to teriflunomide or fingolimod. Any SAE occurred during pregnancy must be reported on the SAE report form.

7.8 Medical Emergencies

In case of confirmed PML, treatment with any disease-modifying drug must be terminated immediately and the patient should be hospitalized in the ICU of the hospital.

8. STUDY OVERSIGHT AND MONITORING

8.1 Principal Investigator

The investigator will be responsible for study oversight, including monitoring safety, ensuring that the study is conducted according to the protocol and ensuring data integrity. The PI will review the data for safety concerns and data trends at regular intervals, and will promptly report to the IRB of the University Hospital of Larissa any unanticipated problem, protocol deviation, or any other significant event that arises during the conduct of the study.

8.2 Independent Data and Safety Monitoring Board

In addition to the PI's responsibility for oversight, study oversight will be under the direction of a DSMB – composed of members with expertise in ethics in clinical research including a representative of the Orthodox Church, a lawyer, a clinician, a representative from the HFPMS and a mathematician specialized in statistics – in consultation with the HNS. The DSMB will meet every 3 weeks during the first 12 months and every 2 months for the remainder of the study to assess safety, study progress and data integrity for the study. If safety concerns arise, more frequent meetings may be held. The DSMB will operate under the rules of a HNS-approved charter that will be approved at the organizational meeting of the DSMB. At this time, most data elements that the DSMB needs to assess will be clearly defined. The DSMB will provide recommendations to the HNS. Should serious safety concerns, overwhelming benefit of either cohort or futility issues arise, the DSMB might recommend termination of the study.

9. CLINICAL SITE MONITORING

Clinical site monitoring is conducted to ensure that the rights of human subjects are protected, that the study is implemented in accordance with the protocol and/or other operating procedures, and that the quality and integrity of study data and data collection methods are maintained. Monitoring for this study will be performed by HNS's CROMS contractor. The monitor will evaluate study processes and documentation based on HNS standards and the International Conference on Harmonization (ICH), E6: GCP guidelines.

Details of clinical site monitoring will be documented in a CMP developed by the CROMS contractor, in collaboration with the HNS OCTOM and the HNS Program Official. The CMP will specify the frequency of monitoring, monitoring procedures, the level of clinical site monitoring activities (e.g., the percentage of subject data to be reviewed), and the distribution of monitoring reports. Some monitoring activities may be performed remotely, while others will take place at the study sites. Staff from the CROMS contractor will conduct monitoring activities and provide reports of the findings and associated action items in accordance with the details described in the CMP. Documentation of monitoring activities and findings will be provided to the site study team, the study PIs, OCTOM, and the HNS. The HNS reserves the right to conduct independent audits as necessary.

10. STATISTICAL CONSIDERATIONS

Purpose of the study is primarily to explore the effectiveness of teriflunomide, as far as the progression of disability is concerned, in patients with relapsing forms of MS, secondarily to assess the risk of disability progression and improvement in terms of number of relapses and tertiarily to assess the tolerability of the drug, MRI quantitative changes and the quality of life of our patients.

10.1 Primary Endpoint

The primary endpoint will be the change in EDSS and MSFC between baseline during enrollment visit and the last visit/end of the study with intermediate measurements.

10.2 Secondary Endpoints

Secondary endpoints will include the following:

- Report of ARR and proportion of patients with a relapse for each of the two years of the duration of the study.
- The change in ARR and proportion of patients with a relapse between Year 1 and Year 2 of the study.

- The change in Rio score between baseline during enrollment visit and termination of the study.

10.3 Tertiary Endpoints

Tertiary endpoints will include the following:

- The quantitative changes in MRI images between baseline and screening visit and the end of the study with intermediate evaluations.
- The change in the MSQOL-54 questionnaire between baseline and enrollment visit and the end of the study with intermediate evaluations.
- Safety and tolerability of teriflunomide based on AEs.

10.4 Control of Bias and Confounding

In order to deal with the presence of bias because of the absence of randomization, the following precautions have been taken:

- The MRI images will be evaluated in a blinded manner at a central MRI reading center.
- Both patients and investigators will be blinded as to the treatment given.
- An independent neurologic committee will be responsible for the confirmation and reporting of the AEs which could reveal the treatment.

10.5 Statistical Methods

For the statistical processing of the data the IBM® SPSS v23.0 will be used. Summary statistics for continuous variables (results from the EDSS, the MSFC, the Rio score, the ARR, proportion of patients with a relapse, number of new or enlarging hypointense lesions on T1–weighted scans, hyperintense lesions on T2–weighted scans and gadolinium-enhancing lesions on T1–weighted scans and the MSQOL-54) will include N, mean, median, standard deviation, minimum, maximum, lower and upper quartile; the t-test will be used for comparisons between the two cohorts and paired t-test for the analysis of the endpoints (change between baseline and last visit). Likewise, for discrete variables (AEs) absolute and relative frequencies will be used.

For selected safety outcomes (AEs), incidence rates with respective 95 % CIs will be calculated when applicable. All results will be reported separately for the 2 cohorts (teriflunomide and the other two oral disease-modifying drugs).

10.5.1 Patient Demographic and other Baseline Characteristics

Baseline and demographic characteristics will be summarized by standard descriptive summaries (e.g. means and standard deviations for continuous variables such as age and percentages for categorical variables such as gender).

10.6 Analysis of the Main Variables

10.6.1 Analysis of the Primary Outcome of Interest

A paired t-test will be used to compare EDSS and MSFC between baseline and termination of study while a t-test will be used to compare mean of the same variables between the two cohorts. A linear regression model for each of the two variables will be used adjusted for baseline scores in both of them, age, sex with the aim to include the number of new or enlarging hypointense lesions on T1–weighted scans, hyperintense lesions on T2–weighted scans and gadolinium-enhancing lesions on T1–weighted scans as a continuous predictor and ARR as a binomial predictor in the model.

10.6.2 Analysis of the Secondary Outcomes of Interest

The Rio score for the risk of disability progression will be assessed as EDSS and MSFC; a paired t-test for comparison between baseline and termination of study, a t-test for comparison between the two cohorts and a linear regression model adjusted for baseline score, age and sex with the inclusion of EDSS and MSFC score as continuous predictors.

The ARR will be analyzed with the use of a binary(logistic) regression model adjusted for age, sex and baseline EDSS score as long as with a Kaplan-Meier analysis for patients with a relapse while for the proportion of patients with a relapse a Cox proportional-hazards model will be used to assess the difference in the risk for a relapse between the two cohorts, adjusted for EDSS score, age, sex, relapse rate and number of new or enlarging hypointense lesions on T1–weighted scans, hyperintense lesions on T2–weighted scans and gadolinium-enhancing lesions on T1–weighted scans if possible.

10.6.3 Analysis of the Tertiary Outcomes of Interest

The incidence of adverse events (new or worsened from baseline) will be summarized as frequency count and percentage of patients with adverse events by primary system organ class, and preferred term. In addition, the incidence of death, SAEs, AEs leading to discontinuation, and other significant AEs will be summarized separately by primary system organ class and preferred term. All information pertaining to adverse events noted during the study will be listed by center and patient number.

The MRI endpoints will be utilized not only as continuous variables (number of new or enlarging hypointense lesions on T1–weighted scans, hyperintense lesions on T2–weighted scans and gadolinium-enhancing lesions on T1–weighted scans) with comparisons between the two cohorts with the aid of a t-test, but also as percentages where the percent of patients free from new or enlarging hypointense lesions on T1–weighted scans, hyperintense lesions on T2–weighted scans and gadolinium-enhancing lesions on T1–weighted scans in the teriflunomide cohort will be compared to its correspondent one in the parallel cohort by using a x^2 test.

As far as the MSQOL-54 is concerned, the mean score of each category for each cohort could be compared with its correspondent one from the other cohort but it is highly preferable to compare percentages for each category after its segregation into one, two, three, four, five, six or seven sub-categories.

10.7 Sample Size and Power

The power approach was used in order to estimate the appropriate sample size for our study. Normally, the primary outcome would be used for the estimation of the sample size. But in our case, the primary outcome (disability in terms of the EDSS and MSFC) is rather difficult to "quantify". In order to recompensate for that, we decided that disability would still be the principal determinant for the sample size estimation; thus disability progression would be used instead of disability in terms of the EDSS and MSFC.

We traced back at the results of the Phase III trials about our three drugs of choice that we presented in the introduction and picked the significant results. According to them, only teriflunomide in the dose of 14 mg reduced disability progression significantly vs. placebo; that took place in the TEMSO trial (by 30%) and the TOWER trial (by 32%). Thus we used the mean of those two rates (31%) as the percentage of success with our study test drug.

The same procedure was followed for fingolimod and dimethyl fumarate; for fingolimod only the FREEDOMS trial produced a significant decrease in disability progression by 30% with the approved dose of 0.5mg vs. placebo. Likewise, only in the DEFINE trial a significant reduction in disability progression was acquired with dimethyl fumarate (38% with the approved dose of 240 mg vs. placebo). In order to have a percentage of success for our control drugs we accepted conventionally the mean (34%) or used both rates to calculate the minimum and maximum for our sample size.

We accepted arbitrarily that a change/decrease in disability progression by 10% is clinically important. Furthermore, we accepted 5% as our Type I error rate (p-value/false positive) and 10% as our Type II error rate(false negative); in conclusion, we accepted a power of 90%.

We used the following formula for the estimation of our sample size:

$$n \geq \left(\frac{p1*(1-p1)+p2*(1-p2)}{\Delta^2}\right)*(a+b)^2$$

where n stands for sample size, p1 stands for percentage of success with test drug (teriflunomide), p2 stands for percentage of success with control drugs (fingolimod and dimethyl fumarate), Δ stands for change/decrease in disability progression that is deemed as clinically important, a stands for Type I error rate (1.96→5% point of the normal distribution) and b stands for power (1.28→10% point of the normal distribution).

For p2=30% (minimum) the desirable sample size per treatment must be greater or equal to 445 (for both treatments 890), for p2=38% (maximum) the desirable sample size per treatment must be greater or equal to 472 (for both treatments 944) and for p2=34% (mean) the desirable sample size per treatment must be greater or equal to 460 (for both treatments 920). If we add to the rates we acquired the 25% of them to recompensate for potential dropouts, patients lost to follow-up and to compulsory termination of participation due to SAEs, the corresponding rounded rates for the whole sample size are 1113 for p2=30%, 1180 for p2=38% and 1150 for p2=34%. To conclude, we chose to have a sample size of about 1150 with approximately 575 patients in each of the two parallel cohorts.

11. SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

Study staff will maintain appropriate medical and research records, such as laboratory results and MRI images, for this study, in compliance with ICH E6, Section 4.9 and regulatory and institutional requirements for the protection of confidentiality of subjects. Study staff will permit authorized representatives of HNS and regulatory agencies to examine (and when required by applicable law, to copy) research records for the purposes of quality assurance reviews, audits, and evaluation of the study safety, progress and data validity.

12. QUALITY MANAGEMENT AND QUALITY ASSURANCE

QM activities will be conducted at each subject study visit, as well as on a quarterly and annual schedule. Additional QM activities and reviews will be conducted on an as-needed basis in response to staff or process changes. CQMP will be a living document and will be reviewed for applicability

and accuracy and updated as necessary every 12 months by the Lead SC. Additional QM needs identified at a study site will be communicated to the study team. The study team will evaluate the need to update the CQMP, tools, and logs.

12.1 Quality Management Activity Schedule and Tools

The following tools will be used to document Quality Management (QM) activities for this study:

- QM Essential Documents Review Tool
- QM Subject Data Review Tool
- QM Quarterly Review Tool
- QM Annual Review Tool

In addition the following checklists and reminders have been developed for this study's QM process:

- Eligibility Checklist
- Calibration Check Log

12.2 Quality Management Documentation and Reporting

Each clinical site from the 9 of this study will maintain the CQMP and QM tools/logs either in a QM binder (for paper documents) or electronic folder. Site SCs will be responsible for site-specific QM activities. The Lead SC will be responsible for QM activities at the University Hospital of Larissa and will provide oversight for QM activities for the entire study.

Each site SC will provide a Quality Management Summary Report to the Lead SC one month before the MMOR is due. The Lead Study SC will compile the site reports into a comprehensive, study-wide report that will be provided to the HNS. The Lead Study SC will also summarize the information for inclusion in the MMOR. This summary will document the following:

- QM activities completed since the prior report submittal, including:
    - Frequency of reviews
    - Number of charts reviewed
    - Items covered by the review

- Identification of problem areas
- Corrective Action Plan(s)
- Possible need for revision to CQMP

12.3 Ad Hoc Quality Management Activities

12.3.1 New Study Personnel

The site SC will ensure that all study personnel have completed all required institution-specific and protocol-specific trainings and that these trainings are documented appropriately on the Training Log. The site SC will also ensure that new personnel are appropriately documented on the DRL. While training should be completed and documented in real time, the Lead SC will verify that all training is current and appropriately documented on a quarterly basis.

12.4 Subject Study Visit Quality Management Activities

Procedures and processes to ensure protocol adherence at each subject study visit are documented in the MOP. The following is a detailed description of QM activities that will be performed prior to subject study visits and upon visit completion.

12.4.1 Subject Record Review

Prior to confirming subject study visits, the site SC will verify that the subject is scheduled for the appropriate appointment as listed on the Visit Window Calculator and will assess the Protocol Visit Compliance (missed and unscheduled visits, patients lost to follow-up).

During the visit, the site SC will ensure that the subject still meets the eligibility requirements.

At the completion of each visit, the site SC will review the subject record to identify any AE/SAE/unanticipated problem that needs reporting and any protocol deviations.

12.4.2 Consent Process Completion and Documentation

Prior to the screening and baseline visits, the site SC will verify that the most current IRB-approved study consent documents are available for use. If re-consenting is required throughout the subject's participation in the study, the site SC will verify that the most current IRB approved consent is available prior to the study visit.

Before the subject leaves the clinic, the site SC will review the consent documentation and confirm adherence to the consent processes described in the MOP.

12.4.3 Source Document Completion

At visit completion, a reviewer will complete a checklist that captures the required elements of the visit. Gaps and errors will be corrected as soon as time allows following the visit.

12.4.4 Case Report Form Completion

Procedures for completion of CRFs as well as data error detection and correction procedures are documented in MOP. At visit completion, a reviewer will complete a checklist that captures the required elements of the visit. Gaps and errors will be filled and corrected as soon as time allows following the visit.

12.5 Quarterly Quality Management Activities

Quarterly Quality Management Activities will be documented on the QM Quarterly Review Tool.

12.5.1 Consent Process Completion and Documentation

The site SC or designee will review 20% of the site's executed consents using the Quality Management Subject Data Review Tool.

12.5.2 Laboratory Specimens

MOP describes the labeling, collection, handling and storage of the clinical specimens obtained in this study. Every 3 months, the site SC will review laboratory checklists and temperature logs for completeness (no shipment will be taking place so there is no need for it to be reviewed).

12.5.3 Equipment Set-up and Calibration

MOP describes the procedures for setting up, checking calibration, and calibrating the automated sphygmomanometers and ECG recorders. Equipment calibration checks and recalibrations will be noted on the Calibration Check Log. The site SC will review the Calibration Check Log every 3 months.

12.5.4 Source Document Completion

The site SC will use the Quality Management Subject Data Review Tool to review completion and accuracy of the source documents and the CRFs for 100% of subjects at the site every 3 months.

12.5.5 Case Report Form Completion

The site SC will cross-check CRF data for accuracy and completeness every 3 months.

12.5.6 Study Drug

MOP describes the pharmacy's processes for the ordering, maintenance, and dispensing of the study drug. The pharmacy maintains all logs associated with the study drug. The site SC will obtain log information from the pharmacist quarterly and review accountability records for accuracy and completeness. This review will be documented on the QM Quarterly Review Tool.

12.5.7 Staff Training / Qualifications

Training Logs will be reviewed by the site SC every 3 months to verify training is current and properly documented. This will include a review for institution-specific and protocol-specific trainings.

12.6 Annual Quality Management Activities

Annual Quality Management review activities will be documented on the QM Annual Review Tool.

12.6.1 Process Documents

The procedures and processes to ensure protocol adherence among the study personnel are set forth in the MOP. The MOP is reviewed by the Lead SC every 12 months for applicability and accuracy.

13. ETHICS/PROTECTION OF HUMAN SUBJECTS

13.1 Ethical Standard

The investigator will ensure that this study is conducted in full conformity with all applicable federal and state laws and regulations, the Good Clinical Practice: Consolidated Guideline approved by the International Conference on Harmonisation (ICH) and the ethical principles laid down in the Declaration of Helsinki. All episodes of noncompliance will be documented.

13.2 Institutional Review Board

The protocol, informed consent form(s), recruitment materials and all participant materials will be submitted to the IRB of the University Hospital of Larissa for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB of the University Hospital of Larissa before the changes are implemented in the study.

13.3 Informed Consent Process

A screening consent form must be signed by the prospective participant, or his/her appointed legal representative in case of disability, before screening. Once screening is over extensive discussion of risks and possible benefits of study participation will be provided to participants and their families. Prior to his/her enrollment in the study, a study consent form describing in detail the study procedures and risks will be given to the participant. Consent forms will be approved by the University Hospital of Larissa IRB and the participant is required to read and review the document or have the document read to him or her. The investigator or designee will explain the research study to the participant and answer any questions that may arise. The participant or his/her appointed legal representative will sign the informed consent document prior to any study-related assessments or procedures.

Participants will be given the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. They may withdraw consent at any time throughout the course of the study. A copy of the signed informed consent document will be given to participants for their records. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their clinical care will not be adversely affected if they decline to participate in this study.

The consent process will be documented in the clinical or research record.

13.4 Participant Confidentiality

Participant confidentiality is strictly held in trust by the investigators, study staff, clinical monitor, representatives of HNS and University Hospital of Larissa IRB representatives. This confidentiality is extended to cover testing of blood and urine samples in addition to any study information relating to participants.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

The clinical site monitor and the DSMB may inspect all study documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) for the study participants. The clinical study site will permit access to such records.

14. DATA HANDLING AND RECORD KEEPING

The investigators are responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. The investigators will maintain adequate case histories of study participants, including accurate case report forms (CRFs), and source documentation.

14.1 Data Management Responsibilities

Data collection and accurate documentation are the responsibility of the study staff under the supervision of the investigator. All source documents and laboratory reports must be reviewed by the study team and data entry staff, who will ensure that they are accurate and complete. Unanticipated problems must be reviewed by the PI.

14.2 Data Capture and Document Retention

Site staff will enter protocol defined data into paper CRFs following CRF Completion Guidelines entailed in the MOP after appropriate training. At the end of the study, the treating physician must certify that the data entered into the CRF are complete and accurate.

In all scenarios, the physician must maintain source documents for each patient in the study, consisting of case and visit notes (hospital or clinic medical records) containing demographic and medical information, and the results of any other tests or assessments. All information entered in the CRF must be traceable to these source documents in the patient's file. The physician must also keep the original informed consent form signed by the patient (a signed copy is given to the patient). No information in source documents about the identity of the patients will be disclosed.

14.3 Protocol Deviations

A protocol deviation is any non compliance with the clinical study protocol, GCP, or MOP. The noncompliance may be on the part of the subject, the investigator, or study staff. As a result of deviations, corrective actions are to be developed by the study staff and implemented promptly.

These practices are consistent with investigator and sponsor obligations in ICH E6:

- Compliance with Protocol, Sections 4.5.1, 4.5.2, 4.5.3, and 4.5.4.
- Quality Assurance and Quality Control, Section 5.1.1
- Noncompliance, Sections 5.20.1 and 5.20.2.

All deviations from the protocol must be addressed in study subject source documents and promptly reported to HNS and the University Hospital of Larissa IRB, according to their requirements.

15. PUBLICATION DATA/SHARING POLICY

This study will comply with the HNS Public Access Policy, which ensures that the public has access to the published results of HNS funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from HNS funds to the digital archive PubMed Central upon acceptance for publication.

16. LIMITATIONS

Because this is an observational non-randomized study, bias may occur in many aspects of it. Treating physicians may assign the participants into one of the two parallel cohorts based on severity and duration of disease, concomitant comorbidities and medications and other factors. This can lead to systematic bias and confound the true association between treatment and disability progression.

17. REFERENCES

1. Chan A, de Seze J, Comabella M. "Teriflunomide in Patients with Relapsing–Remitting Forms of Multiple Sclerosis". *CNS Drugs*. 2016;30:41-51.

2. Fred D. Lublin, Stephen C. Reingold, Jeffrey A. Cohen et al.(2014). "Defining the clinical course of multiple sclerosis. The 2013 revisions". Neurology July 15, 2014 vol. 83 no. 3 278-286,

3. Wingerchuk Dean M., Weinshenker Brian G. "Disease-modifying therapies for relapsing multiple sclerosis". BMJ 2016; 354:i3518

4. Torkildsen Ø., Myhr K-M., and Bø L., "Disease-modifying treatments for multiple sclerosis – a review of approved medications". Eur J Neurol. 2016 Jan; 23(Suppl 1): 18-27.

5. Tsivgoulis G, Katsanos AH, Grigoriadis N, et al. "The effect of disease modifying therapies on brain atrophy in patients with relapsing-remitting multiple sclerosis: a systematic review and meta-analysis". PLoS One 2015;10:e0116511.

6. Okuda DT, Siva A, Kantarci O, et al. Radiologically Isolated Syndrome Consortium (RISC) Club Francophone de la Sclérose en Plaques (CFSEP). "Radiologically isolated syndrome: 5-year risk for an initial clinical event". PLoS One2014;9:e90509.

7. Dubey D, Kieseier BC, Hartung HP, et al. "Dimethyl fumarate in relapsing-remitting multiple sclerosis: rationale, mechanisms of action, pharmacokinetics, efficacy and safety". Expert Rev Neurother 2015;15:339-46.

8. Linker RA, Gold R. "Dimethyl fumarate for treatment of multiple sclerosis: mechanism of action, effectiveness, and side effects". Curr Neurol Neurosci Rep. 2013 Nov; 13(11):394.

9. Fox RJ, Miller DH, Phillips JT, et al. "CONFIRM Study Investigators. Placebo-controlled phase 3 study of oral BG-12 or glatiramer in multiple sclerosis". N Engl J Med2012;367:1087-97.

10. Gold R, Kappos L, Arnold DL, et al. "DEFINE Study Investigators. Placebo-controlled phase 3 study of oral BG-12 for relapsing multiple sclerosis". N Engl J Med2012;367:1098-107.

11. Longbrake EE, Naismith RT, Parks BJ, Wu GF, Cross AH. "Dimethyl fumarate-associated lymphopenia: Risk factors and clinical significance". Mult Scler J Exp Transl Clin2015;1:2055217315596994.

12. Ermis U, Weis J, Schulz JB. "PML in a patient treated with fumaric acid". N Engl J Med2013;368:1657-8.

13. van Oosten BW, Killestein J, Barkhof F, Polman CH, Wattjes MP. "PML in a patient treated with dimethyl fumarate from a compounding pharmacy". N Engl J Med2013;368:1658-9.

14. US Food and Drug Administration. 2014. FDA Drug Safety Communication: "FDA warns about case of rare brain infection PML with MS drug Tecfidera (dimethyl fumarate)".

15. Rosenkranz T, Novas M, Terborg C. "PML in a patient with lymphocytopenia treated with dimethyl fumarate". N Engl J Med2015;372:1476-8.

16. European Medicines Agency. Updated recommendations to minimise the risk of the rare brain infection PML with Tecfidera. 2015.

17. US Food and Drug Administration. Drug safety labeling changes. 2016.

18. Cohen JA, Chun J. "Mechanisms of fingolimod's efficacy and adverse effects in multiple sclerosis". Ann Neurol2011;69:759-77.

19. Bridel Claire, Lalive H. Patrice, "Update on multiple sclerosis treatments". Swiss Med Wkly. 2014:144:w14012.

20. Kappos L, Radue EW, O'Connor P, et al. FREEDOMS Study Group. "A placebo-controlled trial of oral fingolimod in relapsing multiple sclerosis". N Engl J Med2010;362:387-401.

21. Calabresi PA, Radue EW, Goodin D, et al. "Safety and efficacy of fingolimod in patients with relapsing-remitting multiple sclerosis (FREEDOMS II): a double-blind, randomised, placebo-controlled, phase 3 trial". Lancet Neurol2014;13:545-56.

22. Cohen JA, Barkhof F, Comi G, et al. TRANSFORMS Study Group. "Oral fingolimod or intramuscular interferon for relapsing multiple sclerosis". N Engl J Med2010;362:402-15.

23. Arvin AM, Wolinsky JS, Kappos L, et al. "Varicella-zoster virus infections in patients treated with fingolimod: risk assessment and consensus recommendations for management". JAMA Neurol2015;72:31-9.

24. Jain N, Bhatti MT. "Fingolimod-associated macular edema: incidence, detection, and management". Neurology2012;78:672-80.

25. Amit Bar-Or, Pachner Andrew, Menguy-Vacheron Francois, Kaplan Johanne, Wiendl Heinz. "Teriflunomide and Its Mechanism of Action in Multiple Sclerosis". Drugs. 2014; 74(6): 659–674.

26. O'Connor P, Wolinsky JS, Confavreux C, et al. TEMSO Trial Group. "Randomized trial of oral teriflunomide for relapsing multiple sclerosis". N Engl J Med2011;365:1293-303.

27. Confavreux C, O'Connor P, Comi G, et al. TOWER Trial Group. "Oral teriflunomide for patients with relapsing multiple sclerosis (TOWER): a randomised, double-blind, placebo-controlled, phase 3 trial". Lancet Neurol2014;13:247-56.

28. Miller AE, Wolinsky JS, Kappos L, et al. TOPIC Study Group. "Oral teriflunomide for patients with a first clinical episode suggestive of multiple sclerosis (TOPIC): a randomised, double-blind, placebo-controlled, phase 3 trial". Lancet Neurol2014;13:977-86.

29. Vermersch P, Czlonkowska A, Grimaldi LM, et al. "Teriflunomide versus subcutaneous interferon beta-1a in patients with relapsing multiple sclerosis: a randomised, controlled phase 3 trial". TENERE Trial Group Mult Scler. 2014 May; 20(6):705-16.

30. Amit Bar-Or, Freedman MS, Kremenchutzky M, et al. "Teriflunomide effect on immune response to influenza vaccine in patients with multiple sclerosis". Neurology. 2013 Aug 6;81(6):552-8.

31. Lu Elen, Wei Wang Bing, Guimond Colleen, et al. "Safety of disease-modifying drugs for multiple sclerosis in pregnancy: current challenges and future considerations for effective pharmacovigilance". (2013) Expert Review of Neurotherapeutics, 13:3, 251-261.

32. Oxford Handbook of Neurology

33. http://www.nidcr.nih.gov/Research/toolkit/

34. https://irb.research.chop.edu/protocol-templates

35. http://www.nationalmssociety.org/

36. http://www.nationalmssociety.org/NationalMSSociety/media/MSNationalFiles/Brochures/10-2-3-29-EDSS_Form.pdf

37. http://www.nationalmssociety.org/NationalMSSociety/media/MSNationalFiles/Brochures/10-2-3-30-Functional_Systems_Kurtzke_Form.pdf

38. http://www.nationalmssociety.org/NationalMSSociety/media/MSNationalFiles/Brochures/MSQOL54_995.pdf

39. http://www.nationalmssociety.org/NationalMSSociety/media/MSNationalFiles/Brochures/10-2-3-31-MSFC_Manual_and_Forms.pdf

40. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4444088/table/pone.0129243.t001/