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Secção Autónoma das Ciências da Saúde

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DADOS DE VIDA REAL DO FINGOLIMOD

FINGOLIMOD REAL WORLD EXPERIENCE

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biomedicina Farmacêutica, realizada sob a orientação científica do Professor Doutor Bruno Gago, Professor Associado Convidado da Universidade de Aveiro.

Dissertation presented to the University of Aveiro in partial fulfilment of the requirements for the degree of Master of Science in Pharmaceutical Medicine, under the supervision of Professor Bruno Gago, Invited Associate Professor at the University of Aveiro.

I dedicate this work to my beloved wife and twin sons.

Thesis jury

President Professora Doutora Alexandra Isabel Cardador de Queirós
Professora Coordenadora S/Agregação, Universidade de Aveiro

Doutora Bárbara da Silva Rocha
Investigadora de Pós Doutoramento,Universidade de Coimbra - Faculdade de Medicina

Doutor Bruno Miguel Alves Fernandes do Gago
Professor Auxiliar Convidado, Universidade de Aveiro

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Keywords

Fingolimod, multiple sclerosis, real world evidence, clinical practice

Abstract

Fingolimod is a Multiple Sclerosis treatment licensed in Europe since 2011. Its efficacy has been demonstrated in three large phase III trials, used in the regulatory submissions throughout the world. As usual, in these trials the inclusion and exclusion criteria were designed to obtain a homogeneous population, with interchangeable characteristics in the different treatment arms. Although this is the best strategy to achieve a robust answer to the investigation question, it does not guaranty the treatment efficacy in the clinical practice, since in the real world there are concomitant treatments, comorbidities, adherence and persistence challenges. But, to make informed treatment decision for a real life patient, we need to have evidence of the treatment efficacy, what has been called treatment effectiveness. This work aims to review fingolimod effectiveness, using as source of information abstracts, posters and manuscripts. This unorthodox strategy was developed because more than half of the published experience with fingolimod is still on abstracts and posters. Only a small part of the studies reviewed are already published in peer reviewed journals. Fingolimod seems to be, at least, as effective and safe as it was on clinical trials, and with its long term experience no new safety signals were observed. In the Portuguese hospital perspective, early treatment with fingolimod is expected to result in better clinical outcomes associated with a more efficient healthcare resources allocation.

Palavras-chave

Fingolimod, esclerose múltipla, evidência do mundo real, prática clínica

Resumo

O fingolimod é um tratamento para a Esclerose Múltipla aprovado na Europa desde 2011. A sua eficácia foi demonstrada em três grandes ensaios clínicos de fase III, cujos resultados foram usados para as submissões regulamentares em todo o mundo. Como é habitual, nestes ensaios os critérios de inclusão e exclusão foram desenhados para obter uma população homogénea, com características intermutáveis nos diferentes braços de tratamento. Apesar de esta ser a melhor estratégia para obter uma resposta robusta à pergunta de investigação, não garante a eficácia do tratamento na prática clínica, uma vez que no mundo real existem tratamentos concomitantes, comorbilidades, e dificuldades de adesão e persistência no tratamento. Mas, para poder fazer uma decisão terapêutica informada para um doente de vida real, precisamos de ter evidência da eficácia do tratamento, qye tem sido também chamada de efetividade do tratamento. Este trabalho tem como objetivo rever a efetividade do fingolimod, usando como fontes de informação resumos, pósteres e artigos. Esta estratégia pouco ortodoxa foi usada pois mais de metade da experiência com fingolimod ainda só está publicada através de resumos e pósteres. Apenas uma pequena parte dos estudos revistos estão já publicados em jornais e revistas científicas revistas pelos pares. Esta revisão sugere que o fingolimod é, pelo menos, tão eficaz e seguro quanto o foi nos ensaios clínicos, e os seus dados de longo prazo não vieram demonstrar novos sinais de segurança. Numa perspectiva dos hospitais Portugueses pertencentes ao Sistema Nacional de Saúde, o tratamento precoce com fingolimod resulta em melhores resultados clínicos associados a uma melhor utilização dos recursos disponibilizados para a saúde.

List of Abbreviations

9PHT	9-Hole Peg Test
ARR	Annualized Relapse Rate
AV	Atrioventricular
AVB	Atrioventricular Block
BBB	Blood-Brain Barrier
BBs	Beta Blockers (BBs)
BBs	Beta Blockers (BBs)
BDI-II	Beck Depression Inventory-II
BDNF	Brain-Derived Neurotrophic Factor
BL	Baseline
BP	Blood Pressure
bpm	Beats Per Minute
CBC	Complete Blood Cell counts
CCBs	Calcium Channel Blockers
CCR7+	C-C Chemokine Receptor type 7
CGI-I	Clinical Global Impression – Global Improvement
CIS	Clinically Isolated Syndrome
CNS	Central Nervous System
CS	Cross-Sectional
CSF	Cerebrospinal Fluid
CV	Cardiovascular
DMF	Dimethyl Fumarate
DMTs	Disease Modifying Treatments
EAE	Experimental Autoimmune Encephalomyelitis
ECG	Electrocardiogram
EDSS	Expanded Disability Status Scale
FDO	First Dose Observation
FSS	Fatigue Severity Scale
FTY	fingolimod
FU	Follow Up
GA	Glatiramer Acetate
GCDNF	Glial Cell-Derived Neurotrophic Factor
GC-IP	Ganglion Cell/Inner Plexiform
HADS	Hospital Anxiety and Depression Scale
HAQUAMS	Hamburg Quality of Life Questionnaire in Multiple Sclerosis
HR	Heart Rate
HRQoL	Health-Related Quality of Life

IFN	Interferon
IFNβ	interferonβ
IL-1β	interleukin-1β
IL-6	interleukin-6
IM	Intramuscular
IV	Intravenous
JCV	John Cunningham Virus
MCP-1	Monocyte Chemotactic Protein-1
MMP	Matrix Metalloproteinase
MOG-EAE	Myelin Oligodendrocyte Glycoprotein induced EAE
MP	Methylprednisolone
MP	Methylprednisolone
MPR	Medication Possession Ratio
MRI	Magnetic Resonance Imaging
mRNA	Messenger RNA
MS	Multiple Sclerosis
MSFC	Multiple Sclerosis Functional Composite
MSIS	Multiple Sclerosis Impact Scale
MSPS	Multiple Sclerosis Performance Scales
MSSS	Multiple Sclerosis Severity Score
NA	Non-available/unidentifiable
NEDA	Non-Evidence of Disease Activity
NHS	National Healthcare System
NROs	Neurologist Reported Outcomes
NRS	Scripps Neurological Rating Scale
NS	Non significative
NT	Non-Treated
NTZ	Natalizumab
OPCs	Oligodendrocyte Precursor Cells
OT	Other Treatments
P	Prospective
P*	Presumably Prospective
PCCs	Pre-existing Cardiac Conditions
PDDS	Patient Determined Disease Steps
PHQ-9	Patient Health Questionnaire 9
PML	Progressive Multifocal Leukoencephalopathy
PNTZ	Patients who has natalizumab as previous treatment
PROs	Patient Reported Outcomes
PTs	Pivotal Trials
QoL	Quality of Life
R	Retrospective
R*	Presumably Retrospective
Reg	Registry
RCTs	Randomized Clinical Trials

RNFL	Retinal Nerve Fiber Layer
RRMS	Relapse-Remitting Multiple Sclerosis
S1P	Sphingosine 1-Phosphate receptor
S1PR	Sphingosine 1-Phosphate
SC	Subcutaneous
SCI	Spinal Cord Injury
SDMT	Symbol Digit Modalities Test
SphK	Sphingosine Kinases
SSRIs	Selective Serotonin Reuptake Inhibitors
T	Treated
T1	T1-weighted lesions
T1Gd+	T1 Gadolinium-Enhancing Lesions
T1Gd+	T1 Gadolinium-Enhancing Lesions
T2	T2-weighted lesions
T25FW	Timed 25-Foot Walk
TCM	Central Memory T cells
TEM	Effector Memory T cells
Th17	IL-17-producing effector T helper cells
Tn	Naïve T cells
TNF-α	Tumor Necrosis Factor-α
TSQM	Treatment Satisfaction Questionnaire for Medication
USA	United States of America
w	weeks
WO	Washout

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1. Introduction

Multiple Sclerosis (MS) is a chronic auto-immune disease of the Central Nervous System (CNS), characterized by inflammation and neurodegeneration, with clinic manifestations in the motor, sensory and cognitive domains[1].

Multiple sclerosis is still a disease with unknown etiology, which incidence is greater for females, Caucasians and people living at higher latitudes. The first symptoms usually appear between 20 and 40 years of age.[2–5] MS affects 6,000 Portuguese[6,7] and 2.5 million people in the world.[8] MS is serious and potentially debilitating, affecting young and middle-aged adults in the prime of their lives. It may impact both the life span and the quality of life of the affected individual by increasing overall mortality risk as well as risk of ambulatory disability. MS is the main non-traumatic cause of neurologic morbidity in young and middle-aged adults.[9–11] Fifty percent of MS patients will need walking aid in the first 15 years of the disease.[11]

Relapse Remitting Multiple Sclerosis (RRMS) is the most common phenotype (85% of MS patients) and the actual therapeutic landscape gives some hope to these patients with improved quality of life.[12,13] Most current Disease Modifying Treatments (DMTs) for MS primarily target the immunological inflammatory component of the disease without acting directly on the CNS; such DMTs have been shown to be only partially effective.

Multiple sclerosis was traditionally considered a white matter disease, in which auto-reactive T cells passed through the Blood-Brain Barrier (BBB) to the CNS promoting chronic inflammation. This focal lesions, with time, result in irreversible damage with axonal and neuronal loss. These lesions are usually perivascular, well defined in space, with a clear frontier between damaged and intact tissue [14–16].

A growing body of evidence, from histopathology and imaging, suggests that MS lesions are not restricted to these focal lesions, but they are spread out in the whole CNS. The diffuse damage in MS is not visible through conventional Magnetic Resonance Imaging (MRI) techniques. It is usually located more than 1cm away from the edge of a focal lesion and can be observed in grey matter, white matter and in normal-appearing white matter[17–20]. The

chronic demyelination of the neurons, results in axonal damage and loss, which ultimately ends in loss of function for the MS patient[21].

The pathological hallmark of MS is the plaques, which are focal demyelinated white matter lesions. These lesions are usually very easy to identify in histology or imaging studies because they are normally round or ovoid, located in a perivascular region and have a clear frontier between damaged and healthy tissue. They can be found in periventricular white matter, cerebellum and brain stem, but they also occur in optic nerves and spinal cord. These plaques are inflammation focus, with presence of astrocytes, macrophages and lymphocytes promoting primary demyelination causing axonal and matrix damage and ultimately glial scarring[18,19]. In early stages of MS remyelination is very frequent, more in the subcortical than in the periventricular region, but it decreases with the evolution of the disease[22]. These active lesions evolve sometimes to being chronic and inactive, with far less cells and inflammation and no evidence of demyelination, appearing in the MRI T1 images as hypointense lesions – black holes[14].

The grey matter focal lesions have a different histopathology from the white matter lesions and are difficult to distinguish with conventional MRI techniques[14]. They are less inflammatory and can be the result of axonal damage in the normal-appearing white matter. In these lesions is common to find macrophage-associated demyelination, activated microglia, neuronal apoptosis and, in many cases, meningeal inflammation[14,23,24] and are less associated with extensive immune cell influx, complement activation and blood-brain barrier leakage than in the white matter lesions[25].

In the normal-appearing white matter there is also evidence of some MS activity resulting in diffuse damage[26]. This tissue is more than 1cm away from a plaque's edge and is microscopically normally myelinated, with some unusual characteristics like the presence of macrophage, demyelination, gliosis and small round cell infiltration. Activated astrocytes and microglia are also frequently present[14]. Axonal density is decreased, comparing with normal white matter, and this may be due to axonal damage in focal lesions or due to an independent process[27,28].

This disease has a profound impact in patients and their families, reducing their Quality of Life (QoL)[29,30] and life expectancy, but also increasing the morbidity[10]. MS also has a big

impact on society, not only through high health care costs[9], but also because 50% of MS patients with a 3.0 score in the Expanded Disability Status Scale (EDSS) are unemployed[11].

Multiple sclerosis represents a big economic burden for the National Health Systems. The pharmacotherapy (symptomatic relief treatments and long term treatment with DMTs), hospitalization, rehabilitation and the social cost of work absenteeism and early retirement contribute to the economic burden of the disease.[9,31] In Portugal there are about 2,694 patients treated with interferon beta (IFNβ)[32] and it is estimated that about 30% are poor responders and can benefit from second-line treatment.[33] Fingolimod treatment costs may be seen as an obstacle to treatment escalation, despite the fact that early initiation of fingolimod may impact favourably on long-term clinical outcomes.[34,35] This analysis aimed to assess if the early switch from IFNB to fingolimod impacts MS clinical outcomes and promotes better resource utilization in a Portuguese hospital perspective.

Unfortunately a cure for MS does not exist yet, but a few treatments are used to modify the natural course of the disease. Every Disease Modifying Treatment (DMT) goal is to achieve a state of full Non Evidence of Disease Activity (NEDA-4), which comprises absence of relapses, disability progression, MRI activity and brain volume loss.

Fingolimod is a DMT used to reduce the progression of the disease and thus improve patient's quality of life. It's a small lipophilic molecule, with a novel mechanism of action, which seems to act both through its peripheral and central actions. Peripherally, fingolimod reduces the focal damage [15,16,36] by reducing the entry of pathological lymphocytes into the CNS and reduces diffuse damage, by its direct actions on the CNS[17,18,20,36,37], reducing the activation of pathogenic astrocytes. Through this two independent mechanisms, the central effects of fingolimod are also supported by the efficacy results on relapses, MRI activity, disability and brain volume loss.[38–42] In a Post-hoc analysis of FREEDOMS and FREEDOMS II (patients with high disease activity despite receiving a DMT in the year before the study), fingolimod showed to reduce the 4 key measures of disease activity.[43] (Figure 1) In FREEDOMS, after 2 years, the likelihood of achieving the 4 parameters of non-evidence of disease activity with fingolimod was more than 4-fold higher vs placebo.[44] Furthermore, the extension phases of fingolimod trials showed that there is a significant clinical benefit of initiating the treatment earlier[34] and that fingolimod efficacy endures in long term treatments,[35] which is of utmost importance for a chronic disease like MS.

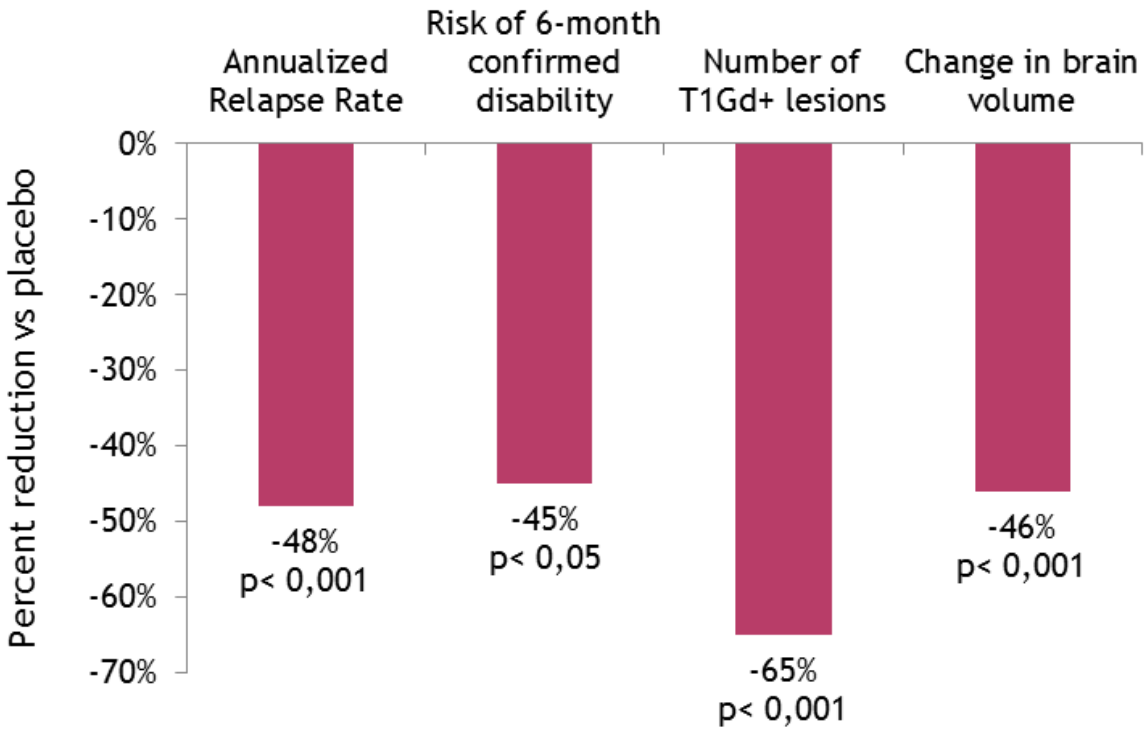

Figure 1. FREEDOMS and FREEDOMS II pooled results at 24 months in patients with high disease activity despite receiving a DMT in the year before the study[43]

Fingolimod achieved a significant reduction in the Annualized Relapse Rate (ARR) in all trials. In patients with the same characteristics as the EU label requires to start fingolimod treatment, with high disease activity despite receiving a DMT in the year before the study, fingolimod achieved a 61% reduction in ARR vs IFNβ-1a.[41,45] Patients that were treated for 1 year with IFNβ-1a and switched to fingolimod, have seen its ARR reduced by 46% [34] and the ARR remained low after 5 years of treatment.[46]

Fingolimod reduced T_1Gd^+ enhanced lesions by 82% in FREEDOMS, and in TRANSFORMS and its extension achieved a 80% reduction in T_1Gd^+ lesions in patients who had high disease activity despite receiving IFNβ-1a IM in the year before study.[34,38] The effect on T_1Gd^+ lesions was sustained over 4 years in FREEDOMS and its extension.[47]

In FREEDOMS fingolimod reduced the disability progression confirmed at 6 months by 37% [38] and in LONGTERMS the EDSS score remained stable over 5 years.[46]

Fingolimod demonstrated to reduce the rate of brain volume loss by 32% ($p<0.001$), 35% ($p<0.001$) and 33% ($p<0.05$) in 3 phase III trials, involving a total of 3,647 patients. [38,42,48]

Results from the extension of these studies,[34,47] and results of seven years from the phase II study,[49] have shown that fingolimod provides a sustained treatment effect, with improved clinical and MRI outcomes, and a tendency of brain atrophy curves from patients treated with fingolimod to resemble the ones from healthy individuals.

Fingolimod most frequent adverse effects are cardiovascular effects, liver toxicity and lymphopenia. Transient bradycardia, frequently asymptomatic, slight increase in blood pressure and small persistent extension of QT interval are the main cardiovascular effects. First degree AV block may occur in the first dose, but it's usually transient and do not require pharmacologic intervention. The liver toxicity occurs mainly in the first months of treatment and requires monitoring. The laboratory values, after interrupting the treatment, revert spontaneously to baseline values after a few weeks. Lymphopenia is a pharmacodynamic effect of fingolimod and only requires treatment interruption if lymphocyte counts are <200/µL. Macular oedema has a very low incidence and after treatment interruption reverts spontaneously. Respiratory and serious herpetic infections were more frequent with fingolimod. Malignancies occurred with the same incidence in all treatment arms. (Table 1) [38,50]

Fingolimod is approved in the USA since 2010 and in Europe since 2011, with some differences in the target label population. Whereas in the USA fingolimod may be used in first-line patients, without any restrictions in terms of disease severity, in Europe fingolimod can only be used in first-line in patients with severe and rapidly evolving disease.

Nowadays, a new treatment has not only to prove its effectiveness, safety and quality, but it has also to prove its cost effectiveness. In Portugal, probably in a more exuberant way than in other European countries, cost has been a serious issue for all new treatments. Thus it is important to prove that, in the Portuguese hospital perspective, using drugs with an higher unit price than the drugs already used, may be in the long run more cost-effective than using less expensive, but less efficacious drugs.

Fingolimod has already a significant experience in clinical practice, with more than 104,700 patients treated and more than 172,500 patient-years of exposure. [51] In this setting, a review of its efficacy and safety in the clinical practice is due, to assess how the drug behaves in the real world, and what impact does it have in patients' lives.

Table 1. Adverse events in FREEDOMS[38]

Adverse events	GILENYA 0.5 mg N=425 (%)	Placebo N=418 (%)
Influenza viral infections	13	10
Herpes viral infections	9	8
Bronchitis	8	4
Sinusitis	7	5
Gastroenteritis	5	3
Tinea infections	4	1
Bradycardia	4	1
Headache	25	23
Dizziness	7	6
Paresthesia	5	4
Migraine	5	1
Diarrhea	12	7
Asthenia	3	1
Back pain	12	7
Alopecia	4	2
Eczema	3	2
Pruritus	3	1
ALT/AST increased	14	5
GGT increased	5	1
Weight decreased	5	3
Blood triglycerides increased	3	1
Cough	10	8
Dyspnea	8	5
Depression	8	7
Vision blurred	4	1
Eye pain	3	1
Hypertension	6	4
Lymphopenia	4	1
Leukopenia	3	<1

2. Fingolimod

Fingolimod was initially investigated as a potential immunosuppressant for kidney transplantation, but the results from a Phase III clinical trial disappointed.[52,53] Fingolimod was not as immunosuppressive as it was needed in a transplantation setting. This led to it being further investigated as a new MS treatment.

Fingolimod is the lead compound in a new class of MS treatments and represents an important therapeutic advance. Its unique mode of action targets both the immune and central nervous systems which explains its efficacy in reducing the disease activity.

Fingolimod is rapidly phosphorylated in vivo by sphingosine kinases, [54,55] to its phosphorylated metabolite, an analogue of sphingosine 1-phosphate (S1P). Fingolimod-phosphate exerts its action through its interaction with S1P receptors.

2.1. Sphingosine 1-phosphate receptor modulation

Sphingosine 1-phosphate (S1P) receptors are G-protein coupled receptors distributed throughout the body [37] and are involved in the regulation of lymphocyte recirculation, neurogenesis, neural cell function, endothelial cell function, vasoregulation and cardiovascular development.[56] Fingolimod is metabolized by Sphingosine Kinases (SphK) to its phosphorylated form, [57,58] which binds to S1P Receptors (S1PR) 1, 3 and 4 located on lymphocytes. After crossing the blood-brain barrier acts on S1PRs 1, 3 and 5 located on CNS cells. Fingolimod phosphate may act as an agonist or a functional antagonist depending on the receptor subtype and its location. [59]

2.2. Immune system

Fingolimod-phosphate, like S1P, exerts its action by binding to S1PRs, which triggers a cellular response. After binding to SP1R, S1P promotes the receptor internalization followed by its recycling back to the cellular membrane. Whereas with fingolimod after the internalization into the endosomal pathway, the S1PR it's not recycled to the cellular membrane and suffers degradation.[60–62] In this way, fingolimod acts initially as a S1PR agonist, but after some time, it starts to act as a S1PR antagonist because it induces the reduction of S1PR available in cell surfaces.

In the lymphoid tissues, the S1P gradient is responsible for controlling lymphocyte trafficking. S1P concentration, which can be sensed by lymphocytes through its S1PR1, is lower in lymphoid tissues and higher on tissues and body fluids. The low S1P concentration in the lymph nodes induces S1PR1 up-regulation and, if the lymphocyte does not find an antigen in the lymph node, will continue in the direction of higher S1P concentrations, which drives it out of the lymph node. However, if an antigen is present, it induces the S1PR down-regulation. The lymphocyte without the S1PR1 does not sense the S1P concentration, and does not follow the higher S1P concentrations. Staying longer in the lymph node allows more time to be in contact with the antigen presenting cells and to become activated. Activated lymphocytes suffer clonal expansion and S1PR1 are up-regulated again allowing its egress from the lymph nodes.[37,58,63–66]

When fingolimod is present, the induced down-regulation of S1PR1, turns T-lymphocytes insensitive to the egress signal provided by S1P gradient. This results in T-lymphocyte retention in the lymph nodes, and subsequent reduced number of T cells in lymph and blood. In MS patients, this means that auto reactive T cells are in lower numbers in the blood stream and thus avoiding its migration into the CNS where they would promote focal damage, which translates clinically in reduced relapse and MRI activity. [37,58,63–66] The lymphocyte functionality, when inside the lymph nodes, remains almost intact [67] and thus fingolimod must be seen as an immunomodulator and not as a pure immunosuppressive. Usually less than 2% of lymphocytes are in blood and lymph, [68] so the average 70% reduction in lymphocyte numbers in the blood, [37,69] does not bring a significant burden to the lymph nodes.

Fingolimod only has an impact on Naïve (Tn) and Central Memory T cells (TCM), and CCR7 expressing B cells. These cells traffic regularly through the lymph nodes and are susceptible to fingolimod effects. CCR7 is a lymph node homing receptor, that is not expressed by all lymphocytes, and can be used to divide T memory lymphocytes in two different subsets, the CCR7+ TCM that express CCR7 and do not have an immediate effector function and the CCR7- Effector Memory T Cells (TEM), that do not express CCR7 and have an immediate effector function. [67,70,71]

In MS, most of the autoreactive T cell subsets belong to the TCM population. Over 90% of Cerebrospinal Fluid (CSF) T cells are CCR7+ TCM, [72] which suggests its important role in MS physiopathology. The proinflammatory Th17 cells, which are augmented in the CSF and inflammatory lesions of MS patients, [70,71] belong mainly to the TCM subset. [73–75] Effector Memory T cells (TEM), which do not recirculate to lymph nodes, are tissue resident cells with an important immune surveillance role. [76,77] Fingolimod does not have an impact in TEM, but reduces drastically Th17 numbers in the bloodstream, [67,70] which can explain both fingolimod efficacy in reducing MS activity and the incidence of infections similar to placebo, almost as if it does not had an effect in the immune competence of the patient.

Fingolimod induced lymphopenia results from a redistribution of T cells, and not from a cytotoxic effect. Thus the lymphopenia induced by fingolimod is readily reversible upon interruption of treatment. After a few weeks, lymphocytes counts return to the normal range,[78] which can be very important to react to a serious infection.

2.3. Central nervous system[79]

2.3.1. S1PRs in the CNS

All S1PRs are present in the CNS, with the exception of S1PR4. However, the type of S1PRs present on different CNS cells is still controversial, given it varies with the cell differentiation stage and activation status. Oligodendrocytes express S1PR1 and S1PR5 and may also express S1PR3, neurons express S1PR1-3 and may also express S1PR5, and astrocytes and microglial cells express all S1P1Rs except S1PR4[80]. S1PR1 and S1PR3 have been found to be upregulated in MS lesions[81] and one report suggests a disturbance in sphingolipid metabolism in MS patients.[82]

2.3.2. Blood-Brain Barrier

Fingolimod seems to have a direct action on the BBB itself. It has been proposed that, by functionally antagonizing S1P1 and S1P3 receptors in astrocytes, fingolimod could reduce the deleterious effects of increased S1P levels and astrocytes on gap junctions between neural cells, thus helping to restore the gap-junctional communication between astrocytes, neurons and endothelial cells of the BBB.[66,83] Fingolimod activation of S1P1 receptors enhances adherens junction assembly and endothelial barrier integrity.[54] BBB permeability can also be altered by S1PR1 and S1PR3 expressed in cerebral capillaries.[84] However, in an in vitro model of human pulmonary endothelial cells, fingolimod's enhancement of barrier function seemed to be independent of both S1P1R and phosphorylation of fingolimod.[85] This apparent variability and heterogeneity of vascular beds raises the question of whether fingolimod actually alters the BBB function.

2.3.3. Oligodendrocytes and OPCs

Oligodendrocyte is the major cell type involved in the remyelination process. Fingolimod exerts direct protective effects on oligodendrocytes,[86] promoting their survival including in situations of serum and glucose deprivation.[87,88] Fingolimod promotes remyelination via direct interaction with S1PRs on oligodendrocytes[89,90], an effect also observed in organotypic cell cultures.[91] In cultures of human mature oligodendrocytes, low

concentrations of fingolimod promote myelin production, stimulate membrane formation and enhance process extension, while high concentrations have the opposite effect.[87,88]

It is thought that the loss of Oligodendrocyte Precursor Cells (OPCs) within MS lesions plays an important role in remyelination failure.[92,93] Fingolimod protects OPCs from apoptosis induced by growth factor deprivation, inflammatory chemokines and microglial activation,[94,95] promotes remyelination via direct interaction with S1PRs in OPCs,[90] regulates OPC differentiation into oligodendrocytes[88] and inhibits OPC migration,[87,96] although the latter effect can be prevented if platelet-derived growth factor is used as a chemoattractant.[88] However, the remyelination process may fail at several stages of oligodendrocyte development, and may be associated to a slow response of astrocytes and/or microglial cells to demyelination, regardless of its association to OPC response failure.[97]

2.3.4. Astrocytes

Astrocytes are the most abundant cells both in the CNS and in MS lesions.[98,99] Astrocytes promote neuron and oligodendrocyte protection,[100] axonal regeneration[98] and myelination.[101] It is thought that astrocytes contribute to MS pathophysiological processes through the secretion of Matrix Metalloproteinase (MMP) and BBB disruption, secretion of adhesion molecules and chemokines, facilitation of inflammatory cells invasion, and secretion of TNFα and lymphotoxin-α, causing oligodendrocytes death and axonal injury. Also, astrogliosis and glial scar formation, a feature of chronic MS lesions, may interfere with the migration of precursor cells, remyelination and axonal regeneration.[102]

Activation of S1PRs leads to astrocyte proliferation, inhibition of inflammatory chemokines release[81,103,104] and increase in the unphosphorylated form of connexin-43, an important protein for neuron survival.[105] In astrocytes, fingolimod decreases the production of the inflammatory chemokine Monocyte Chemotactic Protein-1 (MCP-1),[81] mediates neuroinflammation relevant effects,[106] promotes migration[107] and reduces astrogliosis.[83,90] In an adult rat model of lithium-pilocarpine induced epilepsy, fingolimod decreased activation of astrocytes in the hippocampus.[108] These effects seem to be mediated by S1PR1, and suggest a beneficial neurobiological effect of fingolimod, independent of its immunomodulatory mechanism.[109]

2.3.5. Microglial cells

The primary function of microglia is the maintenance of tissue homeostasis and the support of regeneration from the earliest stages in the development of demyelinating lesions. Microglia supports remyelination and produces several cytokines and chemokines involved in the activation and recruitment of endogenous OPCs to the lesion site, also providing trophic support during remyelination.[110]

Microglial activation is involved in the neurodegeneration of MS progressive types,[3] and fingolimod inhibits persistent activation of microglial cells after a demyelinating event, thus promoting remyelination.[111] Moreover, activated microglial cells release proinflammatory cytokines known to be involved in neuroinflammation,[110] and fingolimod, via S1PR1, downregulates the production of interleukin-6 (IL-6), interleukin-1beta (IL-1β) and Tumor Necrosis Factor-alpha (TNF-α), while upregulating microglial production of Brain-Derived Neurotrophic Factor (BDNF) and Glial Cell-Derived Neurotrophic Factor (GCDNF),[112] thus suggesting a direct effect in microglia's neuroprotective actions.[113]

Administration of fingolimod to Spinal Cord Injury (SCI) models reduces T-lymphocyte infiltration without affecting neutrophil infiltration and microglia activation,[114] and in an adult rat model of lithium-pilocarpine induced epilepsy, fingolimod reduced microglial activation in the hippocampus.[108]

Taken together, these data suggest a positive role of fingolimod in microglia and consequently in microglia's functions.

2.3.6. Neuroprotection

The S1P signalling pathway has neuroprotective and pro-cell survival effects during the development phase. S1PRs play a role in neurogenesis and induce proliferation of neuronal progenitor cells in cultures from rat embryonic hippocampus,[115] and S1PR1-knockout mice show defects in neurogenesis, while the double SphK1/2 knockout increases apoptosis in the developing nervous system, disrupts neurogenesis and increases embryonic mortality.[116] S1PR1 enhances neurite extension, while S1PR2 inhibits it.[117] Given fingolimod only occupies S1PR1, having no affinity for S1PR2, it may be speculated that it promotes endogenous

repair processes mediated by S1PR1 while inhibiting the detrimental effects mediated by S1PR2 activation.

Within the last few years, both *in vitro* and *in vivo* models have provided evidence in favour of fingolimod neuroprotection ability.

Fingolimod may have a neuroprotective action, having a positive role in neurite outgrowth and neurogenesis.[89] *In vitro*, fingolimod protects cortical neurons against excitotoxic death[118] and against oligomeric amyloid beta-induced neurotoxicity, being the latter mediated by upregulation of neuronal BDNF levels.[119] Fingolimod also decreases production of Amyloid-β peptide by neuronal cells.[120] In a rat model of Alzheimer Disease (AD), fingolimod significantly attenuated the $A\beta_{42}$-induced learning and memory impairment, and prevented hippocampus neuronal damage and caspase-3 activation.[121]

Fingolimod has been studied in several variants of Experimental Autoimmune Encephalomyelitis (EAE), preventing the development of clinical and histological disease when used prophylactically, and reversing its manifestations when administered therapeutically after disease onset. Clinical benefits include decreased inflammation,[122,123] electrophysiological anomalies ,[124] demyelination and axonal loss, [122,123,125–127] synaptic dysfunction[128] and dendritic injury,[89] while improving axial and radial diffusivity, which correlate with clinical scores in EAE mice.[127] Genetic knockdown of astrocyte S1PR1 reduces EAE values and prevents the development of astrogliosis, inflammation, demyelination and neuronal loss in the MOG-EAE model.[109] Taken together, these results suggest that fingolimod functional antagonism of S1PR1 is effective in EAE.

In an adult rat model of lithium-pilocarpine induced epilepsy, fingolimod has been shown to be neuroprotective by reducing neuronal loss, increasing neuronal nuclei positive cells and decreasing Fluoro-Jade B positive cells in the hippocampus.[108]

Administration of fingolimod to Spinal Cord Injury (SCI) models shortly after injury, significantly increases motor function recovery without affecting the mRNA expression of inflammatory cytokines, reduces vascular permeability and astrogliosis, having similar effects in severely immuno-compromised SCI model mice.[114]

In a Lewis mice model of delayed type hypersensitivity, whose later stages resemble MS in that there is damage to CNS components behind an intact BBB, fingolimod has been

shown to reduce the CNS inflammatory response, decreasing demyelination and inhibiting microglial activity.[129]

In a model of experimental cerebral malaria, fingolimod inhibited vascular leakage and neurological signs and prolonged survival.[130]

Fingolimod has shown preclinical anti-cancer activity in neuroblastoma, acting synergistically with topotecan,[131] and increases viability and neurogenicity of hippocampal neural stem cells after irradiation.[132]

However, there is one report in which fingolimod did not promote remyelination in cuprizone and lysolecithin models of demyelination,[133] suggesting that fingolimod remyelination ability depends on the mechanisms leading to demyelination.

Taken together, these results suggest that fingolimod has direct neuroprotective effects in several disease models, and not only in MS. Figure 2 summarizes fingolimod most relevant effects on the main cell types of the CNS.

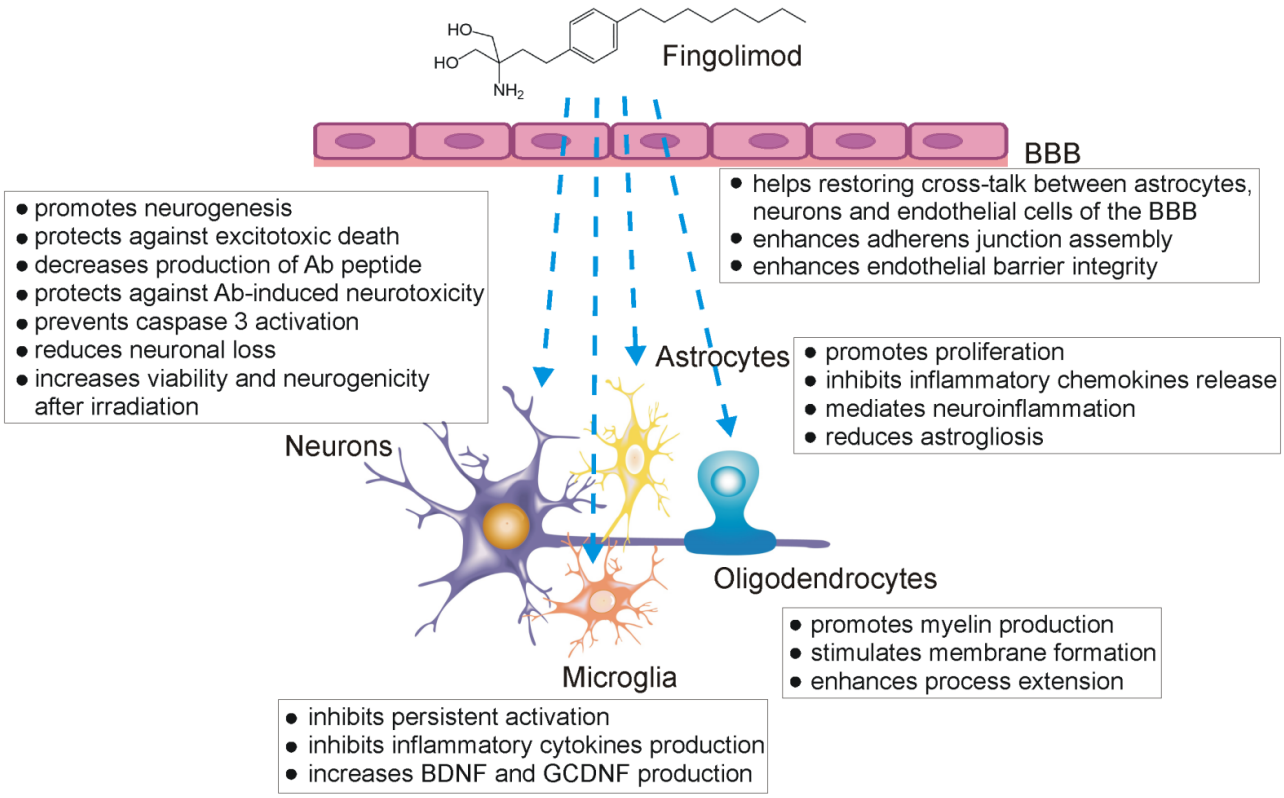

Figure 2. Fingolimod most relevant effects on the main cell types of the CNS

2.3.7. Clinical Trials evidence

The central effects of fingolimod have also been supported by the efficacy results demonstrated by clinical trials, showing a significant efficacy in reducing relapse rate, disability progression and disease activity as assessed by MRI.[38–42,48,49] The confirmed 37% decrease in disability progression at 6 months, reported by the FREEDOMS study,[38] and the significant reduction in inflammatory activity as early as at the second month of treatment[40] are consistent with a mechanism of action that includes direct effects in CNS cells. However, the most relevant data from clinical trials that confirms this possibility is the reduction of brain volume loss rate, already demonstrated in 3 large studies. In fact, the TRANSFORMS,[48] FREEDOMS[38] and FREEDOMS II[42] studies, involving a total of 3,647 patients, have shown that fingolimod reduced the rate of brain volume loss by 32% ($p<0.001$), 35% ($p<0.001$) and 33% ($p<0.05$), respectively. The fingolimod-induced reduction of brain volume loss was shown to be independent of the presence of inflammatory lesions at baseline - Figure 3.

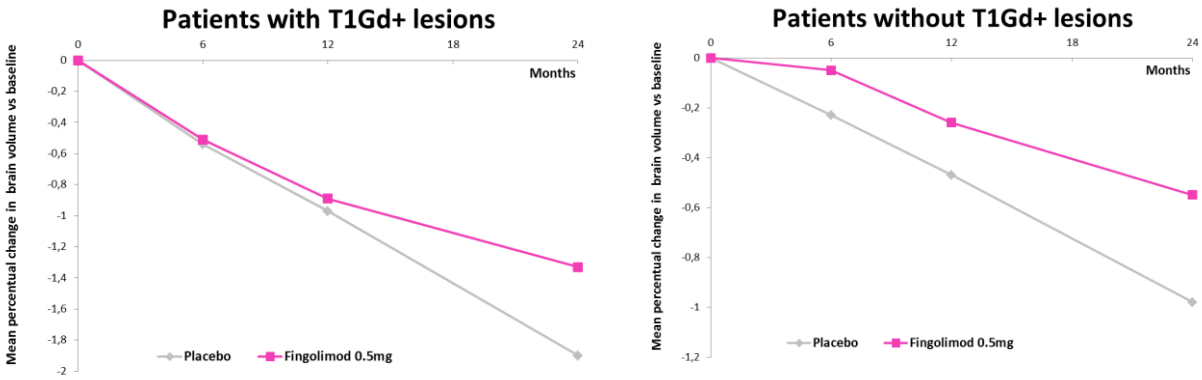

Figure 3. Fingolimod reduction in brain volume loss is independent of the presence of inflammatory lesions at baseline

Results from the extension of these studies,[35,47] and results of seven years from the phase II study,[49] have shown that fingolimod provides a sustained treatment effect, with improved clinical and MRI outcomes, and a tendency of brain atrophy curves from patients treated with fingolimod to resemble the ones from healthy individuals.

Despite its side effects, fingolimod shows efficacy, real-world adherence and tolerability, and some authors have suggested that it is precisely the differential modulation of S1PRs in different cells of the immune, cardiovascular and central nervous systems that may be responsible for fingolimod efficacy and side effects,[134,135] thus supporting the central

effects of fingolimod. Nevertheless, continuous research is warranted, not only to unravel fingolimod underlying mechanisms of action but also to determine its long-term effects.

3. Fingolimod cost-effectiveness analysis[136]

3.1. Objective

The present analysis estimates the cost-effectiveness of early versus delayed initiation of fingolimod based on results of the double-blind extension of the TRANSFORMS trial over the 4.5-year duration of the core and extension phases.

3.2. Methods

3.2.1. Model

This analysis was based on the ARR phase III trial extension.

A cost-effectiveness model was developed to assess the impact associated with 2 treatment strategies in MS patients (Figure 4): [34,35,48]

a. early treatment: 4.5 years of continuous treatment with fingolimod;
b. delayed treatment: 1 year of treatment with IFNβ followed by 3.5 years of treatment with fingolimod.

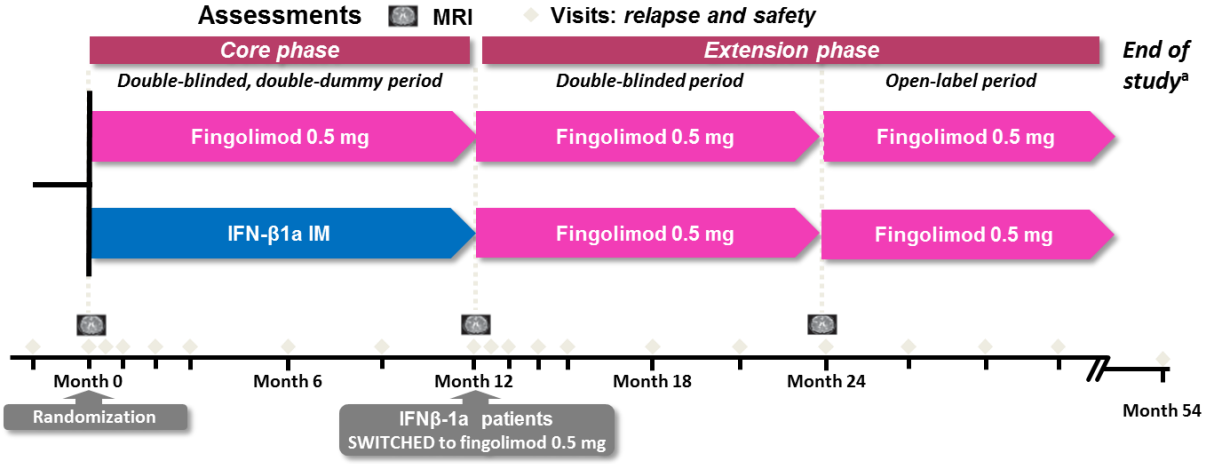

Figure 4. TRANSFORMS and extension study design. MRI – magnetic resonance imaging. [a]The optional extension phase continued until all the patients either discontinued or were transferred to the LONGTERMS study

The model calculates the number of relapses associated with the different treatment strategies, the total treatment costs, and the cost per relapse avoided.

3.2.2. Perspective

A Portuguese National Healthcare System (NHS) hospital perspective was adopted addressing only direct medical costs: drug acquisition, monitoring and relapses' treatment.

3.2.3. Time Horizon

The time horizon is 54 months (4.5 years): 1 year of TRANFORMS core phase and 3.5 years of TRANSFORMS extension phase. [34,35,48]

3.2.4. Data Sources

This model used the phase III 12-month core and 42-month extension phases of the TRANSFORMS trial to compare cost-effectiveness of continuous fingolimod therapy vs IFNβ-1a for 1 year followed by fingolimod therapy. The complete methods of the TRANSFORMS study program have been previously described. The TRANSFORMS core study enrolled patients aged 18 to 55 years with a diagnosis of relapsing–remitting MS and who had experienced at least 1 documented relapse in the 2 years before randomization.

In the core study, patients were randomized to receive 1 of 3 treatments: 0.5 mg/d oral fingolimod, 1.25 mg/d oral fingolimod, or 30 µg/wk IM IFN-β1a. During the extension phase, patients who had received fingolimod during the core stage continued to receive the same assigned dose, whereas patients who had originally received IFN-β1a were randomized to receive either 0.5 or 1.25 mg/d oral fingolimod.

Of the 1292 patients randomised in the Core phase, 1,030 entered the extension phase of TRANSFORMS and a total of 772 patients completed the 54 months of treatment. The aggregate ARR at end point was 0.17 in the early 0.5-mg/d fingolimod group, and 0.27 in the delayed group, resulting in a 39% reduction in ARR with early treatment with fingolimod versus delayed fingolimod after IFN-β1a ($p<0.001$).[35] A real-world, observational,

retrospective analysis determined that in patients newly initiating IM IFN-β1a therapy, the relapse rate over the previous year was 0.77.[137] On the basis of this rate of relapse in the untreated population, the number of relapses avoided with early treatment with fingolimod was calculated.

Drug acquisition costs for fingolimod were obtained from Infarmed's Approval Report for Gilenya®,[138] while for IM IFN-β1a these were obtained using Avonex® 2013 acquisition cost listed in the Portuguese web-based pricing catalogue for hospitals.[139] To obtain the net drug acquisition cost, prescribing information was used to determine the number of doses per package and the number of packages used per year.[50,140] The model did not include product rebates, discounts, or patient co-pays or co-insurances, and assumed all patients were 100% adherent to treatment. Drug acquisition costs are presented in Table 2.

Table 2. Drug acquisition costs

Medication	Fingolimod	IFNβ-1a
Posology	0.5 mg / day	0.03 mg / week
Unit cost (€)	1,648.43	778.10
Annual cost (€)	21,488.46	10,115.30

Monitoring requirements differed between treatments, and were based on each agent's product prescribing information, clinical guidelines and empirical clinical practice, validated by an experts' panel. On the basis of prescribing information for fingolimod, all patients new to fingolimod therapy were assumed to undergo a 6-hour in-office observation for signs and symptoms of bradycardia after the first dose and an ECG before receiving the first dose and at the end of the 6-hour first-dose observation period. All patients new to fingolimod must have 6 Complete Blood Cell counts (CBC) and 6 liver function test based during the first year. One ophthalmologic visit was also included in the year 1 fingolimod arm per the monitoring recommendations of the package insert.

Unit monitoring costs were obtained from 2 different sources of information:

1. both the 6-hour in-office observation and the outpatient consultation costs were derived from the database for Analytical Accounting of the Portuguese Hospitals for 2009 and; [141]
2. procedure costs were obtained from the Portuguese Diagnosis Related Groups tariff (Portaria n.º 20/2014).[142]

The cost of other potential monitoring requirements for patients at high risk, such as dermatology visits and pregnancy tests, and tests commonly required for diagnosis of MS, were not included in the model. Similarly, costs of extended first-dose monitoring for patients receiving fingolimod who had some pre-existing conditions were not included because of lack of clarity on the prevalence of such patient populations. Resource use and unit costs considered are included in Table 3.

Table 3. Resource use and monitoring costs

Monitoring	Early treatment		Delayed treatment		Unit cost (€)
	FTY year 1	FTY year 2	IFNβ year 1	FTY year 1	
In-office observation	1	0	0	1	615.77[141]
Neurologist visit	6	2	2	6	214.26[141]
Ophthalmologic visit	1	0	0	1	42.32[141]
Complete blood count [a]	6	2	4	6	4.70[142]
Liver function tests [b]	6	2	3	6	15.10[142]
Thyroid panel [c]	0	0	1	0	17.00[142]
MRI [d]	1	1	1	1	127.90[142]
ECG [e]	3	0	0	3	6.50[142]
Total costs (€)	**2,209.85**	**596.02**	**637.52**	**2,209.85**	–

FTY – fingolimod; MRI – magnetic resonance imaging; ECG – electrocardiogram; DRG – diagnosis related groups [a] DRG 24209; [b] DRG (21140, 21220, 21217, 21935, 21344, 22035, 21665, 24347, 24359); [c] DRG (22253, 22925, 22897, 22900); [d] DRG 18010; [e] DRG 40301

The estimate on the cost of relapse was derived from a study performed by Mateus *et al*.[143] This author estimate the cost of relapses in patients with relapsing–remitting MS based on hospitalizations, medications used to treat relapses, ambulatory care and the use of other resources. The costs associated with hospitalization and ambulatory care represent around 95% of total costs, regardless of the level of EDSS.

Low-intensity relapses were defined as those requiring a physician office visit, treatment with symptom-related medication, and a follow-up visit. Medium-intensity relapses were defined as those requiring a physician office or emergency department visit, treatment with IV methylprednisolone and symptom-related medications, and a follow-up office visit or consultations with a physical, occupational, or speech therapist. High-intensity relapses were defined as those requiring a physician office or emergency department visit, treatment requiring hospital admission, and post discharge services including outpatient follow-up, rehabilitation, home health care, skilled nursing facility care, and short-stay

nursing home care, or hospital readmission within 30 days. The relative incidence of each relapse severity was obtained from the EVIDENCE (Evidence for Interferon Dose-response: European North American Comparative Efficacy) trial.[144]

Based on such information, and the potential distribution of patients by EDSS level, the cost of a relapse was estimated to be € 5,852.40 per relapse (Table 4).

Table 4. Relapses' treatment costs

Relapses	EDSS<3	3.5<EDSS>4,5	5>EDSS>6	EDSS>6,5	Total
Total cost / relapse[143]	3,986	5,139	7,212	7,556	–
% of patients[6]	25%	30%	25%	20%	–
Estimated cost / relapse (€)	996.50	1,541.70	1,803.00	1,511.20	5,852.40

EDSS – Expanded Disability Status Scale

3.3. Results

Assuming there are 819 patients treated with IFNβ that are poor responders, the early treatment with fingolimod resulted in more relapses avoided, 2,211, when compared with delayed treatment with fingolimod, 1,843 – an additional 368 relapses avoided. From year 1, the number of relapses avoided is higher for early treatment group compared with delayed treatment. This difference increases over time (Figure 5).

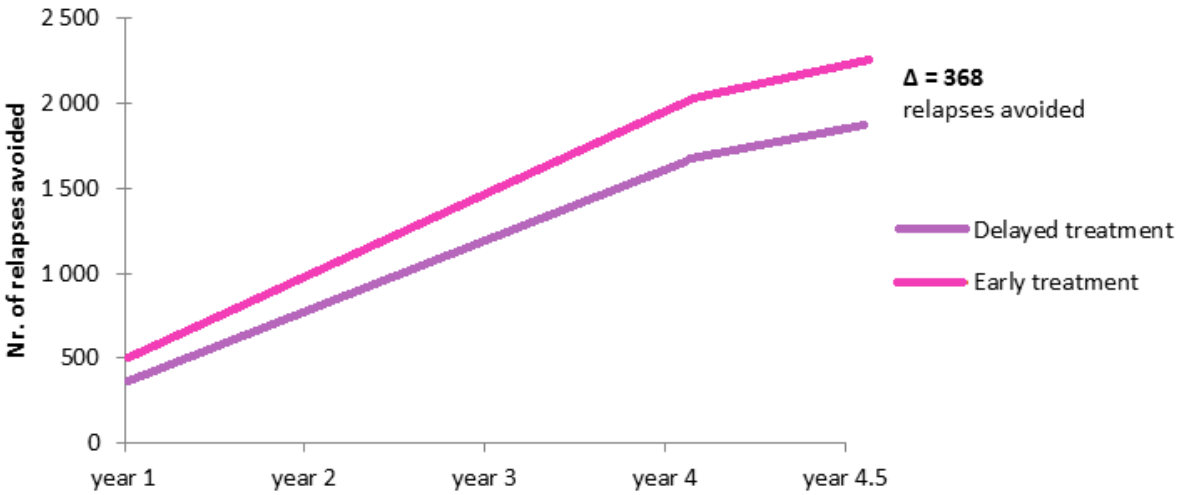

Figure 5. Number of relapses avoided over 4.5 years with early treatment vs. delayed treatment

3.3.1. Incremental costs

The early treatment with fingolimod led to an increase of drug acquisition costs, but reduced costs associated to monitoring and relapses' treatment. The total costs were

86,380,820€ for early treatment versus 79,257,091€ for delayed treatment. This represents an average incremental investment of 1,933€ per patient per year.

3.3.2. Cost-Effectiveness Analysis

The early strategy resulted an incremental cost effectiveness ratio of 19,358€ per relapse avoided when compared with the delayed strategy (Table 5).

After three years, the early treatment with fingolimod is associated with lower costs per relapse avoided compared with delayed treatment.

Table 5. Analysis of a cohort of 819 patients in a 4.5 years' time horizon

	Early treatment	Delayed treatment	Difference
Medication costs (€)	79,195,729	69,881,108	+ 9,314,620
Monitoring costs (€)	3,518,358	3,552,347	– 33,989
Relapses' treatment costs (€)	3,666,733	5,823,635	– 2,156,902
Total costs (€)	86,380,820	79,257,091	+ 7,123,730
Relapses avoided	2,211	1,843	368
ICER (€ / relapse avoided)	–	–	+ 19,358

The estimated cost per relapse avoided was € 39,063 in the early treatment group compared to €43,010 in the delayed group.

The early treatment strategy had a cost per relapse avoided € 3,947 lower than the delayed treatment strategy (Figure 6).

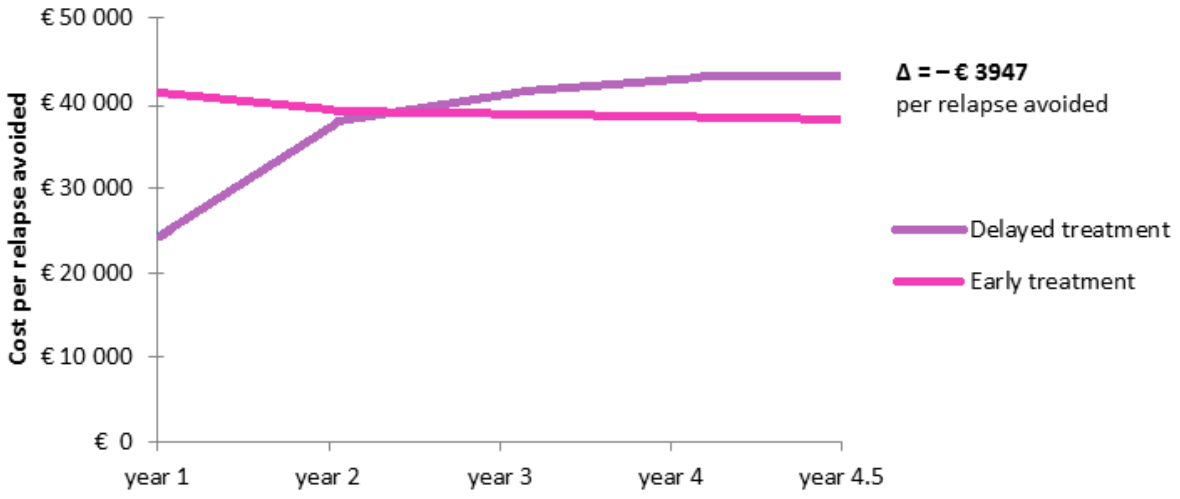

Figure 6. Cost per relapse avoided variation over 4.5 years of early treatment vs. delayed treatment

4. Fingolimod real world effectiveness

4.1. Introduction

Nowadays a new MS drug usually has at least one phase II and two Phase III Clinical trials in order to get the required Regulatory Authorities' approvals. But, as it is commonly accepted, Randomized Clinical Trials (RCTs) have a very homogeneous population without any big issues in terms of comorbidities and co-medication. This is necessary in order to meet the trial's endpoints, in the shortest timeframe and with the smaller sample size possible. However, these perfect patients are rare in the hospitals and more often than not, MS outpatient clinics have many patients that although can be treated on-label by the drug, have very different characteristics from the trials' population. There are also factors inherent to treatments, which may influence its application in clinical practice. The outcomes of using the drug in a real world setting maybe very different from the ideal scenario that happens in clinical trials. (Table 6)

Table 6. Potential reasons for treatment results being different between clinical practice and the clinical trials[145]

Study design	Trial protocol forces uncommon clinical scenarios Comparison group does not represent current standard of care
Patient selection	Biases in the patients who are eligible for a therapy Biases in patients who are ultimately treated with the therapy
Therapeutic implementation	Complexity of therapies makes them challenging to implement Procedural experience of providers influences outcomes
Environment of the healthcare delivery system	Limited availability of providers or resources Inadequate levels of reimbursement

That's the reason why it is of utmost importance to share the experiences, present abstracts, posters or communications in congresses and publish clinical practice results. Furthermore, important drug outcomes, like resource utilization, treatment satisfaction, quality of life or other Patient Reported Outcomes (PROs) aren't usually included in the

regulatory trials' outcomes. Safety issues are also a good reason to share experiences, because the causal association between very rare safety events and a drug can only be made if everyone makes an effort to report to the Market Authorization Holder and/or Health Authorities. When these reports aren't made, if physicians publish and present posters the information will be captured when the pharmacovigilance departments do the scientific literature review.

Efficacy is the treatment result the drug can achieve in clinical trials and effectiveness is the result obtained in clinical practice. Efficiency is a third concept, which has gain a lot of importance especially in these last years, and it brings the economic factor to the equation, where the effectiveness is put in perspective with the economic aspects of using the drug in the real setting.

Real World Evidence, as defined by ISPOR, is data used for clinical, coverage and payment decision-making that are not collected in conventional randomized controlled trials.[146] This kind of data is gaining importance for coverage and payment decisions and its need has been rising in last years.[146] Several methods of capture this data can be used:

- Observational studies
    - Prospective
    - Retrospective
    - Cross-sectional
- Patient registries
- Surveys
- Electronic health records
- Administrative claims data

Although randomized clinical trials are still considered the gold standard, RWE can answer questions, which clinical trials haven't done it. Many physicians don't consider RWE studies as a reliable source of information to serve as basis for therapeutic decisions, but some reports suggest that observational studies have a good reproducibility and its results have a strong correlation with the results obtained in randomized clinical trials.[147–149] Although there are also reports pointing some differences between RCTs and observational trials, these differences can be explained in some cases by confounding factors that were not

included in the analysis. [150] Observational studies have been getting increasing importance and recognition in comparative effectiveness research.[151]

Observational studies are much closer to clinical practice than RCTs and are less expensive to run. These studies can be prospective, if the data will be collected as it occurs, by following up the patients; retrospective if the data collected is already registered, in clinical charts for instance; or cross-sectional if it is a one-time assessment which collects data specific for that moment, much like a photography captures an image in that specific moment.

Registries can be as wide as population based registries, like the ones that exist in the Nordic countries,[152] or disease specific as MSBase[153] or drug specific as the adalimumab registry.[154] The population based registries have millions of subjects and it's easy to find controls, making the external validity very high, but since they are not drug or disease specific important information may not be captured. Treatment and drug registries can be a good reliable source of information not only for assessing the effectiveness and safety of a drug, but also to do comparative effectiveness analysis, frequently required by health technology assessment groups. Registries don't have a finishing date, like observational trials, which can be useful to access drug effects with a considerable delay between exposure and outcome, as it is usually the case with malignancies.

Observational studies and registries both can have bias issues, as indication bias, information bias and confounding bias. These situations occur when there is a preference for enrolling patients with certain characteristics in one of the treatments, there is information easier to detect in one group than in the other, or exists a confounding factor that it is both associated with the exposure and outcome. If the bias is well identified, several methods can be used to overcome this issue, both in the study design, like new user design[155] or excluding patients with contraindications for any of the studied drugs,[156,157] and in the data analysis, like multivariable adjustment through regression models[158,159] or propensity analysis.[160] If there are unidentified or unmeasured biases only instrumental variable methods can diminish its effects,[161,162] even though some author argue that these are equivalent, or even inferior to standard multivariate adjustment methods.[163,164] Study design and the quality of data used are, like in RCTs, crucial factors to study validity.

Surveys and interviews may also be used to gather subjective patients and physicians opinions. Poor recall by subjects, low response rates or intentional misinformation, due to the topic sensitivity for instance, are the main limitations of these studies. Additionally, interviews also have limitations because they are expensive, do not provide anonymity and bias can be unintentionally introduced by the interviewer.

The use of Electronic health records and Administrative claims data warrants generalizability because of the huge number of subjects, it's usually also associated with reduced research costs and faster results. However, these databases have a different objective and thus must be used with caution. When doing studies using this data, one must be aware that some limitations may be present as lack of data accuracy, measurement error, diagnostic error or miscoding, interventions miscoding, undetected cointerventions, missing vitals, biometrics and lab values, etc. These databases are particularly useful to research drug adherence and persistence, resource utilization and outcomes in rare diseases. In order to do comparative studies, statistical methods can be used to match the different treatment arms.[165]

Fingolimod is approved in the United States of America since 2010 and in Europe since 2011. It has been used in more than 104,700 patients worldwide and it has an exposure of more than 172,500 patient-years.[51] Since 2011 we have assisted to a growing number of RWE studies presented at congresses and published in peer reviewed journals. I think it is important now to take a moment to reflect how the drug has been used in clinical practice and what the efficacy outcomes are.

4.2. Methods

A literature search was done to identify fingolimod congress abstracts (2013 and 2014) posters and manuscripts (since January 1st 2011) about its use in the clinical practice. The databases used were Medline, Embase and Biosis previews. Case reports and studies with small samples (less than 8 patients) were excluded as well as publications with data from blinded clinical trials, because they were not considered real world data.

To avoid considering duplicate data an effort has been made to only include in the analysis the latest report of each series of patients excluding older abstracts, manuscripts with the same pool of patients and outcome measures. Whenever there was available an abstract and a published paper the information was extracted from the later. Reviews and manuscripts or abstracts reporting subgroup analysis with the same outcomes of the original study were also excluded.

For the safety data analysis, in order to avoid overestimating adverse events incidence due to small samples, it was used a cut-off of 500 patients in the cohort. In order to have a broader perception of fingolimod safety, it was decided to include in the analysis the five-year interim results of the LONGTERMS extension study. The search was limited to references published after January 1st 2013.

After selecting the references to include the review, the data was collected using Microsoft Excel 2010, version 14.0.7128.5000 (32-bit). In this spreadsheet it was predefined to use these columns to fill as needed per study:

- Status: Processed, Duplicate, Excluded, Accepted
- File: File number with the PDF
- Cohort: Identification of the cohort
- Year
- Focus: Efficacy, FDO, FTY vs DMTs, FTY vs DMFs, FTY vs NTZ, Neurologist Reported Outcomes (NRO), NTZ switch, Large Open trials, Other, Patient Reported Outcomes (PRO), Registry and Safety
- Number of patients (FTY patients; other therapy patients)
- Baseline demographics
- Time since diagnosis of MS (years)
- Time since onset of symptoms of MS (years)

- Percentage of patients with natalizumab (NTZ) as previous treatment (PNTZ)
- Washout period from NTZ (weeks)
- Number of NTZ infusions: whenever this number was not reported, it was assumed that the number of infusions was equal to the number of months in treatment
- Baseline ARR: previous 12 months where possible, but other measures also have been registered
- Baseline MRI activity
- Baseline EDSS
- Source of data: Registry, Claims database, Observational prospective, Observational presumably prospective, Observational retrospective, Observational presumably retrospective, Cross-sectional and NA (impossible to classify)
- Mean Follow-up (months)
- Persistence [Adherence]
- ARR
- Relapses
- Percentage of relapse-free patients
- MRI
- MRI activity free
- EDDS difference
- EDSS unchanged or improved
- Percentage of patients free of relapse and EDSS progression
- Percentage of patients free of relapse and T1Gd+
- Percentage of patients free of relapse and MRI activity
- Percentage of patients free of EDDS progression and MRI activity
- Percentage of patients free of relapse, EDDS progression and MRI activity
- PRO/Other
- NRO
- First Dose Observation (FDO)
- Comments

In comparative studies (FTY vs DMTs, FTY vs DMF, FTY vs NTZ) and whenever there was available subgroup data, the values for each cohort or subgroup were also registered in the same cell.

Whenever more than one publication was accepted for the same cohort, because they were reporting different outcome measures, the data was merged in the same row.

4.3. Results

4.3.1. Publications

4.3.1.1. Source selection

The references were identified using several databases. The data was extracted from abstracts, manuscripts and posters. The study references, abstracts, posters and manuscripts, were scrutinized to achieve the final pool of references which are included in this review. From the 244 references identified 148 were rejected and classified as: (Figure 7)

- **Duplicates** if it had the same pool of patients, with similar outcomes. The most common situation was abstracts or poster presented in different congresses where it was possible to observe the growing number of patients in the cohort;
- **Study design** if it was a poster or abstract reporting the open study design and\or baseline patient demographics;
- **Letter to the editor** if it did not report anything new. One Letter to the editor was used to complement the information gathered from one cohort, for which the original posters\abstracts missed some details;
- **Clinical trials** if it was the result reporting of blinded clinical trials;
- **<8 patients** if the cohort was small. The cut-off of 8 patient does not have any specific rational;
- **Review** if it was a review of published results;
- **Subgroup analysis** if it was the report of subgroup analysis with the same outcome measures as the original work;
- **Without relevant** data if it did not have relevant data for the objective of this review

For the safety analysis the data was extracted from 9 references representing 7 cohorts.

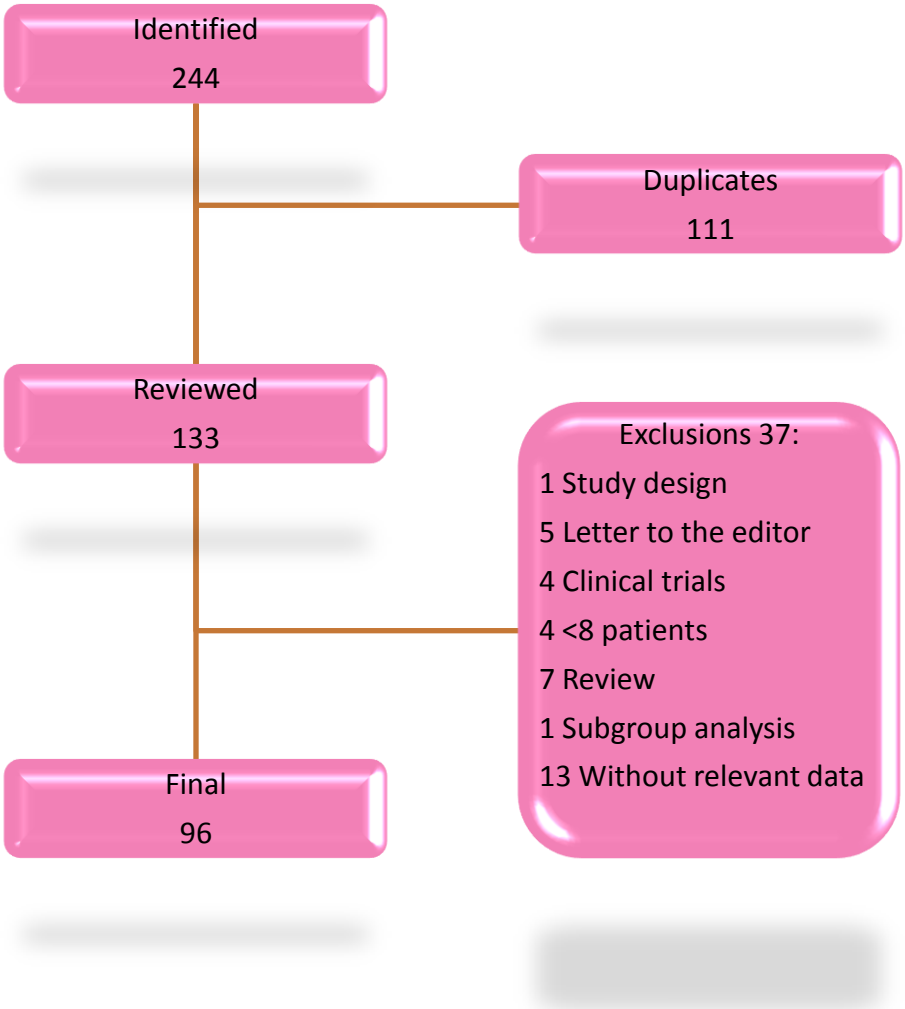

Figure 7. Selection of studies for inclusion in the overall review. A total of 96 reports were included for the overall review and 7 studies for the safety review

4.3.1.2. Patients

During this work it became clear that physicians' publication habits were very different from country to country and even from region to region. 72 different cohorts with published results were identified. Unfortunately is not possible to completely identify in an individual manner many cohorts, because there are many publications where the cohort is defined by a registry, a network of physicians\clinics, an administrative region, or a few MS centres who merge together their data in order to obtain a more robust database. There are published results from 23 different countries, and some cohorts are considered Multinational whenever more than 2 countries are involved. (Figure 8)

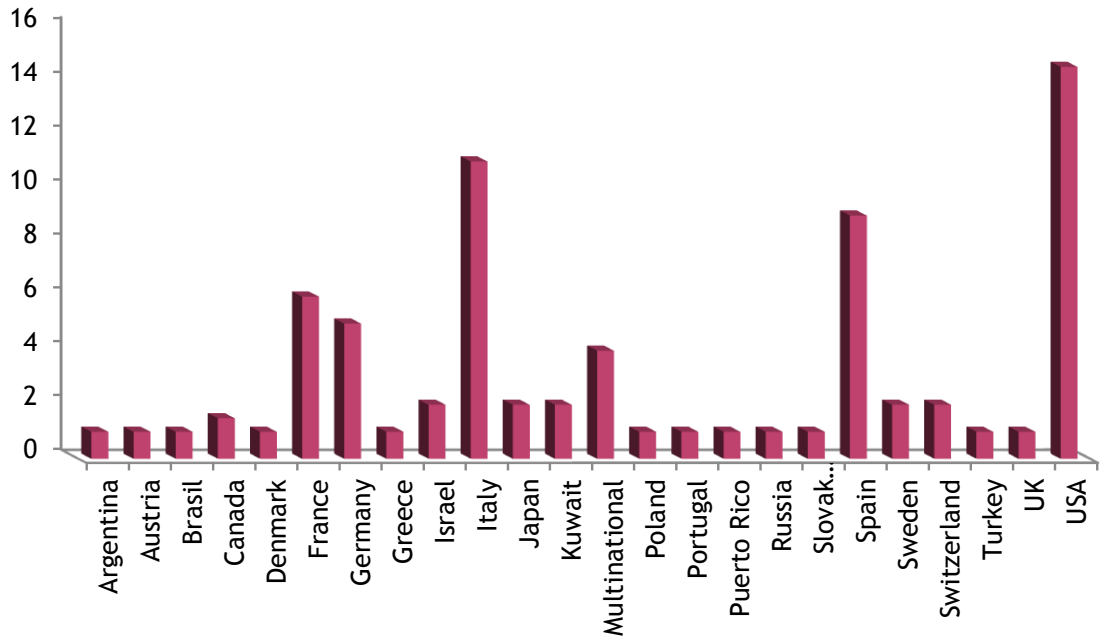

Figure 8. Cohorts studied per nationality

The number of patients per cohort varies wildly, from 8 to 3,641, with a total of 31,199 patients studied. Each study has an average of 330 patients. Unfortunately, it isn't possible from the data collected to estimate accurately how many unique patients this represents, because there is some overlap of different studies, using the same patient pool with different inclusion and exclusion criteria, or aggregating data from three different centres and in a different study using two of these centres aggregated with another different centre, etc.

In a very conservative approach, the minimum unique patients involved in these studies should be 14,582. This was estimated considering for each cohort the patient number was equal to the number of patients reported in its biggest study and only 50% of patients in multicentre studies were not studied in another study. The calculation of patients for Italy, as an example, is on Table 7. The EPOC study, the only one with less than three countries, and not included in the multinationals, reported a maximum number of 976 patients in 152 USA and 6 Canada centres. So it was considered that USA enrolled 96.2% of patients and Canada enrolled the other 3.8%.

The maximum number of unique patients is 25,916, which was calculated by summing the maximum unique patients in each cohort.

Table 7. Patient calculation example for Italy

Cohort	Patients	Maximum	Conservative
Ferrara, Parma Modena, Reggio-Emilia, Fidenza and Piacenza	127	127	127
Gallarate	32		0
Gallarate	55	55	55
iMedWeb registry	106	106	53
L' Aquila, Pozzilli, Foggia	142	142	142
L' Aquila, Pozzilli, Foggia	100		0
Milan	61	61	61
Milan	37		0
Multicenter Italy	906	906	453
Naples	112	112	112
Orbassano	35	35	35
Padua	21	21	21
Rome	55	55	55
Turin	35	35	35
Total	**1824**	**1655**	**1149**

Germany, USA and Canada are the countries which enrolled more unique patients in these studies, followed by Italy, Japan and Sweden. (Figure 9)

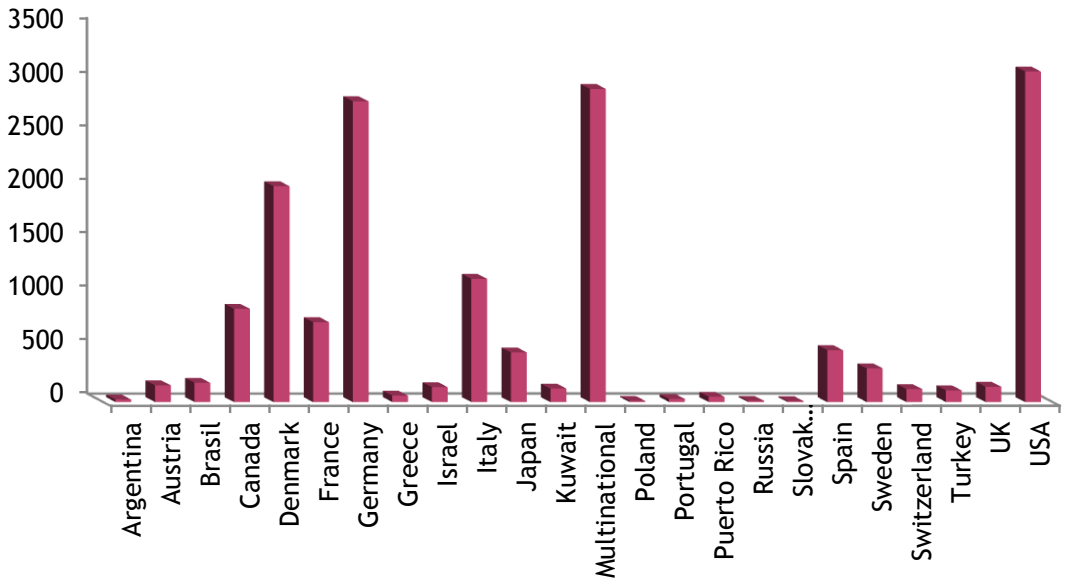

Figure 9. Conservative number of patients enrolled in studies per country

4.3.1.3. Study design

The studies included in this review have different designs and were classified according to the source of data. It is not rare to forget to publish the complete information about the study design and I have found 11% studies impossible to classify and another 9% studies that the reported methodology suggests that is a retrospective or a prospective study, but it is not explicit. Even considering this uncertainty prospective studies dominate, closely followed by retrospective studies. Even if the impossible to classify are all prospective studies, it would not be enough to reach a 50% share of studies. Registries and claim databases represent 13% of the studies. (Figure 10)

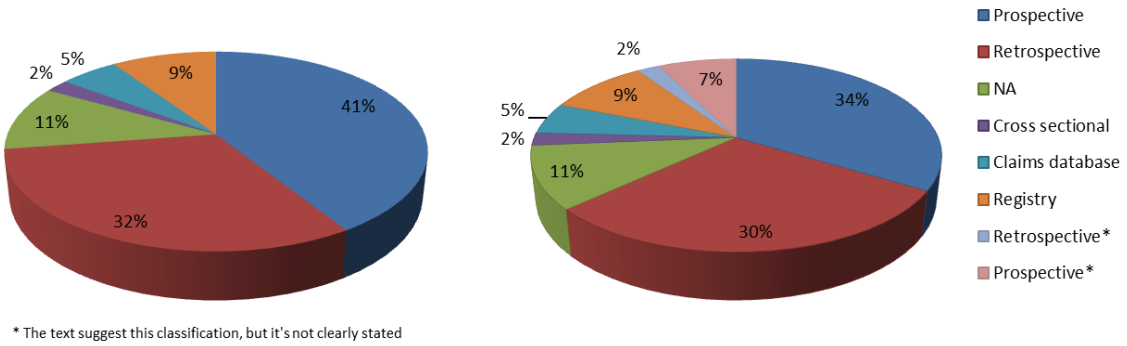

Figure 10. Study classification according to source of data

It is also interesting to notice that some study groups do a better utilization of the cohort data and publish results focusing in different subjects, thus from 72 cohorts there is a total of 95 different studies, resulting in 1.32 published studies per cohort. (Figure 11)

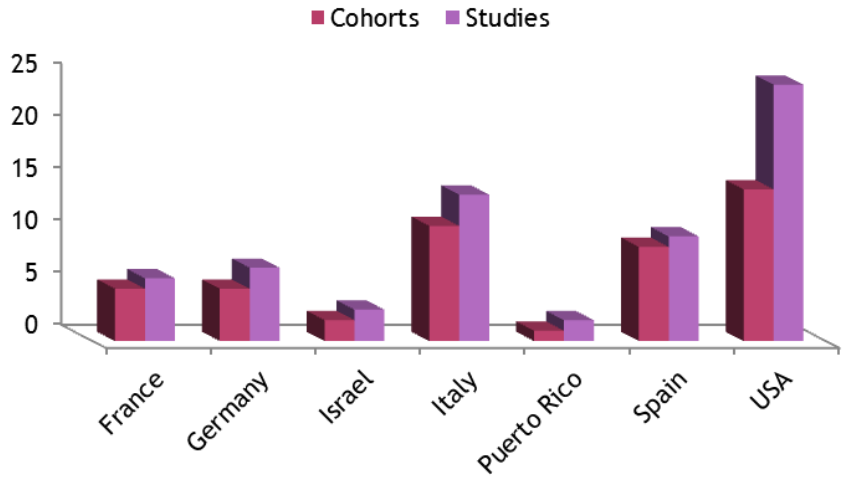

Figure 11. Studies in countries with more than one study published by cohort

As it was expected *Natalizumab switch, First Dose Observation* and *Efficacy* are the three favourite themes, but *Patient Reported Outcome* and *First Dose Observation* have the biggest number of patients. (Figure 12) The category *Other* was composed of studies focusing on:

- Effect of FTY on macular volume and retinal nerve fibre layer thickness
- Use of blood lymphocyte count as a biomarker of FTY efficacy
- FTY influence in changes in frequency and intensity of pre-existing headaches
- Impact of FTY on comorbid migraine
- Brain volume changes
- Cognition

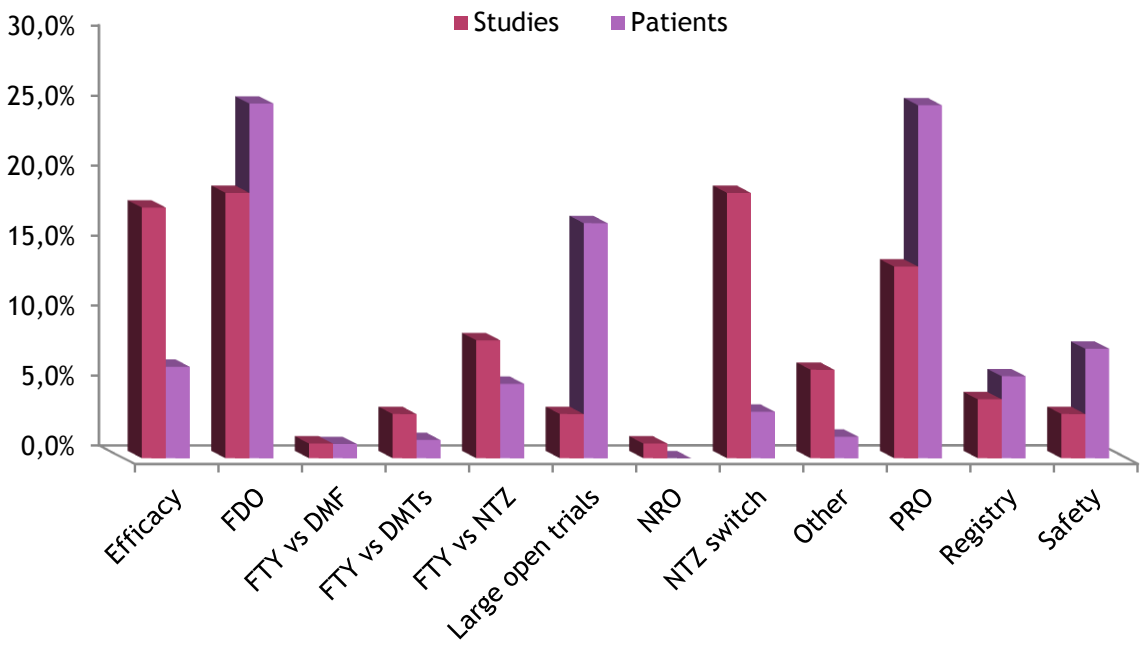

Figure 12. Cohorts and patients stratified by study main focus

4.3.1.4. Type of publications

Being fingolimod a recently approved treatment, the usual approach of doing a literature review considering only published papers would result in a small source of data, consciously I decided to do an unorthodox work, including abstracts, posters and manuscripts. This resulted in 96 publications to review, dated since 2012. (Figure 13)

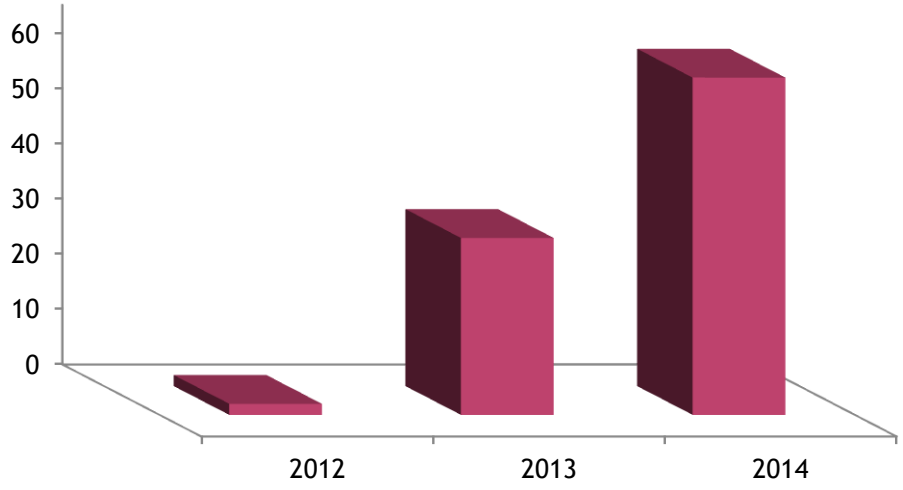

Figure 13. Growing number of publications per year

The big proportion of abstracts is due to the correspondent posters being unavailable online. There were only two exceptions, one abstract from a Russian manuscript[166] and from a Polish manuscript[167], which only the abstract was available in English and one abstract from a French thesis[168], which I did not succeed in obtaining a copy of the full thesis. As far as I am aware I searched everything that was available on the databases in Portuguese, Spanish, English and French languages. (Figure 14)

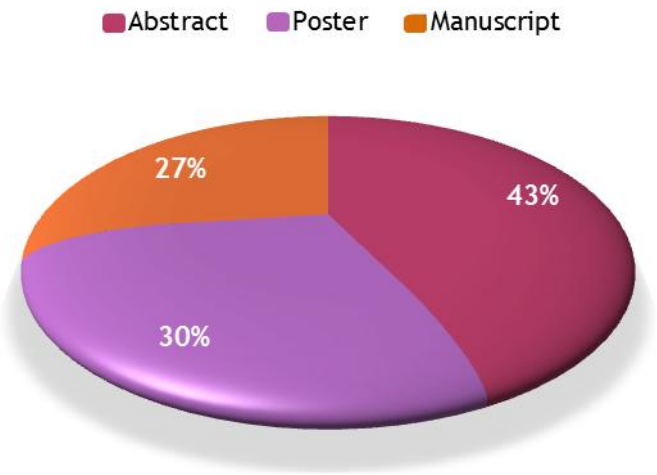

Figure 14. Publications reviewed

Only countries with a substantial amount of published work have published papers in peer reviewed journals, being Kuwait, Poland and Russia the only exceptions. (Figure 15)

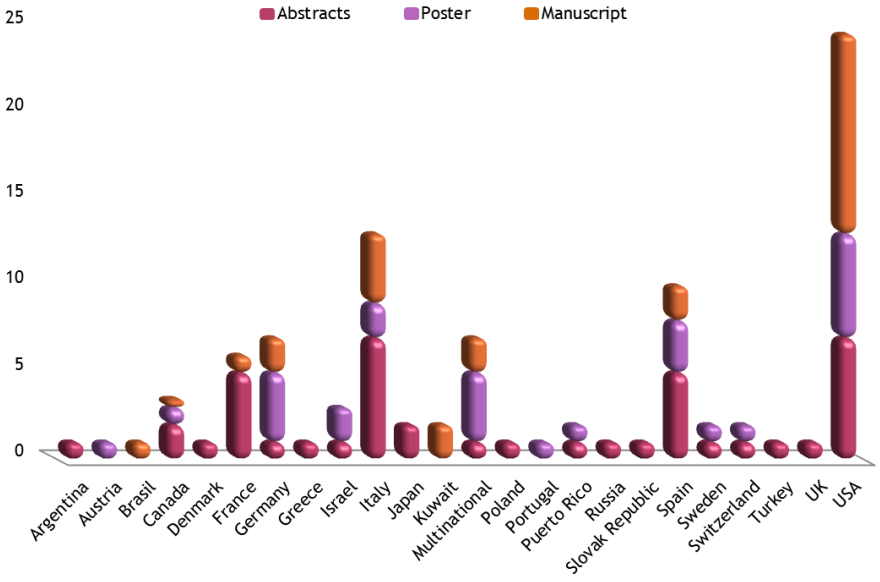

Figure 15. Publications by country

4.3.2. Baseline characteristics

Baseline characteristics are summarized in Table 8

Table 8. Baseline characteristics

Cohort	Country	Focus	Patients	Time since diagnosis of MS (years)	Patients with NTZ as previous treatment	Washout period from NTZ (weeks)	NTZ infusions	Baseline ARR (previous 12 months)	Baseline MRI activity	Baseline EDSS	Source of data	FU (months)
REAL[169]	Argentina	Registry	48	9.1						2.7	Reg	8.5
Multiple Sclerosis Treatment Registry[170]	Austria	FTY vs NTZ	312 (NTZ 1208)	8.9 (NTZ 7.8)	54.2%			1.8 (NTZ 2.3)		2.6 (NTZ 3.2)	Reg	21.5 (NTZ 35.7)
São Paulo[171]	Brazil	FDO	180								R	
Gilenya* Go Program(TM)[172]	Canada	PRO	1700								P	
Multiple Sclerosis Registry Denmark[173]	Denmark	FDO	496	10.6	32.3%			0.37 (PNTZ patients 0.46)			R	> 3
Alsace[174]	France	NTZ switch	59		100%	6.8					R	7
Alsace[175]	France	Efficacy	290							3.6	R	12
Bordeaux[176]	France	FDO	24								P	3
ENIGM[177]	France	NTZ switch	333		100%	17 (55% patients without MP or other DMTs)	31			3.7 (pre-NTZ 3.6; post-NTZ 3.6)	P	6
GRACE[178]	France	PRO	198	8.1				1.6		2.6	P	
Reims[168]	France	Efficacy	66								P	11 (51.5% >12 months)
Dresden[179]	Germany	FDO	36	4							P*	
Munich, Bochum, Marburg[180]	Germany	NTZ switch	33		100%		31.8	0.2 (pre-NTZ 2.5)		3.2 (pre-NTZ 3.1)	R	20.3
NeuroTransConcept[181]	Germany	FTY vs NTZ	190 (NTZ 239)	9.89 (NTZ 9.15)				0.34 (NTZ 0.42)		2.3 (NTZ 3.3)	R	12
PANGAEA[182–184]	Germany	Large open trials	3641 (PEARL active 457; PEARL inactive 1248)	8.2 (PEARL active 6.7; PEARL inactive 7.3)	18.4%	31.9% Patients with WO <12		1.5 (PNTZ 0.9; PEARL active 1.23; PEARL inactive 0.37)		3 (PEARL active 2.60; PEARL inactive 2.20)	P	<24
START[185]	Germany	FDO	1640							2.8	P	
Athens[186]	Greece	NTZ switch	60		33.3%						NA	>12 (MP 8-12)
Ramat-Gann[187]	Israel	Efficacy	110	14.6						3.8	R	20
Tel-Aviv[188,189]	Israel	Other	30					1.2		0.4 increase in previous 12 months	P	12

Cohort	Country	Focus	Patients	Time since diagnosis of MS (years)	Patients with NTZ as previous treatment	Washout period from NTZ (weeks)	NTZ infusions	Baseline ARR (previous 12 months)	Baseline MRI activity	Baseline EDSS	Source of data	FU (months)
Ferrara, Parma Modena, Reggio-Emilia, Fidenza and Piacenza[190]	Italy	Efficacy	127	10.7	30.7%	18		1.2	65,4% (31.5% T1Gd+)	3.1 (PNTZ patients 3.6)	P*	10
Gallarate[191]	Italy	Other	55	12.5	21.8%			1.7 in previous 24 months			NA	12
Gallarate[192]	Italy	NTZ switch	32		100%	15.6	21				NA	73.3% patients with >6 months
iMedWeb registry[193]	Italy	NTZ switch	106 (DMTs 106)	13.15 (DMTs 12.83)	100%		26.41 (DMTs 19.42)			3.79 (DMT 3.95)	R	
L' Aquila, Pozzilli, Foggia[194]	Italy	Efficacy	142		19.7%						NA	15
L' Aquila, Pozzilli, Foggia[195]	Italy	FTY vs NTZ	100 (NTZ 100)					1.2 (NTZ 1.9)	78% of patients (NTZ 90%)	2.6 (NTZ 2.9)	P	12
Milan[196]	Italy	NTZ switch	61		100%	<52		1.2 (0.15 on NTZ; 1.2 WO period)		EDSS increase 0.43 (0.15 on NTZ; 0.43 WO period)	R*	>6
Milan[197]	Italy	FTY vs NTZ	37 (NTZ 160)	11.5 (NTZ 10.1)				1.2 (NTZ 1.3)		2.1 (NTZ 2.5)	P	>12
Multicenter Italy[198]	Italy	FDO	906	8.56	24.3%			1.36		3.06	P	3.14
Naples[199]	Italy	FDO	112								P*	
Orbassano[200]	Italy	NTZ switch	35 (T 19; NT 24)			>12					R	
Padua[201]	Italy	NTZ switch	21	10.7	100%	12				2.9	P*	
Rome[202]	Italy	FDO	55							1.7	P	
Turin[203]	Italy	Other	35	11.3							P	6
Multicenter Japan[204]	Japan	Registry	837	9.3				0.98			Reg	
Tokyo[205]	Japan	Efficacy	44	13.1	0%						R	
Kuwait city[206]	Kuwait	Efficacy	76	7.82	2.6%			13.2% relapse free	22.4% MRI activity free	2.93	R	18.5
MS registry Kuwait[207]	Kuwait	Efficacy	175	7.2	5.7%			32.6% relapse free	23.3% MRI activity free	2.6	Reg	21.7
FIRST[208]	Multinational	FDO	2417		10.5%			1.1		2.4	P	4
LONGTERMS[209,210]	Multinational	Safety									P	

Cohort	Country	Focus	Patients	Time since diagnosis of MS (years)	Patients with NTZ as previous treatment	Washout period from NTZ (weeks)	NTZ infusions	Baseline ARR (previous 12 months)	Baseline MRI activity	Baseline EDSS	Source of data	FU (months)
MSBase Registry[211]	Multinational	NTZ switch	89	12.8	100%	95% with WO < 4 months; 2.6 months		0.26		4	R	7.6
MSBase Registry[212]	Multinational	FTY vs NTZ	171 (NTZ 407)	9.5 (NTZ 9.4)				1.29 (NTZ 1.53)		3 (NTZ 3.5)	P	12
MSBase Registry[213]	Multinational	FTY vs DMTs	148 (DMTs 379)	7.2 (DMTs 7.3)				1.3 (DMT 1.2)		2.5 (DMT 2.5)	P	11
PASSAGE[214]	Multinational	Large open trials	1596	8.56	19.6%			1.0		2.72	P	9
Warsaw[167]	Poland	Efficacy	11	>3							NA	12
Coimbra[215]	Portugal	NTZ switch	29	10.4	100%	9.2	28.7	0.5		2.7	R	17.4
Guaynabo[216]	Puerto Rico	Efficacy	50								R	12
Guaynabo[217]	Puerto Rico	FDO	25								R	6
Moscow[166]	Russia	Efficacy	11								NA	
Prešov[218]	Slovak Republic	NTZ switch	9		100%	3.3	24.1	0 (pre-NTZ >2)			NA	
Alicante[219]	Spain	NTZ switch	8	11.1	100%	12	29.4	pre-NTZ 1.6; pre-FTY 0.13;		2.4	P*	9.35
Granada[220]	Spain	FDO	42	9							R	
Hospital Ntra Sra Valme, Seville[221]	Spain	NTZ switch	49		100%		>12	59.2% free of relapses			NA	
Hospital Universitario Virgen Macarena, Seville[222]	Spain	Efficacy	47		36.5%			0.88 previous 24 months		3.89	R	16.3
Multicenter1 Spain[223]	Spain	FDO	148	9.1							Reg	6.1
Multicenter2 Spain[224]	Spain	Registry	267	9.3	27%						Reg	13.1
Murcia[225]	Spain	NTZ switch	12	9.1	100%	12 (with MP)	31	0.3		3.2	NA	15.4
Murcia[226]	Spain	FDO	18	7.5							P	
Santiago de Compostela[227]	Spain	Efficacy	101	9.54	30%			2.04			R	
Valencia[228]	Spain	NTZ switch	11	6.8	100%	8	37.9	pre-NTZ 1.5		3.5	P	11.7
MS registry Sweden[229]	Sweden	FTY vs NTZ	628 (NTZ 447; NTZ-naïve 306)	7.3 (NTZ 4.5; NTZ-naïve 6.4)	48.1%					2.63 (NTZ 2.35; NTZ-naïve 2.48)	Reg	>12
Multicenter Sweden[230]	Sweden	Registry	674 (186 with 12month FU)		33%						Reg	12

Cohort	Country	Focus	Patients	Time since diagnosis of MS (years)	Patients with NTZ as previous treatment	Washout period from NTZ (weeks)	NTZ infusions	Baseline ARR (previous 12 months)	Baseline MRI activity	Baseline EDSS	Source of data	FU (months)
Basel[231]	Switzerland	NTZ switch	15	11.8	100%	8	41.4	0.4	100% free of T1Gd+	3.5	P*	8
SWISSASCENT[232]	Switzerland	PRO	212	6.6	28%			0.8		2.1	P	8.3
Istanbul[233]	Turkey	Efficacy	109	9.0				1.9		3.5	P	5.5
Wimbledon[234]	UK	FDO	143								P*	
Boston[235,236]	USA	Efficacy	177	11	15.3%			0.5		1.9	R	>12
Boston[237]	USA	FDO	305								R	
Cleveland Clinic[238]	USA	FDO	317	12.1	11.6%						R	3.3
Cleveland Clinic[239]	USA	Efficacy	306								R*	11.1
Cleveland Clinic[240]	USA	Efficacy	196	11.5							R	8.6
Cleveland Clinic[241]	USA	Other	232	10.5							P	6.4
Cleveland Clinic[242]	USA	FTY vs DMF	317 (DMF 426)	14.1 (DMF 12.3)	12.4% (DMF 11.7%)						R	3
Commercial health plan or Part D program[243]	USA	PRO									R	
Cullman[244]	USA	NTZ switch	75		100%						NA	>6
Houston[245]	USA	FDO	59								R	
Medco Health Solutions[246]	USA	PRO	248								R	12
Multicenter1 USA[247]	USA	PRO	205								P	6
Multicenter2 USA[248]	USA	PRO	380	9.8	8.4%						CS	
Multicenter3 USA[249]	USA	NRO	102 Neurologists								CS	
NARCOMS[250]	USA	PRO	50 (NTZ 50)					84% relapse free in previous 6 month (FTY and NTZ)			Reg	54 (NTZ 48)
Partners MS Center[251]	USA	FTY vs NTZ	36 (NTZ 69)	7.9 (8.4; p= 0.71)				0.92(NTZ 0.8; p= 0.38)	1.56 T1Gd+ in previous 12 months (1.39; p= 0.78)	1.75 (NTZ 1.5; p= 0.42)	R	18

Cohort	Country	Focus	Patients	Time since diagnosis of MS (years)	Patients with NTZ as previous treatment	Washout period from NTZ (weeks)	NTZ infusions	Baseline ARR (previous 12 months)	Baseline MRI activity	Baseline EDSS	Source of data	FU (months)
PharMetrics Plus™[252]	USA	PRO	889 (GA 1233; IFN 1341; NTZ 287)					58.6% relapse free (GA 68.0%; IFN 68.2%; NTZ 54.7%); ARR 0.67 (GA 0.39; IFN 0.39; NTZ 0.74)			Claims	12
PharMetrics Plus™[253]	USA	FTY vs NTZ	185 (NTZ 185)					1.56 (NTZ 1.52)			Claims	12
PharMetrics Plus™[254]	USA	FTY vs DMTs	132 (GA 132)					0.46; 66.7% relapse-free (GA 0.49; 66.7%)			Claims	12
PharMetrics Plus™[255]	USA	FTY vs DMTs	128 (DMT 397)					41.4% with >1 relapse (DMTs 15.1%; p< 0.0001); ARR 1.7 (DMTs 1.26)			Claims	18
Seattle[256]	USA	Other	126								R	7
US LRx™ and PharMetrics Plus™[257]	USA	PRO	1553 (DMF 9363)								Claims	>6
EPOC[258]	USA, Canada	FDO	976	12.1				0.8		2.4	P	6
EPOC[259]	USA, Canada	PRO	847								P	6
EPOC[260]	USA, Canada	PRO	790 (DMTs 263)	12.1 (DMT 11.7)	0.1% (DMTs 0%)			0.8 (DMT 0.8)		2.4 (DMT 2.4)	P	6
EPOC[261]	USA, Canada	PRO	790 (DMTs 263)	12.1 (DMTs 11.7)				0.8 (DMT 8.8)		2.4 (DMTs 2.4)	P	6
EPOC[262]	USA, Canada	Safety	783 (DMTs 245)								P	6

CS - Cross-Sectional; DMTs - Disease Modifying Treatments; EDSS - Expanded Disability Status Scale; FDO - First Dose Observation; FTY – Fingolimod; FU – Follow-up; MP – Methylprednisolone; MRI - Magnetic Resonance Imaging; MS – Multiple Sclerosis; NA - Non-available/unidentifiable; NT – Non treated; NTZ – Natalizumab; P – Prospective; P* - Presumably Prospective; PRO - Patient Reported Outcomes; R – Retrospective; R* - Presumably Retrospective; Reg – Registry; T – Treated; T1Gd+ - T1 Gadolinium-Enhancing Lesions; WO - Washout

4.3.3. Relapse outcomes

Relapse outcomes are summarized in Table 9.

Table 9. Relapse outcomes

Cohort	Focus	Patients	Time since diagnosis of MS (years)	Patients with NTZ as previous treatment	Washout period from NTZ (weeks)	Baseline ARR (previous 12 months)	FU (months)	Persistence [Adherence]	ARR	Relapses	Relapse-free
REAL	Registry	48	9.1				8.5				97.9%
Multiple Sclerosis Treatment Registry	FTY vs NTZ	312 (NTZ 1208)	8.9 (NTZ 7.8)	54.2%		1.8 (NTZ 2.3)	21.5 (NTZ 35.7)	59% (NTZ 60%)	Patients with >12 month FU 0.4 (NTZ 0.35)	77.8% ARR reduction	
Alsace	NTZ switch	59		100%	6.8		7	94.9%			81.4% (WO > 12w 50%; WO< 12w 93%, p= 0.02))
Alsace	Efficacy	290					12	89.7%	0.17	4.1 months to 1st relapse; 70.5% ARR reduction	82.4%
Bordeaux	FDO	24					3	91.7%			82.6%
ENIGM	NTZ switch	333		100%	17 (55% patients without MP or other DMTs)		6	97%			80%
Reims	Efficacy	66					11 (51.5% >12 months)			59.1% reduction vs baseline	68.3%
Munich, Bochum, Marburg	NTZ switch	33		100%		0.2 (pre-NTZ 2.5)	20.3		0.7 (if EDSS<3 0.24; if EDSS> 3 1.35)		52% (if EDSS<3 75%; if EDSS> 3 18%)
NeuroTransConcept	FTY vs NTZ	190 (NTZ 239)	9.89 (NTZ 9.15)			0.34 (NTZ 0.42)	12		0.1 (NTZ 0.06)	70.6% ARR Reduction	75.8% (NTZ 71.3%)
PANGAEA	Large open trials	3641 (PEARL active 457; PEARL inactive 1248)	8.2 (PEARL active 6.7; PEARL inactive 7.3)	18.4%	31.9% Patients with WO <12	1.5 (PNTZ 0.9; PEARL active 1.23; PEARL inactive 0.37)	<24	83.4% (PEARL active 63.2%; PEARL inactive 85.9%); [Adherence 0.5 days without MS medication within 2 weeks (PEARL active 1.6)]	1st year 0.47; 2nd year 0.34 (PNTZ 1st year 0.7; 2nd year 0.34) (PEARL active 1st year 1.2; 2nd year 0.94)	68.7% ARR reduction	64.08%

Cohort	Focus	Patients	Time since diagnosis of MS (years)	Patients with NTZ as previous treatment	Washout period from NTZ (weeks)	Baseline ARR (previous 12 months)	FU (months)	Persistence [Adherence]	ARR	Relapses	Relapse-free
Athens	NTZ switch	60		33.3%			>12 (MP 8-12)				93.3% (MPL 88.9%)
Ramat-Gann	Efficacy	110	14.6				20	76.4% at 7.8 months of treatment	0.35		
Ferrara, Parma Modena, Reggio-Emilia, Fidenza and Piacenza	Efficacy	127	10.7	30.7%	18	1.2	10		0.28	Time to 1st relapse 148 days; 76.7% ARR reduction	69.3% (PNTZ patients 59%)
Gallarate	NTZ switch	32		100%	15.6		73.3% patients with >6 months	86.7%			28.1%
iMedWeb registry	NTZ switch	106 (DMTs 106)	13.15 (DMTs 12.83)	100%						4.72% IR (DMTs 8.44; p= 0.02)	
L' Aquila, Pozzilli, Foggia	Efficacy	142		19.7%			15				90.8% (PNTZ 82.1%)
L' Aquila, Pozzilli, Foggia	FTY vs NTZ	100 (NTZ 100)				1.2 (NTZ 1.9)	12				93% (NTZ 87%)
Milan	NTZ switch	61		100%	<52	1.2 (0.15 on NTZ; 1.2 WO period)	>6		0.5		
Milan	FTY vs NTZ	37 (NTZ 160)	11.5 (NTZ 10.1)			1.2 (NTZ 1.3)	>12	At 24 month 81% (NTZ 58%)	0.26 (NTZ 0.04, p< 0.001)	72.9% ARR reduction;	
Orbassano	NTZ switch	35 (T 19; NT 24)			>12						68.6% (OT 58.2%)
Padua	NTZ switch	21	10.7	100%	12						71.4%
Multicenter Japan	Registry	837	9.3			0.98			0.3	69.4% ARR reduction	
Tokyo	Efficacy	44	13.1	0%				75%			95,5%
Kuwait city	Efficacy	76	7.82	2.6%		13.2% relapse free	18.5	94.7%			77.6%
MS registry Kuwait	Efficacy	175	7.2	5.7%		32.6% relapse free	21.7	88.6%			86.3%

Cohort	Focus	Patients	Time since diagnosis of MS (years)	Patients with NTZ as previous treatment	Washout period from NTZ (weeks)	Baseline ARR (previous 12 months)	FU (months)	Persistence [Adherence]	ARR	Relapses	Relapse-free
MSBase Registry	NTZ switch	89	12.8	100%	95% with washout < 4 months; 2.6 months	0.26	7.6		0.38 (vs 0.26 BL, p= 0.002; vs pre-NTZ 1.54)		79.8%
MSBase Registry	FTY vs NTZ	171 (NTZ 407)	9.5 (NTZ 9.4)			1.29 (NTZ 1.53)	12		0.36 (NTZ 0.19)	72.1% ARR reduction (NTZ 87.6%)	
MSBase Registry	FTY vs DMTs	148 (DMTs 379)	7.2 (DMTs 7.3)			1.3 (DMT 1.2)	11	Persistence on FTY was better compared to DMT (hazard ratio 0.39)	0.3 (DMTs 0.45)	76.9% ARR reduction (DMTs 62.5%)	
Warsaw	Efficacy	11	>3				12	100%			100%
Coimbra	NTZ switch	29	10.4	100%	9.2	0.5	17.4	79.3%	0.7 (p= 0.4 vs BL)	69% with similar or improved ARR vs BL	55.2%
Moscow	Efficacy	11									82%
Prešov	NTZ switch	9		100%	3.3	0 (pre-NTZ >2)					44.4%
Alicante	NTZ switch	8	11.1	100%	12	pre-NTZ 1.88; pre-FTY 0.13;	9.35		0.8		37%
Hospital Ntra Sra Valme, Seville	NTZ switch	49		100%		59.2% free of relapses					75.5%
Hospital Universitario Virgen Macarena, Seville	Efficacy	47		36.5%		0.88 previous 24 months	16.3		0.25 (PNTZ patients 0.49)	71.6% ARR reduction (PNTZ 44.3%)	
Multicenter2 Spain	Registry	267	9.3	27%			13.1	96.6%			80%
Murcia	NTZ switch	12	9.1	100%	12 (with MP)	0.3	15.4				86.4%
Santiago de Compostela	Efficacy	101	9.54	30%		2.04		96%	2.14		
Valencia	NTZ switch	11	6.8	100%	8	pre-NTZ 1.5	11.7	90.9%	0.09	9.1% patients with 1 mild relapse	90.9%
Basel	NTZ switch	15	11.8	100%	8	0.4	8	86.7%			46%
SWISSASCENT	PRO	212	6.6	28%		0.8	8.3	96%	0.2	75% ARR Reduction	88%
Istanbul	Efficacy	109	9.0			1.9	5.5	94.5%			90%
Boston	Efficacy	177	11	15.3%		0.5	>12				83.6%
Cleveland Clinic	Efficacy	306					11.1	76.1%	0.12		64%
Cleveland Clinic	FTY vs DMF	317 (DMF 426)	14.1 (DMF 12.3)	12.4% (DMF 11.7%)			3	92% (DMF 82%)		0.03 relapses/patient (DMF 0.12)	97% (DMF 89%)

Cohort	Focus	Patients	Time since diagnosis of MS (years)	Patients with NTZ as previous treatment	Washout period from NTZ (weeks)	Baseline ARR (previous 12 months)	FU (months)	Persistence [Adherence]	ARR	Relapses	Relapse-free
Partners MS Center	FTY vs NTZ	36 (NTZ 69)	7.9 (8.4; p= 0.71)			0.92(NTZ 0.8; p= 0.38)	18	77.8% (NTZ 63.8%)		patients treated with NTZ had a longer time to relapse (2.20 p = 0.095) in the unadjusted analysis; patients treated with NTZ had a significantly lower hazard of a relapse in each model;	
PharMetrics Plus™	FTY vs NTZ	185 (NTZ 185)				1.56 (NTZ 1.52)	12	71.9% (NTZ 76.2%; p=0.3427)			68.1% (NTZ 68.6%; p= 0.9110)
PharMetrics Plus™	FTY vs DMTs	132 (GA 132)				0.46; 66.7% relapse-free (GA 0.49; 66.7%)	12	73.5% (GA 62.9%; p= 0.0643)	0.19 (GA 0.51)	59% reduction in the probability of having a relapse vs GA (p =0.0091); 360 days median time to 1st relapse (GA 274); Patients treated with FTY had 62% fewer relapses per year (p =0.0013)	87.1% (GA 75%; p= 0.012)
PharMetrics Plus™	FTY vs DMTs	128 (DMT 397)				41.4% with >1 relapse (DMTs 15.1%; p< 0.0001); ARR 1.7 (DMTs 1.26)	18	57.0% (DMTs 45.1%; p=0.0187)	0.32 (DMTs 0.64; p= 0.0006)	52% reduction in the probability of having a relapse in the post-index persistence period compared with IFN/GA (p=0.0097); 50% fewer relapses per year during the persistence period than those treated with IFN or GA (p=0.0006)	68.7%

ARR – Annualized Relapse Rate; BL – Baseline; DMTs - Disease Modifying Treatments; EDSS - Expanded Disability Status Scale; FTY – Fingolimod; FU – Follow-up; GA – Glatiramer Acetate; MP – Methylprednisolone; MRI - Magnetic Resonance Imaging; MS – Multiple Sclerosis; NT – Non treated; NTZ – Natalizumab; T – Treated; w – weeks;

4.3.4. Disability outcomes

Disability outcomes are summarized in Table 10.

Table 10. Disability outcomes

Cohort	Focus	Patients	Time since diagnosis of MS (years)	Patients with NTZ as previous treatment	Washout period from NTZ (weeks)	Baseline ARR (previous 12 months)	Baseline EDSS	FU (months)	Baseline EDSS	EDDS difference
Multiple Sclerosis Treatment Registry	FTY vs NTZ	312 (NTZ 1208)	8.9 (NTZ 7.8)	54.2%		1.8 (NTZ 2.3)	2.6 (NTZ 3.2)	21.5 (NTZ 35.7)	2.6 (NTZ 3.2)	
Alsace	Efficacy	290					3.6	12	3.6	0.1
Munich, Bochum, Marburg	NTZ switch	33		100%		0.2 (pre-NTZ 2.5)	3.2 (pre-NTZ 3.1)	20.3	3.2 (pre-NTZ 3.1)	0.1
NeuroTransConcept	FTY vs NTZ	190 (NTZ 239)	9.89 (NTZ 9.15)			0.34 (NTZ 0.42)	2.3 (NTZ 3.3)	12	2.3 (NTZ 3.3)	-0.14 (NTZ -0.13)
PANGAEA	Large open trials	3641 (PEARL active 457; PEARL inactive 1248)	8.2 (PEARL active 6.7; PEARL inactive 7.3)	18.4%	31.9% Patients with WO <12	1.5 (PNTZ 0.9; PEARL active 1.23; PEARL inactive 0.37)	3 (PEARL active 2.60; PEARL inactive 2.20)	<24	3 (PEARL active 2.60; PEARL inactive 2.20)	-0.07 (PEARL active 0.5)
Tel-Aviv	Other	30				1.2	0.4 increase in previous 12 months	12	0.4 increase in previous 12 months	0
Ferrara, Parma Modena, Reggio-Emilia, Fidenza and Piacenza	Efficacy	127	10.7	30.7%	18	1.2	3.1 (PNTZ patients 3.6)	10	3.1 (PNTZ patients 3.6)	0 (PNTZ patients 0.1, p= 0.14)
L' Aquila, Pozzilli, Foggia	Efficacy	142		19.7%				15		
L' Aquila, Pozzilli, Foggia	FTY vs NTZ	100 (NTZ 100)				1.2 (NTZ 1.9)	2.6 (NTZ 2.9)	12	2.6 (NTZ 2.9)	
Milan	FTY vs NTZ	37 (NTZ 160)	11.5 (NTZ 10.1)			1.2 (NTZ 1.3)	2.1 (NTZ 2.5)	>12	2.1 (NTZ 2.5)	0 (NTZ 0)
Orbassano	NTZ switch	35 (T 19; NT 24)			>12					0.2 (OT 0.5; p NS)
Kuwait city	Efficacy	76	7.82	2.6%		13.2% relapse free	2.93	18.5	2.93	-0.98 (p< 0.0001)
MS registry Kuwait	Efficacy	175	7.2	5.7%		32.6% relapse free	2.6	21.7	2.6	-0.34 (p= 0.03)
MSBase Registry	FTY vs NTZ	171 (NTZ 407)	9.5 (NTZ 9. 4)			1.29 (NTZ 1.53)	3 (NTZ 3.5)	12	3 (NTZ 3.5)	area under disability-time curve 0.04 (NTZ -0.12)
MSBase Registry	FTY vs DMTs	148 (DMTs 379)	7.2 (DMTs 7.3)			1.3 (DMT 1.2)	2.5 (DMT 2.5)	11	2.5 (DMTs 2.5)	no difference in the post-switch disability between the two groups
Warsaw	Efficacy	11	>3					12		
Coimbra	NTZ switch	29	10.4	100%	9.2	0.5	2.7	17.4	2.7	0.1 (p= 0.6 vs BL)
Alicante	NTZ switch	8	11.1	100%	12	pre-NTZ 1.6; pre-FTY 0.13;	2.4	9.35	2.4	1.0

Cohort	Focus	Patients	Time since diagnosis of MS (years)	Patients with NTZ as previous treatment	Washout period from NTZ (weeks)	Baseline ARR (previous 12 months)	Baseline EDSS	FU (months)	Baseline EDSS	EDDS difference
Hospital Ntra Sra Valme, Seville	NTZ switch	49		100%		59.2% free of relapses				
Hospital Universitario Virgen Macarena, Seville	Efficacy	47		36.5%		0.88 previous 24 months	3.89	16.3	3.89	0.04
Multicenter2 Spain	Registry	267	9.3	27%				13.1		
Murcia	NTZ switch	12	9.1	100%	12 (with MP)	0.3	3.2	15.4	3.2	0
Santiago de Compostela	Efficacy	101	9.54	30%		2.04				
Valencia	NTZ switch	11	6.8	100%	8	pre-NTZ 1.5	3.5	11.7	3.5	-0.3
Multicenter Sweden	Registry	674 (186 with 12month FU)		33%				12		0
Boston	Efficacy	177	11	15.3%		0.5	1.9	>12		

BL – Baseline; DMTs - Disease Modifying Treatments; EDSS - Expanded Disability Status Scale; FTY – Fingolimod; FU – Follow-up; GA – Glatiramer Acetate; MP – Methylprednisolone; MS – Multiple Sclerosis; NS – Non Significative; NT – Non treated; NTZ – Natalizumab; OT – Other Treatments; PNTZ- Patients Previously Treated with NTZ; T – Treated;

4.3.5. MRI outcomes

MRI outcomes are summarized in Table 11.

Table 11. MRI outcomes

Cohort	Focus	Patients	Time since diagnosis of MS (years)	Patients with NTZ as previous treatment	Washout period from NTZ (weeks)	Baseline MRI activity	FU (months)	Persistence [Adherence]	MRI activity free	MRI
Reims	Efficacy	66					11 (51.5% >12 months)		T2 84.8%	
Ferrara, Parma Modena, Reggio-Emilia, Fidenza and Piacenza	Efficacy	127	10.7	30.7%	18	65,4% (31.5% T1Gd+)	10		54.9%; T1Gd+ 83.1%; (PNTZ 45.8%; T1Gd+ 66.7%);	
Gallarate	Other	55	12.5	21.8%			12		69.1%	
Gallarate	NTZ switch	32		100%	15.6		73.3% patients with >6 months	86.7%	96.7%	
L' Aquila, Pozzilli, Foggia	Efficacy	142		19.7%			15		82.8%	
L' Aquila, Pozzilli, Foggia	FTY vs NTZ	100 (NTZ 100)				78% of patients (NTZ 90%)	12		75% (NTZ 93%)	
Padua	NTZ switch	21	10.7	100%	12				52.4%	
Kuwait city	Efficacy	76	7.82	2.6%		22.4% MRI activity free	18.5	94.7%	82.9%	
MS registry Kuwait	Efficacy	175	7.2	5.7%		23.3% MRI activity free	21.7	88.6%	81.7%	
Guaynabo	Efficacy	50					12			86% free of new black holes; 90% free of Brain Volume Loss; 2% with Brain volume loss and black hole;
Hospital Ntra Sra Valme, Seville	NTZ switch	49		100%					44.9%	
Murcia	NTZ switch	12	9.1	100%	12 (with MP)		15.4		T1Gd+ 100%	
Valencia	NTZ switch	11	6.8	100%	8		11.7	90.9%	T1Gd+ 81.8%	

Basel	NTZ switch	15	11.8	100%	8	100% free of T1Gd+	8	86.7%	46.2%
Boston	Efficacy	177	11	15.3%			>12		85.9%
Cleveland Clinic	FTY vs DMF	317 (DMF 426)	14.1 (DMF 12.3)	12.4% (DMF 11.7%)			3	92% (DMF 82%)	T1Gd+ 95% (DMF 94%)
Partners MS Center	FTY vs NTZ	36 (NTZ 69)	7.9 (8.4; p= 0.71)			1.56 T1Gd+ in previous 12 months (1.39; p= 0.78)	18	77.8% (NTZ 63.8%)	

DMF – Dimethyl Fumarate; DMTs - Disease Modifying Treatments; FTY – Fingolimod; MP – Methylprednisolone; NTZ – Natalizumab; PNTZ- Patients Previously Treated with NTZ; T1Gd+ - T1 Gadolinium-Enhancing Lesions; T2 - T2-weighted lesions

4.3.6. Composed outcomes

Composed outcomes are summarized in Table 12.

Table 12. Composed outcomes

Cohort	Focus	Patients	Time since diagnosis of MS (years)	Patients with NTZ as previous treatment	Washout period from NTZ (weeks)	Baseline ARR (previous 12 months)	FU (months)	Free of relapse and EDSS progression	Free of relapse and T1Gd+	Free of relapse and MRI activity	Free of EDDS progression and MRI activity	Free of relapse, EDDS progression and MRI activity
Reims	Efficacy	66					11 (51.5% >12 months)		89.1%			
NeuroTransConcept	FTY vs NTZ	190 (NTZ 239)	9.89 (NTZ 9.15)			0.34 (NTZ 0.42)	12	71.1% (NTZ 62.0%)				
Gallarate	Other	55	12.5	21.8%		1.7 in previous 24 months	12	67.3%				
Gallarate	NTZ switch	32		100%	15.6		73.3% patients with >6 months	70%				
L' Aquila, Pozzilli, Foggia	Efficacy	142		19.7%			15	79.7%				72.5%
L' Aquila, Pozzilli, Foggia	FTY vs NTZ	100 (NTZ 100)				1.2 (NTZ 1.9)	12					70% (NTZ 79%)
Milan	FTY vs NTZ	37 (NTZ 160)	11.5 (NTZ 10.1)			1.2 (NTZ 1.3)	>12	68% (NT> 92%, p= 0.0003)			42% (NT> 93%, p= 0.0003)	
Padua	NTZ switch	21	10.7	100%	12					47.6%		
Hospital Universitario Virgen Macarena, Seville	Efficacy	47		36.5%		0.88 previous 24 months	16.3	63.8%				
Multicenter2 Spain	Registry	267	9.3	27%			13.1	56.3%				
Boston	Efficacy	177	11	15.3%		0.5	>12			87.6%		50.3%
Cleveland Clinic	Efficacy	306					11.1			59.3%		
Cullman	NTZ switch	75		100%			>6		97.3%			

ARR – Annualized Relapse Rate; EDSS - Expanded Disability Status Scale; FTY – Fingolimod; FU – Follow-up; MRI - Magnetic Resonance Imaging; NT – Non treated; NTZ – Natalizumab; T1Gd+ - T1 Gadolinium-Enhancing Lesions;

4.3.7. PRO, FDO and Other outcomes

PRO, FDO and Other outcomes are summarized in Table 13.

Table 13. PRO, FDO and Other outcomes

Cohort	Patients	Patients with NTZ as previous treatment	FU (months)	Persistence [Adherence]	PRO/Other	Neurologists Reported Outcomes	FDO
São Paulo	180						Pre-FTY (2.8% high blood pressure; 2.2% right or left branch block; 1.1% sinus tachycardia; 1.1% sinus bradycardia); 89.4% discharged at 6h; 1.7% ICU
Gilenya* Go Program(TM)	1700			85.5%			0.29% AV block; 0.17% bradycardia; 99.3% continued on treatment post-FDO;
Multiple Sclerosis Registry	496	32.3%	> 3	89.2%			3.9% required prolonged cardiac monitoring (2.2% bradycardia; 2.7% 2nd degree grade AVB type I; 0.5% patient sinus arrest); 2.3% respiratory complaints, mostly dyspnoea;
Bordeaux	24		3	91.7%			8.3% symptomatic bradycardia; 4.2% treatment discontinuation after FDO
GRACE	198				HADS 8.1 (baseline 8.8) (p=0.001)		
Dresden	36						9.5% maximum decrease of heart rate between 3rd and 5th hour after FTY first dose
PANGAEA	3641 (PEARL active 457; PEARL inactive 1248)	18.4%	<24	83.4% (PEARL active 63.2%; PEARL inactive 85.9%); [Adherence 0.5 days without MS medication within 2 weeks (PEARL active 1.6)]	>96.9% of patients rated FTY tolerability as good or very good; >89.5% of patients and physicians rated FTY effectiveness as good or very good. 89.5% stabilized or better QoL (Eq5D)	>94.1% physicians rated FTY tolerability as good or very good; >90.7% physicians rated FTY effectiveness as good or very good.	1.7% cardiac AEs (0.03% 1st degree AVB; 0.3% 2nd degree AVB; 0.3% bradycardia; 0.45% lowest HR at 6h; 0.03% QTc interval ≥ 500 msec; 0.7% other clinical findings); 0.77% prolonged monitoring;
START	1640						91.2% discharged after 6h; 1% required overnight monitoring; 12bpm average maximum decrease of heart rate; 4bpm average decrease of heart rate 6h after FTY first dose; nadir HR at 4.1; 99% with heart rate >45bpm at any time; Within the first 6 hours the HR returned to 93 % of the baseline level; 3.6% with de novo 1st degree AVB; 1.6% 2nd degree or higher AVB;

Cohort	Patients	Patients with NTZ as previous treatment	FU (months)	Persistence [Adherence]	PRO/Other	Neurologists Reported Outcomes	FDO
Tel-Aviv	30		12		FTY treatment demonstrated preservation of structural thickness over one year of treatment. A numerical increase has been observed in all but one cognitive measure. The lack of statistical significance may be due to the very small sample. FTY treatment demonstrated preservation of structural thickness over one year of treatment. no difference in EDSS vs BL.		
Gallarate	55	21.8%	12		Active patients had more relapses before FTY (1.8 Vs 1.5, p = 0.043), a lower mean white blood cell count (5043.9/mm3 Vs 5328.8/ mm3, p = 0.027) and a lower mean lymphocyte count (653.8/mm3 Vs 756.8/mm3 , p = 0.05) compared to disease-free patients		
Milan	37 (NTZ 160)		>12	At 24 month 81% (NTZ 58%)	26% patients improved in cognitive tests (NTZ 7%)		
Multicentre Italy	906	24.3%	3.14	96%			95.3% patients with uneventful FDO; 1.3% bradycardia; 0.1% 1st degree AVB; 0.2% 2nd degree AVB; 0.1% palpitations; 0.1% sinus arrhythmia; 0.1% ventricular premature beats; 95.7% discharged after 6h;
Naples	112						20.5% with ECG abnormalities pre-FTY; 75.4 bpm pre-FTY; -6.3 bpm at 5h; -4.6 bpm at 5h; 5ms PR interval increase; 0.5ms QTc decrease; 0.9% symptomatic bradycardia (with headache and lower limbs weakness); 1.8% 2nd degree AVB; 16.4% sinus bradycardia, 9.1% right bundle branch block, 9.1% ventricular extrasystole, 3.6% 1st degree AV block;
Rome	55						-14.8% HR at nadir; nadir at 4h; 5.5% 1st degree AVB; 1.8% 2nd degree AVB;
Turin	35		6		2.9% de novo headache;		
Multicentre Japan	837						3.1% bradycardia, 1.4% HR decreased, 0.9% 2nd degree AVB;

Cohort	Patients	Patients with NTZ as previous treatment	FU (months)	Persistence [Adherence]	PRO/Other	Neurologists Reported Outcomes	FDO
FIRST	2417	10.5%	4	94.4%			96.7% discharged at 6h; 1.3% HR< 45bpm; 0.6% Palpitations; 0.6% Bradycardia; 0.1% Tachycardia; 0.1% Cardiovascular disorder; 0.1% Angina pectoris; 0.2% 2nd degree AVB; 0.2% Ventricular extra systoles; 0.04% Ventricular tachycardia;0.04% Cardiac disorder;0.04% Sinus bradycardia; 0.04% AVB
PASSAGE	1596	19.6%	9				3.5% bradyarrhythmia (1.3% bradycardia; 0.3% 1st degree AVB; 0.1% 2nd degree AVB)
Guaynabo	25		6				no significant CV events; 100% discharged after 6H;
Granada	42			98%			95.2% asymptomatic bradycardia; 11.9% required prolonged monitoring after the 6h;
Multicenter1 Spain	148		6.1	100%			9.5% average decrease of heart rate 6h after FTY first dose; 1.2mmHg decrease in systolic BP; 3.5mmHg decrease in diastolic BP;
Multicenter2 Spain	267	27%	13.1	96.6%			88.4% discharged at 6h; mean monitoring time 7.3 hours;
Murcia	18						5.56% symptomatic bradycardia; asymptomatic ECG changes were observed at 6 (44%) and 24 hours (20%) after first dose of FTY; no clinical or ECG effects requiring FTY discontinuation.
Multicentre Sweden	674 (186 with 12month FU)	33%	12	89.6%	-12.2% MSSS; +8.7% SDMT; -7.4% MSIS-29 physical score; -13.3% MSIS-29 psychological score; +6.3% EQ-5D		0.59% cardiac disorders (bradycardia, AV-block, hypertension);
SWISSASCENT	212	28%	8.3	96%	QoL remained stable over time in patients switching from other DMTs but those patients showed a significant improvement in the mental subscore; Treatment satisfaction improved 14 points upon switch from other DMTs to FTY (p= 0.001). This was true for all domains of the TSQM-9 scale.		99.5% patients did not require any medical intervention, nor monitoring on day two (98%), and continued FTY treatment after the FDO (99.5%)

Cohort	Patients	Patients with NTZ as previous treatment	FU (months)	Persistence [Adherence]	PRO/Other	Neurologists Reported Outcomes	FDO
Istanbul	109		5.5	94.5%			
Wimbledon	143			99.4%			
Boston	305			84.59%			-14 bpm at nadir; 98.7% uneventful; 0.66% 2nd degree AVB;
Cleveland Clinic	317	11.6%	3.3	90.5%	There were no significant differences in T25FW, PHQ-9, MSPS or EQ5D (n=164) compared to baseline.		Uneventful in 97.8%; 0.9% symptomatic bradycardia; 0.6% Chest tightness; 0.3% Hypertension; 0.3% Sensory symptoms; −11.5 bpm at 3 h; −12.0 bpm at 6 h; −1.8 mmHg Systolic BP at 3h and at 6 h; −3.3 mmHg Diastolic blood pressure at 3 h and −3.7 mmHg at 6h;
Cleveland Clinic	306		11.1	76.1%	MS performance scale, PHQ9, and EQ5D remained stable at 12 month follow-up (p>0.2 for all). T25FW was significantly slower at 12 months compared with BL (difference=1.16 seconds, paired t-test p = 0.013).		
Cleveland Clinic	196		8.6		85% stable or improved in T25FW; 91.6% stable or improved in 9PHT; -0.03 mean change in PHQ9 (p= 0.06); -0.005 mean change in EQ-5D (p= 0.02); 0.54 mean change in MSPS (p= 0.20);	Clinician-derived and patient-reported disability scores and patient-reported QoL measures were stable	

Cohort	Patients	Patients with NTZ as previous treatment	FU (months)	Persistence [Adherence]	PRO/Other	Neurologists Reported Outcomes	FDO
Cleveland Clinic	232		6.4		Mean GCIP thickness at BL was 70.64mum, with mean change of -0.37mum (p = 0.0038). Mean macular RNFL thickness at BL was 27.95mum with mean change of 0.12mum (p = 0.1456). The mean outer retina thickness at BL was 125.8mum, with mean change of 0.406mum (p = 0.0355), and the mean peripapillary RNFL thickness was 81.8mum with mean change of -0.97mum (p = 0.0053).		
Houston	59						5% rhythm disturbances (3% bradycardia secondary to atrioventricular conduction abnormality; 1.7% sinus bradycardia with accelerated idioventricular rhythm.)
Multicenter1 USA	205		6		TSQM improvement 17.57 vs 2.10 (IM IFN a), 24.7 vs 2.29 (SC IFN a) and 22.34 vs 4.45; TSQM subscale scores: Effectiveness, 13.31 vs 1.37 (IM IFN a), 15.07 vs 1.62 (SC IFN a), 17.59 vs 0.68 (IFN b); Side Effects, 30.62 vs 6.56 (IM IFN a), 27.83 vs -0.42 (SC IFN a), 21.50 vs -1.24 (IFN b); Convenience, 43.83 vs 5.70 (IM IFN a), 42.36 vs 1.66 (SC IFN a), 41.57 vs 1.31; FSS improvement: -0.33 vs -0.07 (IM IFN a), -0.44 vs 0.15 (SC IFN a), -0.46 vs 0.08 (IFN b). BDI-II improvement -3.21 vs -2.26 (IM IFN a), -2.73 vs -0.10 (SC IFN a), -4.16 vs 0.14 (IFN b); CGI-I improvement 3.25 vs 4.06 (IM IFN a), 3.11 vs 3.98 (SC IFN a) and 3.35 vs 3.97 (IFN b)		

Cohort	Patients	Patients with NTZ as previous treatment	FU (months)	Persistence [Adherence]	PRO/Other	Neurologist Reported Outcomes	FDO
Multicenter2 USA	380	8.4%			80% reported their first dose of FTY was moderately, very, or extremely manageable, convenient, and easy to take; 80% reported experiencing a side effect; 42.4% reported the side effect was “not at all” or only “a little” difficult to tolerate; 2.9% reported a “not at all satisfied” first-dose experience; TSQM scale scores (79.4 side effect, 71.7 convenience, 70.1 effectiveness, 68.9 global satisfaction);		
Multicenter3 USA	102 Neurologists				85.3% with >1 FTY patient; 5.2% of patients under FTY treatment; 94% reported that "All/Most” of their patients were highly adherent with FTY; 31% reported that their patients were “Very Satisfied” or “Extremely Satisfied” with FTY;		
NARCOMS	50 (NTZ 50)		54 (NTZ 48)		0.5 increase in PDSS (NTZ 0.1; p=0.02)		
Seattle	126		7		Trend of RNFL and GC-IP thinning was noted but did not reach statistical significance. The observed trend in macular volume change was independent of age, gender, time since MS diagnosis, or prior history of optic neuritis.		
EPOC	976		6				8.1 maximum HR decrease (at 5h); 7.2 average decrease of heart rate 6h after FTY first dose; 0.01% with HR< 40bpm; 18.2% with new ECG abnormalities (8.8% 1st degree AVB; 7.2% sinus bradycardia; 0.6% Left anterior hemiblock; 1.7% Atrial premature complex; 0.6% Biphasic Twaves); 98.5% discharged at 6h; 1.3% Required extended observation after 6h on day 1; 0.5% Required extended observation on day 2; 1.3mmHg decrease in systolic BP; 3.1mmHg decrease in diastolic BP; 1.2% Symptomatic bradycardia
EPOC	847		6		50.5% recovered from depression (DMT 25.3%); 8.1% became depressed (DMT 9.3%);		

Cohort	Patients	Patients with NTZ as previous treatment	FU (months)	Persistence [Adherence]	PRO/Other	Neurologists Reported Outcomes	FDO
EPOC	790 (DMTs 263)	0.1% (DMTs 0%)	6	90.4% (DMTs 87.1%)	TQSM scores were significantly greater with FTY than with DMT (Global satisfaction, Effectiveness, Side Effects, and Convenience; p< 0.0001); Improvements in FSS and BDI-II score were significantly greater with FTY than with DMT (p< 0.0001); PRIMUS Activities score were numerically reduced (better outcomes in fatigue and depression) with FTY versus DMT; SF-36 scores were significantly greater with FTY than with DMT (p< 0.0001), except for the domains 'Physical functioning' and 'General health perceptions'		
EPOC	790 (DMTs 263)		6		BDI-II; Somatic subdomain, 7.5 (at BL) to 5.4 (at Month 6) (DMTs 6.9 to 6.7) (p<0.0001); Affective subdomain, 4.2 (at BL) to 2.9 (at Month 6)(DMTs 4.0 to 3.8) (p=0.0001)		

AEs – Adverse Events; AV – Atrioventricular; AVB – AV Block; BDI-II - Beck Depression Inventory-II; BL – Baseline; Bpm – beats per minute; DMTs - Disease Modifying Treatments; ECG – Electrocardiogram; EDSS - Expanded Disability Status Scale; FDO – First Dose Observation; FTY – Fingolimod; FU – Follow-up; GCIP - Ganglion Cell/Inner Plexiform; HADS - Hospital Anxiety and Depression Scale; HR – Heart Rate; IFN – Interferon; IM – Intramuscular; MSIS - Multiple Sclerosis Impact Scale; MSPS - Multiple Sclerosis Performance Scales; MSSS - Multiple Sclerosis Severity Score; PDSS - Patient Determined Disease Steps; PHQ-9 - Patient Health Questionnaire 9; PRO - Patient Reported Outcomes; QoL - Quality of Life; RNFL - Retinal Nerve Fibre Layer; SC – Subcutaneous; SDMT - Symbol Digit Modalities Test; T25FW - Timed 25-Foot Walk; TSQM - Treatment Satisfaction Questionnaire for Medication

4.3.8. Safety outcomes

Analysing real world evidence for safety outcomes is somehow tricky, because most of the studies have less than 500 patients, and thus incidence rate of rare events can be unrealistically overestimated. A cut-off of 500 patients in each study was used to avoid this artefact. This resulted in a substantial drop of the number of studies available to review.

The results can be found on Table 14 where in the last columns are the results of the pooled analysis of the phase III trials, and the pooled analysis of only FREEDOMS, FREEDOMS II and TRANSFORMS, to be used as reference.

Table 14. Safety outcomes

Cohort	EPOC[260,262]	FIRST[208]	Gilenya* Go Program(TM)[172]	LONGTERMS[209, 210]	Multicentre Sweden[230]	Multicentre Japan[204]	PASSAGE[214]	Phase III studies[44]	FREEDOMS[38]	TRANSFORMS[48]
Comment				Incidence per 100 patient-years				9,070 patient-years		
Patients	783	2417	1700	1655	674	809	1419	1640	425	429
Any AE	78.8%			296.7	3.1%	40.9%	33.6%	95.8%	94,4%	86,0%
AEs leading to study drug discontinuation	5.2%				5.9%	11.4%	1.27%	7.6%	7,5%	5,6%
AEs leading to study drug discontinuation after 1st dose			0.6%							
Abnormal laboratory value leading to study drug								4.8%		
Any SAEs	4.0%				1.93%	17.2%	4.2%	11.9%	10,1%	7,0%
Infections				68.3			9.3%	74.5%		
Infections and infestations	30.1%				0.44%					
Infections in patient-years								114.7		
Any serious infection								2.9%		
Any severe infection								5.2%		
Appendicitis (SAE)								0.3%		
Herpes infection (SAE)								0.4%		0,2%
Any Herpes infection								11.2%	8,7%	2,1%
Herpes Zoster						1.2%	0.8%	3.0%		
Herpes Zoster (SAE)						0.9%		0.1%		
Influenza				4.4				12.8%	12,9%	6,8%
Lower respiratory tract and lung infection								13.2%	9,6%	
Bronchitis	1.5%			3.5				9.2%	8,0%	
Pneumonia									0,9%	
Upper respiratory tract infection	6.5%			6.2		0.7%	0.8%	19.1%	49,9%	7,2%
Nasopharyngitis	5.5%			11.9		0.6%	1.3%	30.4%	27,1%	20,5%

Cohort	EPOC[260,262]	FIRST[208]	Gilenya* Go Program(TM)[172]	LONGTERMS[209, 210]	Multicentre Sweden[230]	Multicentre Japan[204]	PASSAGE[214]	Phase III studies[44]	FREEDOMS[38]	TRANSFORMS[48]
Sinusitis	3.3%			3.3					6,6%	
Rhinitis									5,9%	
Pharyngitis									6,4%	
Reactivation of chronic viral infections				5.3						
Urinary tract infection	5.1%			4.3			1.5%	12.1%	8,0%	6,1%
Urinary tract infection (SAE)								0.2%		
Leucopenia and lymphopenia				6.9%						
Leucopenia									2,8%	
Lymphocyte count decreased						12.4%				
Lymphocyte count decreased (SAE)						4.2%				
Lymphopenia			1.24%	3.8		1.6%	2.3%	12.0%	4,2%	0,2%
Lymphopenia (SAE)	0.3%					0.5%		0.2%	0,5%	
Lymphadenopathy										
White blood cell count decreased						5.7%				
White blood cell count decreased (SAE)						2.6%				
Angina pectoris (SAE)		1 (0.0%)								
Atrioventricular block			0.25%							
Atrioventricular block (SAE)						0.5%				
Atrioventricular block first degree (FDO)							0.3%			
Atrioventricular block first degree							0.3%		0,5%	
Atrioventricular block first degree (SAE)						0.5%		0.1%		0,2%
Atrioventricular block second degree						0.9%	0.1%			
Atrioventricular block second degree (SAE)		0.2%				0.9%		0.1%		0,2%
Blood pressure decreased						0.6%				

Cohort	EPOC[260,262]	FIRST[208]	Gilenya* Go Program(TM)[172]	LONGTERMS[209, 210]	Multicentre Sweden[230]	Multicentre Japan[204]	PASSAGE[214]	Phase III studies[44]	FREEDOMS[38]	TRANSFORMS[48]
Bradyarrhythmia occurring post-first dose				2.0			3.5%			
Bradycardia FDO										
Symptomatic bradycardia FDO										
Bradycardia	2.2%	0.6%	0.2%			3.1%	1.3%			
Bradycardia (SAE)		1 (0.0%)		0.1		1.5%	0.4%	0.4%	0,9%	0,5%
Bradycardia, bradyarrhythmia, or sinus bradycardia									2,1%	0,5%
Cardiac disorder	4.3%		0.06%		0.37%					
Cardiac disorder (SAE)		1 (0.0%)			0.73%					
Cardiac flutter	0.3%									
Cardiovascular disorder		0.1%								
Cardiovascular disorder (SAE)		1 (0.0%)								
Chest pain (SAE)								0.2%	0,9%	
Hypertension	5.5%			3.0%			1.3%	8.8%	6,1%	3,7%
Diastolic blood pressure ≥ 15mmHg increase vs BL								29.6%		
Diastolic blood pressure increase ≥ 100mmHg								7.6%		
Systolic blood pressure ≥ 20mmHg increase vs BL								34.1%		
Systolic blood pressure increase ≥ 160mmHg								4.8%		
Heart rate decreased						1.4%				
Heart rate decreased (SAE)						0.4%				
Ischaemic and haemorrhagic strokes (SAE)								0.2%		
Palpitations	1.5%	0.6%								
QT interval prolongation				0.7						
Sinus bradycardia						0.6%				
Sinus bradycardia (SAE)		1 (0.0%)				0.4%				
Tachycardia		0.1%								

Cohort	EPOC[260,262]	FIRST[208]	Gilenya* Go Program(TM)[172]	LONGTERMS[209, 210]	Multicentre Sweden[230]	Multicentre Japan[204]	PASSAGE[214]	Phase III studies[44]	FREEDOMS[38]	TRANSFORMS[48]
Thromboembolic events				0.9			0.5%			
Ventricular extra systoles		0.2%								
Posterior reversible encephalopathy syndrome (SAE)								0.1%		
Pulmonary edema				0.1						
Alanine amino transferase increased	2.4%			2.7		1.8%	1.3%	11.2%		6,5%
Aspartate aminotransferase increased						0.6%	0.6%			
Bilirubin								8.7%		
Liver disorder						1.5%				
Liver dysfunction						5.1%				
Liver dysfunction (SAE)						1.4%				
Liver enzyme elevation				5.8			3.5%			
Liver function test abnormal						2.5%			15,8%	
Liver function test abnormal (SAE)						0.4%			0,5%	
γ-glutamyltransferase increased				1.8		3.5%	1.1%			
γ-glutamyltransferase increased (SAE)						0.4%				
Bronchoconstriction				2.1	0,13%					
Cough								12.8%	10,1%	4,7%
Dyspnea				2.0					7,1%	1,9%
Oropharyngeal pain									6,8%	
Nasal congestion										
Basal-cell carcinoma (SAE)				0.5				1.6%	0,9%	0,7%
Breast cancer (SAE)							0.2%	0.3%		0,7%
Malignant melanoma (SAE)								0.2%		0,7%

Cohort	EPOC[260,262]	FIRST[208]	Gilenya* Go Program(TM)[172]	LONGTERMS[209, 210]	Multicentre Sweden[230]	Multicentre Japan[204]	PASSAGE[214]	Phase III studies[44]	FREEDOMS[38]	TRANSFORMS[48]
Melanocytic nevus				2.8						6,5%
Other malignant neoplasms				0.4			0.5%			
Ovarian epithelial cell cancer (SAE)								0.1%		
Pancreatic carcinoma							0.1%			
Skin cancer				0.7			0.1%			
Squamous cell carcinoma of the skin (SAE)								0.1%		
Thyroid malignancy (SAE)				1 (0.0)						
Uterine leiomyoma (SAE)								0.4%		
Fatigue	11.5%			3.7			2.0%	10.7%	11,3%	10,3%
MS relapse				0.6		1.1%	0.6%			
MS Relapse (SAE)	0.6%			0.3		0.9%	0.6%	0.8%	0,9%	
MS symptoms worsening			0.76%							
Headache	12.4%		0.71%	9.9		0.7%	2.5%	26.5%	25,2%	23,1%
Migraine				1.5						
Migraine (SAE)	0.3%									
Dizziness	6.4%			2.5					7,3%	5,6%
Paraesthesia									5,4%	
Syncope (SAE)										
Convulsion (SAE)										
Epilepsy (SAE)								0.1%	2,1%	
Diarrhea	5.2%			4.2		1.0%		13.6%	11,8%	7,5%
Nausea	6.1%			3.5		1.2%		()/(425+	8,9%	9,3%
Vomiting										

Cohort	EPOC[260,262]	FIRST[208]	Gilenya* Go Program(TM)[172]	LONGTERMS[209, 210]	Multicentre Sweden[230]	Multicentre Japan[204]	PASSAGE[214]	Phase III studies[44]	FREEDOMS[38]	TRANSFORMS[48]
Dyspepsia										
Arthralgia				3.0					7,1%	2,8%
Back pain				4.2				12.0%	11,8%	6,1%
Back pain (SAE)								0.2%		
Pain in extremity									6,6%	
Myalgia										3,3%
Limb pain										4,9%
Neck pain										
Depression				3.4					7,8%	4,9%
Depression (SAE)									0,5%	
Major depression (SAE)										
Anxiety										
Insomnia				2.4					4,9%	
Abdominal pain (SAAE)										
Cholelithiasis (SAE)								0.4%		
Nephrolithiasis (SAE)				0.1						
Decreased renal function				0.3						
Hypercholesterolemia									5,6%	
Weight increase									3,3%	
Vertigo									4,2%	
Death (SAE)							0.1%	0.2%		
Fever (SAE)					1,04%	0.4%				4,2%
Influenza-like illness										3,5%

Cohort	EPOC[260,262]	FIRST[208]	Gilenya* Go Program(TM)[172]	LONGTERMS[209, 210]	Multicentre Sweden[230]	Multicentre Japan[204]	PASSAGE[214]	Phase III studies[44]	FREEDOMS[38]	TRANSFORMS[48]
Itchiness			0.06%							
Macular edema	0.9%		0.35%	0.2		0.7%	0.6%	0.4%		
Macular edema (SAE)						0.5%		0.2%		
Patients treated for macular edema								0.2%		
Muscular weakness						0.6%				
Muscular weakness (SAE)						0.5%				
Non-cardiac chest pain (SAE)	0.3%							0.2%		
Physical weariness						1.7%				
Pregnancy							0.3%			
Reproductive toxicity				0.9						
Abortion										

AEs – Adverse Events; BL – Baseline; FDO – First Dose Observation; SAEs – Serious Adverse Events

4.4. Discussion

4.4.1. Studies analysed and limitations of this review

When well designed a retrospective study is as valid as a prospective one. If in everyday clinical practice all the right evaluations are systematically registered, there is no reason to obtain different results between a prospective and retrospective study following the same cohort in the same time window. The (bad) reputation that sometimes retrospective studies have is mainly due to poor designs or incomplete data in the source documents. The high percentage of retrospective studies reviewed here, representing approximately 40% of the patients, shouldn't bring *per se* any reduction in the validity of its results.

Cohorts from well identified centres, from studies done individually or with other well identified centres, account for 54.7% of the studies, but only for 14.8% of the patients. The remaining studies and patients are multicentre studies, national or multinational, and registries.

Due to the nature of studies done in the clinical practice setting it is easier and financially more feasible to do comparative studies than active controlled randomized trials. But, at the end of the day, is the evidence that everyone wants to know. I was surprised to see that only 12.6% of the studies reviewed, had a control cohort. These studies followed 2,384 fingolimod patients. Without any surprise, most of these studies included natalizumab as control. (Figure 16)

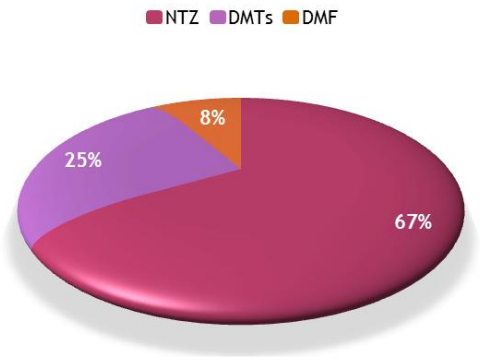

Figure 16. Control used in comparative studies

In this review I wanted to gather as much real world data as possible and thus very inclusive criteria was used to select the studies to be reviewed. In clinical trials we have a very homogeneous population, which guarantees high precision of the results and high internal validity. This

strategy results, most of the times, in studies with a less than perfect external validity. On the other hand, studies in the clinical practice setting usually have a more heterogeneous population, resulting in a lower internal validity, but with a higher external validity. My belief is that, even though when considered individually these studies can't be used to reach any conclusion about the treatment effectiveness due mostly to small samples, when considered as a whole they become more robust and credible to be used to reach drug effectiveness deductions.

In this work, I wasn't able to review references in other languages than Portuguese, English, Spanish and French. Poster and abstracts that were not available online, or that I did not have the opportunity to see in the respective congress, were naturally excluded from this review. Regarding oral presentations I have only considered its abstracts, when published online. I believe that, even with all these exclusions, I have captured a big proportion of RWE data shared by clinicians in congresses and peer-reviewed journals.

Individual comments and conclusions of each study are summarized in Table 15.

Table 15. Key takeaways for each study

Cohort	Country	Focus	Comments
REAL	Argentina	Registry	Interim results of Argentinian registry with 48 patients; 8.5 months FU; 97.9% relapse-free
Multiple Sclerosis Treatment Registry	Austria	FTY vs NTZ	Austrian registry; 312 patients (NTZ 1208); 21.5month FU; 54.2% of FTY patients have been previously treated with NTZ. From these patients, the ones treated for at least one year with FTY (n=72), had an increase of ARR from 0.3 to 0.5 and the EDSS changed from 3.6 to 3.9 (NTZ cessation) and stayed stable during FTY treatment at 3.8. 72.9% ARR reduction (inferior vs NTZ); disability stable or improved in 78% (NTZ 85%); 59% of persistence (NTZ 60%)
São Paulo	Brazil	FDO	Retrospective FDO study with 180 patients; 89.4% patients discharged after 6h;
Gilenya* Go Program(TM)	Canada	PRO	Patient program registry with 1,700 patients; 0.29% AV block; 0.17% bradycardia; 99.3% continued on treatment post-FDO; 85.5% of persistence
Multiple Sclerosis Registry	Denmark	FDO	FDO retrospective study with 496 patients; 32.3% with NTZ as previous treatment; >3month FU; Cardiac adverse effects occurred at a similar rate as in clinical trials and did not require treatment. Most respiratory symptoms resolved spontaneously on continued FTY treatment, but 2 patients had to discontinue treatment. 3.9% required prolonged cardiac monitoring; 89.2% of persistence;
Alsace	France	NTZ switch	Data extracted from 1 abstract + 1 letter to the editor; Retrospective study with 59 patients; 6.8 weeks WO; 7month FU; NTZ switch; 81.4% relapse-free patients similar to PTs, but for those patients with WO> 12weeks only 50% were relapse-free; 94.9% of persistence;
Alsace	France	Efficacy	Retrospective regional study with 290 patients; 12month FU; high EDSS at BL (3.6); 0.17 ARR; 70.5% ARR reduction; 4.1 months to 1st relapse; 82.4% relapse-free; +0.1 EDSS difference vs BL; 89.7% of persistence;
Bordeaux	France	FDO	Prospective study with 24 patients; 3month FU; 82.6% relapse-free; 4.2% treatment discontinuation after FDO; 91.7% of persistence;
ENIGM	France	NTZ switch	NTZ switch; survey-based; 333 patients; 31 NTZ infusions; 71% JCV+; 27% patients relapsed in the WO period (17 weeks); 80% relapse-free patients; the percentages of patients who relapsed depending on the WO were 19.9% for less than 3 months, 31.3% for 3 to 6 months, and 59.1% for greater than 6 months; switching from NTZ to FTY was associated with a risk of MS reactivation during the WO or shortly after FTY initiation. The WO should be shorter than 3 months. 97% of persistence;
GRACE	France	PRO	Prospective study with 198 patients; The BL level of anxiety was not high before FTY treatment initiation (HADS 8.8), but decreased even further within 4 months of treatment (HADS 8.1); (8.8 vs 8.1; p=0.001)
Reims	France	Efficacy	Prospective study with 66 patients; 11month FU, 51.5% patients with >12month; 59.1% ARR reduction; 68.3% relapse-free; 84.8% MRI lesions-free; 89.1% Free of relapse and T1Gd+
Dresden	Germany	FDO	36 patients; Most CV effects are only short-lived during the first day of FTY administration.
Munich, Bochum, Marburg	Germany	NTZ switch	Retrospective NTZ switch study with 33 patients; 31.8 NTZ infusions; 20.3month FU; EDSS >3 after switch of NTZ is a predictor for relapses during subsequent FTY therapy; 75% of patients with EDSS ≤3 remained relapse-free; patients with EDSS ≤3 ARR 0.24 vs EDSS>3 ARR 1.35; ARR during NTZ did not significantly influence relapse activity during FTY; +0.1 EDSS difference vs BL

Cohort	Country	Focus	Comments
NeuroTransConcept	Germany	FTY vs NTZ	Retrospective study with 190 patients; 12month FU; Switch from DMT to FTY or NTZ; Clinical efficacy of FTY and NTZ in RRMS second-line-therapy was similar during the first 12 months of treatment. Disability stable or improved in 80.5% (NTZ 79.3%); -0.14 EDSS difference vs BL (NTZ -0.13); 70.6% ARR reduction; 75.8% relapse-free (NTZ 72.3%); 71.1% Free of relapse and EDSS progression (NTZ 62%)
PANGAEA	Germany	Large open trials	Prospective study with 3641 patients; data extracted from 3 different posters; <24month FU; 18.4% with NTZ as previous treatment; NTZ patients 66.92% JCV+; A short NTZ wash-out period is associated with lower clinical reactivation. The relapse activity in the first half year of PANGAEA highly depends on the length of the NTZ WO phase prior the FTY first dose. 2.63% patients hospitalized in the past 3 months (PEARL active 8.02%). 14.02% patients took sick leave in the past 3 months (PEARL active 35.94%). Superior ARR reduction vs IFN/GA; disability stable or improved in 94%; -0.07 EDSS difference vs BL (PEARL +0.5); 1.7% cardiac AEs; 0.77% prolonged monitoring; >96.0% of patients and physicians rated FTY tolerability as good or very good. >94.1% physicians and 96.9% of patients rated FTY tolerability as good or very good; >90.7% physicians 89.5% of patients rated FTY effectiveness as good or very good. 83.4% of persistence; (PEARL active 63.2%; PEARL inactive 85.9%); 0.5 days without MS medication within 2 weeks (PEARL active 1.6)
START	Germany	FDO	Prospective FDO study with 1640 patients; Main analysis + subgroup analysis for patients also treated with SSRIs; 91.2% discharged after 6h; 1% required overnight monitoring; 12bpm average maximum decrease of heart rate; 4bpm average decrease of heart rate 6h after FTY first dose; nadir Heart Rate at 4.1; 99% with heart rate >45bpm at any time; Within the first 6 hours the heart rate returned to 93 % of the baseline level; 3.6% with de novo 1st degree AVB; 1.6% 2nd degree or higher AVB; similar results between the main analysis and the SSRIs subgroup
Athens	Greece	NTZ switch	NTZ switch study with 60 patients; 33.3% with NTZ as previous treatment; >12month FU; 10/60 patients treated with monthly MP in the WO period; 93.3% relapse-free
Ramat-Gann	Israel	Efficacy	Retrospective study with 110 patients; 20month FU; Discontinuation due to adverse events occurred within the first year of treatment. ARR 0.35; 76.4% patients continued on treatment;
Tel-Aviv	Israel	Other	Data extracted from 2 different posters; Prospective study with 30 patients; 12month FU; Gilenya treatment demonstrated preservation of structural thickness over one year of treatment. A numerical increase has been observed in all but one cognitive measure. The lack of statistical significance may be due to the very small sample. Gilenya treatment demonstrated preservation of structural thickness over one year of treatment. no difference in EDSS vs BL.
Ferrara, Parma Modena, Reggio-Emilia, Fidenza and Piacenza	Italy	Efficacy	127 patients; 10month FU; Confirms the efficacy of FTY in reducing relapse rate in patients previously treated with DMTs, while it seems to be less effective in patients discontinuing NTZ. 30.7% patients previously treated with NTZ; 18 weeks WO; ARR 0.28; 76.7% ARR reduction; 20.2% improvement in MRI lesions-free; 14.6% improvement in T1Gd+ lesions-free; WO period may have been too long. No difference in EDSS vs BL. In PNTZ 0.1 difference vs BL, p= 0.14)

Cohort	Country	Focus	Comments
Gallarate	Italy	Other	55 patients; 12month FU; 21.8% patients with NTZ as previous treatment; 67.3% Free of relapse and EDSS progression; Active patients had more relapses before FTY (1.8 Vs 1.5, p = 0.043), a lower mean white blood cell count (5043.9/mm3 Vs 5328.8/ mm3, p = 0.027) and a lower mean lymphocyte count (653.8/mm3 Vs 756.8/mm3 , p = 0.05) compared to disease-free patients. 69.1% MRI lesions-free. 67.33% patients free of relapse and EDSS progression. Maybe, excessive lymphopenia could include "protective" cell subsets.
Gallarate	Italy	NTZ switch	NTZ switch study with 32 patients; 21 NTZ infusions; 15.6 weeks WO; 73.3% patients with >6month FU; 28.1% relapse-free patients; long WO (15.6 weeks); 28.1% relapse-free; 96.7% MRI-lesions free; 70% free of relapse and EDSS progression 86.7% of persistence;
iMedWeb registry	Italy	NTZ switch	Retrospective NTZ switch study with 106 patients; 26.4 NTZ infusions (DMTs 19.4); The relapse risk was 4 and 15 time higher in patients with WO length 1.5-4.4 M and >4.4 M, respectively, in comparison to patients with WO <1.5 M. FTY is more effective than other DMTs in reducing the risk of relapse after NTZ stop (IRR=0.56, p=0.0019). The cumulative probability of first relapse was significantly lower in patients receiving FTY than in those receiving other DMTs (p=0.021).
L' Aquila, Pozzilli, Foggia	Italy	Efficacy	142 patients; 19.7% with NTZ as previous treatment; 15month FU; 90.8% relapse free (NTZ 82.1%); 82.8% MRI-lesions free; disability stable or improved in 87.1%; 72.5% Free of relapse, EDDS progression and MRI; 79.7% Free of relapse and EDSS progression activity
L' Aquila, Pozzilli, Foggia	Italy	FTY vs NTZ	Prospective study with 100 patient; 12month FU; NTZ patients in a more advanced state of disease in the BL (ARR +0.7; MRI activity +12%; EDSS +0.3); FTY numerically superior in relapse-free patients vs NTZ (93% vs 87%); 75 % MRI lesions-free patients (NTZ 93%); no difference in disability outcomes vs NTZ; disability stable or improved in 99% (NTZ98%); 70% Free of relapse, EDDS progression and MRI activity (NTZ 79%)
Milan	Italy	NTZ switch	NTZ switch study with 61 patients; >6month FU; Increase of the ARR in the WO period (1.20 vs 0.15, p < 0.0001); after FTY the ARR had a significant reduction (0.50 vs 1.20, p = 0.0006); after FTY EDSS maintained stable (0.15 on NTZ; 0.43 WO period);
Milan	Italy	FTY vs NTZ	Prospective study with 37 patients; >12month FU; 72.9% ARR reduction (NTZ 96.9%); ARR 0.26 (NTZ 0.04; p< 0.0001) no difference in disability outcomes vs NTZ; 68% Free of relapse and EDSS progression (NTZ > 92%, p= 0.003); 42% Free of EDDS progression and MRI activity (NTZ> 93%, p= 0.0003); At 24 month 81% of persistence (NTZ 58%); 26% patients improved in cognitive tests (NTZ 7%);
Multicentre Italy	Italy	FDO	Prospective FDO study with 906 patients; 24.3% with NTZ as previous treatment; 3.14month FU; 95.3% patients with uneventful FDO; 1.3% bradycardia; 0.1% 1st degree AVB; 0.2% 2nd degree AVB; 0.1% palpitations; 0.1% sinus arrhythmia; 0.1% ventricular premature beats; 95.7% discharged after 6h; 96% of persistence
Naples	Italy	FDO	FDO study with 112 patients; 20.5% with ECG abnormalities pre-FTY; HR decrease was lower than expected with terminal ECGs showing a small increase in PR interval. 0.9% symptomatic bradycardia (with headache and lower limbs weakness); 1.8% 2nd degree AVB; 16.4% sinus bradycardia, 9.1% right bundle branch block, 9.1% ventricular extrasystole, 3.6% 1st degree AV block;

Cohort	Country	Focus	Comments
Orbassano	Italy	NTZ switch	Retrospective NTZ switch study with 35 patients; >12 weeks WO; Treatment arms after NTZ: FTY, No Treatment (NT), and Treatment (T) with other DMTs or immunomodulating agents; OT = NT + T; +0.2 EDSS difference vs BL (OT 0.5, p NS); 68.6% relapse-free (OT 58.2%)
Padua	Italy	NTZ switch	NTZ switch study with 21 patients; 12 weeks WO; 3 month WO period following NTZ exposes patients to disease reactivation particularly during the 1st month of FTY. Only patients having a very active disease seem not to respond to FTY. FTY was capable to control disease activity in the majority of the patients (>70%). 71.4% relapse-free; 52.4% MRI-lesions free; 47.6% Free of relapse and MRI activity;
Rome	Italy	FDO	Prospective study with 55 patients; Significant correlations between measures of parasympathetic function and FTY-induced bradycardia. Subjects with higher Valsalva ratio and HR variation during deep breathing had nadir HR ≤ 50 bpm after the first FTY dose. Significant negative correlations were found between measures of sympathetic function and FTY-induced PR interval increase. Subjects with lower LFnu at rest and less increase of blood pressure on the handgrip test showed a PR interval increase > 20 ms after FTY. -14.8% HR at nadir; nadir at 4h; 5.5% 1st degree AVB; 1.8% 2nd degree AVB;
Turin	Italy	Other	Prospective study with 35 patients; 6month FU; 2.9% de novo headache; FTY might represent an important trigger of transient migraine worsening, but the mechanisms underlying this phenomenon are still unknown.
Multicentre Japan	Japan	Registry	Japanese registry with 837 patients; ARR 0.3; 69.4% ARR reduction; 3.1% bradycardia, 1.4% HR decreased, 0.9% 2nd degree AVB;
Tokyo	Japan	Efficacy	44 patients; 95.5% relapse-free patients; 75% persistence
Kuwait city	Kuwait	Efficacy	Retrospective study with 76patients; 18.5month FU; FTY is safe and effective in reducing clinical and radiological disease activity in relapsing remitting MS patients. 64.4% more patients relapse-free vs baseline (p< 0.001); 60.5% more patients MRI lesions-free vs baseline (p< 0.001); disability stable or improved in 64.5%; -0.98 EDSS difference vs BL (p< 0.0001); 94.7% of persistence
MS registry Kuwait	Kuwait	Efficacy	Retrospective study with 175 patients; 21.7months average FU; FTY treatment was associated with reduced relapse and MRI activity, and an improved EDSS score. Discontinuation/switch rates and adverse events were low. 54.3% more patients relapse-free; 58.4% more patients MRI lesions-free; disability stable or improved in 92%; -0.34 EDSS difference vs BL (p= 0.03); 88.6% of persistence
FIRST	Multinational	FDO	Prospective FDO study with 2,417 patients; 4month FU; Main analysis + subgroup analysis for patients with or without pre-existing cardiac conditions + subgroup analysis for patients with or without concomitant treatment with BBs/CCBs; 9.3 years since onset of symptoms of MS ; 96.7% discharged at 6h; 1.3% HR< 45bpm; 94.4% of persistence; there is no increased risk of symptomatic or serious cardiac events during treatment initiation in patients with PCCs or in those receiving BBs/CCBs
LONGTERMS	Multinational	Safety	Data extracted from 2 different posters; Extension clinical trials; 1665 patients; There were no new safety signals detected with the long-term use of fingolimod.

Cohort	Country	Focus	Comments
MSBase Registry	Multinational	NTZ switch	Retrospective NTZ switch study with 89 patients; 7.6month FU; NTZ switch; 95% with WO < 16 weeks; average WO 11.1weeks; 79.8% relapse-free; 30% of patients with disease activity on NTZ relapsed within the first 6 months on FTY; patients who had a gap in treatment of 8-16 weeks were more likely to have a relapse on FTY than switchers without WO, p = 0.040; recommendation of 2 months maximum WO
MSBase Registry	Multinational	FTY vs NTZ	MS Base registry; 171 patients; 12month; Matched cohort; 72.1% ARR reduction (NTZ 87.6%); ARR 0.36 (NTZ 0.19); disability outcomes favoured NTZ
MSBase Registry	Multinational	FTY vs DMTs	MS Base registry; 148 patients; 11month FU; Matched cohort; 76.9% ARR reduction (DMTs 62.5%); superior proportion of relapse-free patients vs DMTs (HR= 0.74; p= 0.04); ARR 0.3 (DMT 0.45; p= 0.009); superior proportion of patients free from disability progression vs DMT (HR= 0.53; p= 0.02); superior proportion of patients with reduced disability vs DMT (HR= 2; p= 0.005); Persistence on FTY was better compared to DMT (HR 0.55; p= 0.04)
PASSAGE	Multinational	Large open trials	interim analysis Post-Approval Safety Study with 1596 enrolled patients; 19.6% with NTZ as previous treatment; 9month FU; 3.5% bradyarrhythmia (1.3% bradycardia; 0.3% 1st degree AVB; 0.1% 2nd degree AVB)
Warsaw	Poland	Efficacy	11 patients; 12month FU; abstract very incomplete; Polish article; abstract in English; 100% relapse-free; disability stable or improved in 90.9%; 100% of persistence
Coimbra	Portugal	NTZ switch	NTZ switch study with 29 patients; 28.7 NTZ infusions; 9.2 weeks WO; 17.4 months FU; 55.2% relapse-free; a higher ARR previous to FTY it's associated with relapse risk during FTY (p= 0.04); 69% with similar or improved ARR vs BL; +0.1 EDSS difference vs BL (p= 0.6); 79.3% of persistence;
Guaynabo	Puerto Rico	Efficacy	retrospective study with 50 patients; 12month FU; 86% free of new black holes; 90% free of Brain Volume Loss; 2% with Brain volume loss and black hole; FTY can reduce the development of T1 hypo intense-black holes and cerebral atrophy in Hispanic population with MS.
Guaynabo	Puerto Rico	FDO	Retrospective study with 25 patients; 6month FU; At FDO no significant cardiovascular events; 100% discharged after 6H;
Moscow	Russia	Efficacy	11 patients; abstract very incomplete; 82% relapse-free
Prešov	Slovak Republic	NTZ switch	NTZ switch; 9 patients; no report of FU period; 24.1 NTZ infusions; 13.2 weeks WO; 44.4% relapse-free, 11.1% had MRI reactivation and 11.1% had disease rebound during WO;
Alicante	Spain	NTZ switch	NTZ switch; 8 patients; 29.4 NTZ infusions; 12 weeks WO; 9.35month FU; 37% relapse-free; ARR (1.88 pre-NTZ; 0.13 during NTZ; 0.63 during FTY); T2 lesion free 50%; T1Gd+ lesion-free 37.5%; disability stable or improved in 62.5%; +1.0 EDSS difference vs BL
Granada	Spain	FDO	Retrospective study with 42 patients; FDO reported by MS nurse team; 95.2% asymptomatic bradycardia; 11.9% required prolonged monitoring after the 6h; 98% of persistence
Hospital Ntra Sra Valme, Seville	Spain	NTZ switch	NTZ switch study with 49 patients; FTY increased the relapse-free patients from 59.2% to 75.5% and kept 44.9% patients free of MRI activity and 75.5% of patients free of disability progression

Cohort	Country	Focus	Comments
Hospital Universitario Virgen Macarena, Seville	Spain	Efficacy	Retrospective study with 47 patients; 36.5% with NTZ as previous treatment; 16.3 month FU; the response to FTY in real world practice supports FTY efficacy in the clinical practice. 71.6% ARR reduction (PNTZ 44.3%); +0.04 EDSS difference vs BL; 63.8% Free of relapse and EDSS progression
Multicenter1 Spain	Spain	FDO	Prospective study with 148 patients; 6.1month FU; 9.5% average decrease of heart rate 6h after FTY first dose; 1.2mmHg decrease in systolic BP; 3.5mmHg decrease in diastolic BP; 100% of persistence
Multicenter2 Spain	Spain	Registry	Spain registry with 267 patients; 27% with previous NTZ treatment; 13.1month FU; 11.5years since onset of symptoms of MS. 85.3% JCV+; 88.4% discharged at 6h; mean monitoring time 7.3 hours; 80% relapse-free; 69.8% of patients free of disability progression; 56.3% Free of relapse and EDSS progression; 96.6% of persistence;
Murcia	Spain	NTZ switch	NTZ switch study 12month; 15.4month FU; small sample; 31 NTZ infusions; WO with MP monthly; 86.4% relapse-free after 15.4 months; 100% T1Gd+ lesion-free; no difference in EDSS vs BL.
Murcia	Spain	FDO	Prospective FDO study with 18 patients; 5.56% symptomatic bradycardia; asymptomatic ECG changes were observed at 6 (44%) and 24 hours (20%) after first dose of FTY; no clinical or ECG effects requiring FTY discontinuation.
Santiago de Compostela	Spain	Efficacy	Retrospective study with 101 patients; 30% with previous NTZ treatment; FTY demonstrated efficacy in 94% of patients; only six of them (5,9%) had more relapses and/or disability progression. 30% of patients previously treated with NTZ; disability stable or improved in 94%; 96% of persistence
Valencia	Spain	NTZ switch	NTZ switch study with 11 patients; 37.9 NTZ infusions; 9 weeks WO; 11.7month FU; small sample; WO with MP; ARR 0.09 after 11.7 months and 90.9% patients relapse-free; 81.8% T1Gd+ lesions-free; -0.3 EDSS difference vs BL; 90.9% of persistence
MS registry Sweden	Sweden	FTY vs NTZ	Sweden registry; 628 patients; 48.1% with NTZ as previous treatment; >12month FU; Time since onset of symptoms of MS (years) 9.9 (NTZ 7.1; NTZ-naïve 8.9). Treatment discontinuation rate for FTY was higher than for NTZ, which entirely is explained by a higher drop-out rate for NTZ experienced FTY patients. 82.3% of persistence (NTZ 86.6%; NTZ-naïve 86.9%)
Multicentre Sweden	Sweden	Registry	Prospective study with 674 patients; 33% with NTZ as previous treatment; 12month FU; The clinical measures improved vs BL; EDSS (+ 0 %), MSSS (- 12.2 %), SDMT (+ 8.7 %), MSIS-29 physical/psychological scores (-7.4% and -13.3%, respectively) EQ-5D (+ 6.3 %). Blood lymphocytes values were registered throughout the treatment (x 109/L); no difference in EDSS vs BL. 0.59% cardiac disorders (bradycardia, AV-block, hypertension); 89.6% of persistence;
Basel	Switzerland	NTZ switch	NTZ switch study with 15 patients; 41.4 NTZ infusions; 8 weeks WO; 8month FU; 100% JCV+; 46% relapse-free; 46.2% T2 and T1Gd+ lesion-free; Clear pattern of increasing disease activity after alpha4-integrin desaturation. Preliminary analysis revealed increased TNF-α and IL-8 after stopping of NTZ. Autoantigen and viral antigen specific IFNγ production peaked after stopping NAT and decreased upon FTY start. A shorter wash out period is more suitable for switching patients safely. Very small sample; 86.7% of persistence;

Cohort	Country	Focus	Comments
SWISSASCENT	Switzerland	PRO	212 patients; 28% with NTZ as previous treatment; 8.3month FU; ARR 0.2; 75% ARR reduction; 88% relapse-free; QoL (MSIS-29) remained stable over time in patients switching from other DMTs but those patients showed a significant improvement in the mental subscore; Treatment satisfaction improved 14 points upon switch from other DMTs to FTY (p= 0.001). This was true for all domains of the TSQM-9 scale. 75% ARR reduction; 99.5% patients did not require any medical intervention, nor monitoring on day two (98%), and continued FTY treatment after the FDO (99.5%); 96% of persistence;
Istanbul	Turkey	Efficacy	Prospective study with 109 patients; 5.5month FU; 90% relapse-free; 8.3% relapsed in the 1st 3 month, 1.7% relapsed more than 3 month after initiation; 94.5% persistence
Wimbledon	UK	FDO	FDO study with 143 patients; 94% discharged at 6h; 4% extended monitoring and discharged at 8h; 1% overnight stay for symptomatic bradyarrhythmia;
Boston	USA	Efficacy	Retrospective study with 177 patients; 15.3% with NTZ as previous treatment; >12month FU; no demographic feature was significantly associated with time to first event in patients treated with FTY; 83.6% relapse-free; 85.9% free of MRI activity; disability stable or improved in 75.7%; 87.6% Free of relapse and MRI activity; 50.3% free of relapse, EDDS progression and MRI activity
Boston	USA	FDO	Retrospective FDO study with 305 patients; 84.6% of persistence; -14 bpm at nadir; 98.7% FDO uneventful; 0.66% 2nd degree AVB;
Cleveland Clinic	USA	FDO	Retrospective study with 317 patients; 3.3month FU; There were no significant differences in T25FW, PHQ-9, MSPS or EQ5D (n=164) compared to baseline. FDO uneventful in 97.8%; 90.5% of persistence;
Cleveland Clinic	USA	Efficacy	Study with 306 patients; 11.1month FU; MS performance scale, PHQ9 and EQ5D remained stable; T25FW deteriorated; ARR 0.12; 64% relapse-free; 59.3% Free of relapse and MRI activity; 76.1% of persistence;
Cleveland Clinic	USA	Efficacy	Retrospective study with 196 patients; 8.6month FU; 85% stable or improved in T25FW; 91.6% stable or improved in 9PHT; -0.03 mean change in PHQ9 (p= 0.06); -0.005 mean change in EQ-5D (p= 0.02); 0.54 mean change in MSPS (p= 0.20); Clinician-derived and patient-reported disability scores and patient-reported QoL measures were stable
Cleveland Clinic	USA	Other	Prospective study with 232 patients; 6.4month FU; Small magnitude increases in central subfoveal thickness and outer retina thickness may occur on FTY therapy. Decrease in ganglion cell thickness and peripapillary RNFL thickness identified over 6 months may relate to the MS disease process. FTY may selectively affect foveal measures. Further studies with appropriate controls are needed to elucidate the effect of FTY on macular volume and specific retinal layers.
Cleveland Clinic	USA	FTY vs DMF	Retrospective study with 317 patients; 3 month FU; difference in currently available data for each treatment group; 0.03 relapses/patient (DMF 0.12); 97% relapse-free (DMF 89%); similar T1Gd+ lesion-free results; 92% of persistence (DMF 82%);
commercial health plan or Part D program	USA	PRO	US claims database analysis; Adherence rate similar to NTZ; MPR 75.4% (NTZ 79%)

Cohort	Country	Focus	Comments
Cullman	USA	NTZ switch	NTZ switch study with 75 patients; >6month FU; 97.3% Free of relapse and T1Gd+; 75% of persistence;
Houston	USA	FDO	Continuous on-line electrocardiographic telemetry may detect abnormal rhythms in a small number of patients started on FTY. The clinical significance of these is unclear. 3% bradycardia secondary to atrioventricular conduction abnormality; 1.7% sinus bradycardia with accelerated idioventricular rhythm.
Medco Health Solutions	USA	PRO	pharmacy claims; 248 patients; 12month FU; FTY initiators were more compliant, less likely to discontinue treatment, and discontinued later; 68.8% of persistence; adherence >87.4% with MPR>0.8
Multicenter1 USA	USA	PRO	Prospective study with 205 patients; 6month FU; Switching to FTY demonstrated improvements in patient-reported treatment satisfaction, fatigue, depressive symptoms, and physician assessments vs remaining on IFN.
Multicenter2 USA	USA	PRO	Cross-sectional survey with 380 patients; most FTY patients were satisfied with their FDO experience. Satisfaction with FTY was high and observed higher among treatment-experienced compared to treatment-naïve patients.
Multicenter3 USA	USA	NRO	Online survey of 102 neurologists; Among the DMTs available at the time of the survey, neurologists reported that patients were most satisfied with, and adherent to FTY
NARCOMS	USA	PRO	50 patients (NTZ 50); 54month FU; PDDS; Transitioning of natalizumab after 2 years was associated with a statistically significant increase in the likelihood of participant-reported disability progression and increased mean disability. No report of mean washout period. 6 month difference between FTY and NTZ follow up
Partners MS Centre	USA	FTY vs NTZ	Retrospective study with 38 patients; 18month FU; JCV serology to determine therapy; 44% JCV+; patients treated with NTZ had a longer time to relapse (2.20 $p = 0.095$) in the unadjusted analysis; patients treated with NTZ had a significantly lower hazard of a relapse in each model; patients treated with NTZ had a significantly longer time to first inflammatory event (relapse or new GD+ lesion) 2.31 $p = 0.041$; 11.6% JCV seroconversion; FTY had a better persistence on treatment; 77.8% of persistence (NTZ 63.8%)
PharMetrics Plus™	USA	PRO	US claims database analysis; 889 patients; 12 months FU; Persistence with and adherence to FTY compared with NTZ, GA and IFN; 72.1% of persistence (GA 60.5%; IFN 56.3%; NTZ 61%; FTY vs all $p<0.001$); 93.8% with MPR>0.8 (GA 88.1%; IFN 88.1%; NTZ 88.7%; FTY vs all $p<0.001$); 89.7% with PDC>0.8 (GA 84.1%; IFN 85.1%; NTZ 86.0%; FTY vs all $p<0.001$); no safety data reported
PharMetrics Plus™	USA	FTY vs NTZ	Retrospective study with 185 patients (NTZ 185); 12month FU; 68.1% relapse-free (NTZ 68.6%; $p= 0.9110$); 71% reduction of patients who required an MS-related inpatient admission (NTZ 87%; $p= 0.2620$); 58% reduction of patients who visited an emergency department (NTZ 69%; $p= 0.7215$); FTY reduced the MS-related outpatient claims ($p< 0.0001$) and NTZ increased the MS-related outpatient claims ($p< 0.0001$); 71.9% of persistence (NTZ 76.2%; $p=0.3427$)
PharMetrics Plus™	USA	FTY vs DMTs	US claims database analysis; 132 patients; 12month FU; ARR 0.19 (GA 0.51); 87.1% relapse-free (GA 75%; $p= 0.012$); 59% reduction in the probability of having a relapse vs GA ($p =0.0091$); 360 days median time to 1st relapse (GA 274); Patients treated with FTY had 62% fewer relapses per year ($p =0.0013$); 73.5% of persistence (GA 62.9%; $p= 0.0643$);

Cohort	Country	Focus	Comments
PharMetrics Plus™	USA	FTY vs DMTs	US claims database analysis; 128 patients (DMTs 397); 18month FU; relapse outcomes results superior to IFN/GA; 57.0% of persistence (DMTs 45.1%; p=0.0187);
Seattle	USA	Other	Retrospective study with 126 patients; 7month FU; Trend of RNFL and GC-IP thinning was noted but did not reach statistical significance. The observed trend in macular volume change was independent of age, gender, time since MS diagnosis, or prior history of optic neuritis. FTY-treated MS patients experience a decrease in macular volume during the first year of treatment.
US Lord™ and PharMetrics Plus™	USA	PRO	US claims database; 1553 patients; >6month FU; Higher persistence with FTY vs DMF (p< 0.01)
EPOC	USA, Canada	FDO	Prospective study with 976 patients; 6month FU; 18.5% of patients with pre-existing cardiac conditions and did an ECG at 6h post-dose. Patients with pre-existing cardiac conditions (PCCs: history of cardiac arrest, myocardial infarction, unstable ischemic heart disease, coronary spasm, congestive heart failure, HR <55bpm, sick sinus syndrome, sinoatrial heart block, or any factors considered to be cardiac risk factors by the study investigator); 0.01% with HR< 40bpm; 18.2% with new ECG abnormalities; 98.5% discharged at 6h;
EPOC	USA, Canada	PRO	Prospective study with 847 patients; 6month FU; 50.5% recovered from depression (DMT 25.3%); 8.1% became depressed (DMT 9.3%);
EPOC	USA, Canada	PRO	Prospective study with 790 patients; 6month FU; TSQM scores were significantly greater with FTY than with DMT (Global satisfaction, Effectiveness, Side Effects, and Convenience; p< 0.0001); Improvements in FSS and BDI-II score were significantly greater with FTY than with DMT (p< 0.0001); PRIMUS Activities score were numerically reduced (better outcomes in fatigue and depression) with FTY versus DMT; SF-36 scores were significantly greater with FTY than with DMT (p< 0.0001), except for the domains 'Physical functioning' and 'General health perceptions'; no relapse, MRI or disability reported data; 90.4% of persistence (DMTs 87.1%)
EPOC	USA, Canada	PRO	Prospective study with 790 patients; 6month FU; therapy switch from DMTs to FTY may improve depressive symptoms, particularly somatic concerns
EPOC	USA, Canada	Safety	Prospective study with 783 patients; 6month FU; Lymphocyte counts did not significantly differ by occurrence or non-occurrence of specific infection types. The overall incidence of infection was similar in the fingolimod and DMT.

9PHT - 9-Hole Peg Test; ARR - Annualized Relapse Rate; AV – Atrioventricular; AVB - Atrioventricular Block; BBs - Beta Blockers; BDI-II - Beck Depression Inventory-II; BL – Baseline; BP - Blood Pressure; bpm - Beats Per Minute; CCBs - Calcium Channel Blockers; CV – Cardiovascular; DMF - Dimethyl Fumarate; DMT - Disease Modifying Treatments; ECG – Electrocardiogram; EDSS - Expanded Disability Status Scale; FDO - First Dose Observation; FSS - Fatigue Severity Scale; FTY – Fingolimod; FU - Follow-Up; GA - Glatiramer Acetate; GC-IP - Ganglion Cell/Inner Plexiform; HADS - Hospital Anxiety and Depression Scale; HR - Heart Rate; JCV - John Cunningham Virus; MP – Methylprednisolone; MPR - Medication Possession Ratio; MRI - Magnetic Resonance Imaging; MS - Multiple Sclerosis; MSFC - Multiple Sclerosis Functional Composite; MSIS - Multiple Sclerosis Impact Scale; MSPS - Multiple Sclerosis Performance Scales ; MSSS - Multiple Sclerosis Severity Score; NT - Non-Treated; NTZ - Natalizumab; PDSS Patient Determined Disease Steps; PHQ-9 Patient Health Questionnaire 9; PNTZ - Patients who have natalizumab as previous treatment; PTs - Pivotal Trials; QoL - Quality of Life; RNFL - Retinal Nerve fibre Layer; SDMT - Symbol Digit Modalities Test; SSRIs - Selective Serotonin Reuptake Inhibitors; T - Treated; T1Gd+ - T1 Gadolinium-Enhancing Lesions; T25FW - Timed 25-Foot Walk; TSQM - Treatment Satisfaction Questionnaire for Medication; WO - Washout;

4.4.2. Baseline characteristics

Number of patients is the only baseline characteristic that it is reported by virtually every study. Regarding multiple sclerosis outcome measures, a big proportion of studies report disability and relapses, but only 6.3% publish MRI baseline data. Almost half of the studies reported if natalizumab was the previous treatment and, within those studies, the vast around 55% just mention the percentage of patients previously treated with natalizumab. (Figure 17)

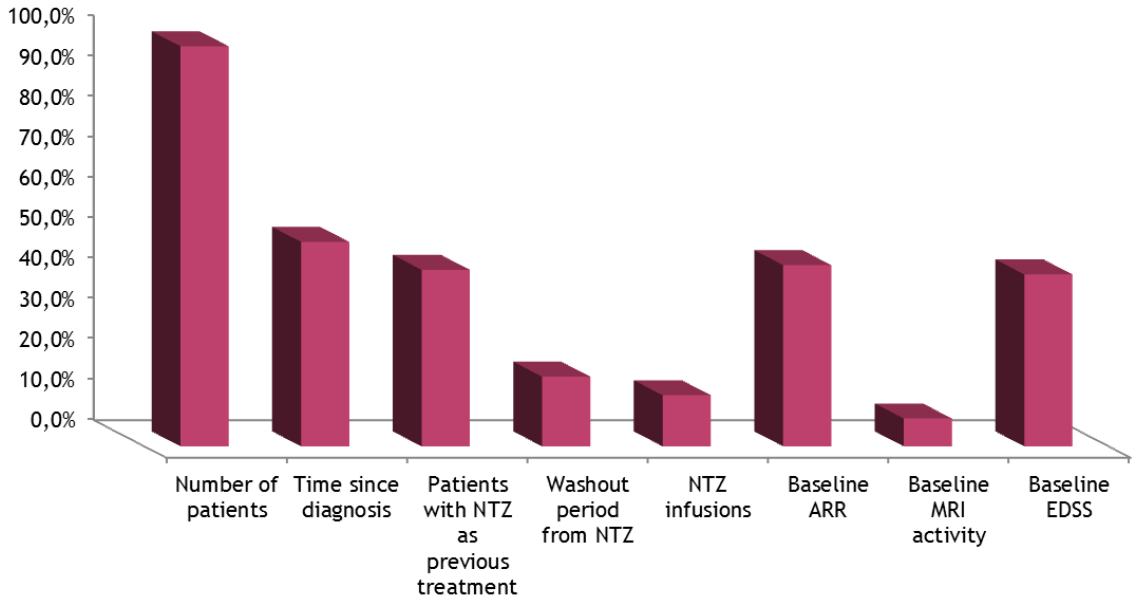

Figure 17 Baseline characteristics reported by studies

Patients in the TRANSFORMS study had, at baseline, mean disease duration of 7.5 years, 1.5 relapses in previous 12 months (2.3 in previous 24 months); EDSS of 2.24 and 67.4% of patients did not have T1Gd+ lesions on the MRI. The average patient enrolled in these studies had longer disease duration and higher EDSS score, but a lower ARR. If we exclude USA studies, considering them as a separate subgroup due to USA's first line label,

the group does not change much, and oddly enough USA patients all have more than 7.5 years of disease duration. (Table 16)

Table 16. Reported disease status at baseline. Studies weighted by patients enrolled

	Review	World	USA only
< Disease duration	8,0%	0,0%	11,0%
< ARR	63,6%	69,9%	61,5%
< EDSS	3,3%	7,7%	2,4%

4.4.3. Relapse outcomes

Relapse outcomes were reported in 48.8% of studies, which enrolled 8,483 patients. Generally, the vast majority of studies confirmed fingolimod efficacy in reducing relapse activity seen in pivotal trials. The ARR reduction weighted average was 70.1%, and the reported ARR reduction was superior to 68% in all but two studies. (Figure 18)

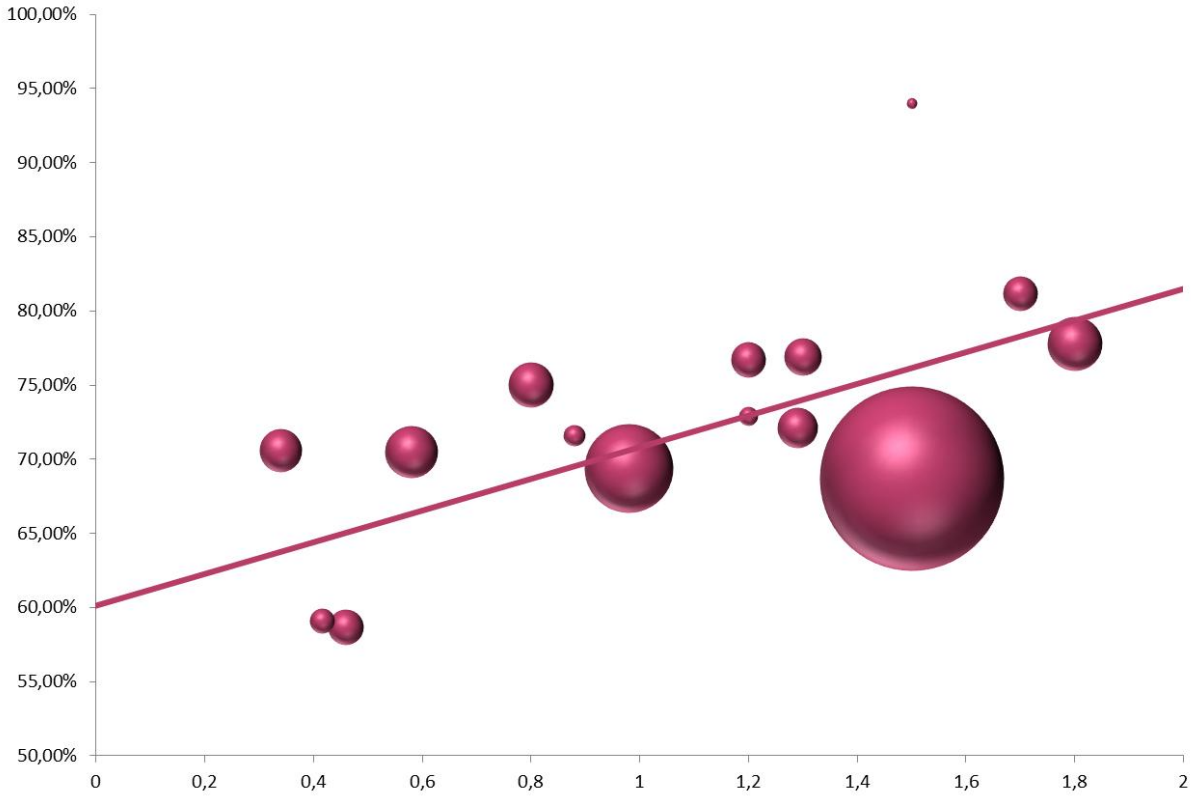

Figure 18. ARR reduction. The bubble size represents the patient numbers

In the studies where natalizumab was used as control, fingolimod seems to be, at least as effective as natalizumab when used in second line therapy. Fingolimod reported similar or

superior results in 50% of the studies. When the control was glatiramer acetate, interferon or DMF, fingolimod always showed superior relapse outcome results.

4.4.4. Disability

It is pretty difficult to summarize disability outcomes in the 29.5% studies that reported it. Main reason being the non-standardized approach of how this should be reported. In clinical trials, the most common endpoints are:[263]

- EDSS score difference vs baseline
- NRS score difference vs baseline
- MSFC score difference vs baseline
- Proportion of patients without confirmed disability progression (confirmed at 3 or 6 months)
- Time to progression of disability as measured by EDSS at 12 weeks

In the clinical practice EDSS is the most commonly used measure of disability. Although it's far from perfect, it's widely used both in clinical trials and clinical practice because it's easy to apply and it is accepted by the regulatory authorities. The main problems of EDSS scale are its dependence on rater judgement and its main focus on physical disability.

A total of 13 studies with 5,249 patients reported proportion of patients free of disability progression, and fingolimod achieved a weighted average of 89.9% after 13.2 months. In the 15 studies that reported EDSS difference from baseline, involving 5,212 patients, the weighted average difference in the EDSS score compared to the baseline was -0.07, and a similar result is obtained if we exclude the natalizumab switch studies.

4.4.5. MRI Outcomes

Measuring MRI outcomes in the clinical practice maybe challenging in some countries, where the access to routine MRI exams is restricted based on budgetary issues. Furthermore, MRI scanning machines have evolved a lot, in the last decades, and are very expensive, and thus aren't upgraded as much as it would be desirable in some MS centres. These two reasons may help to understand why MRI outcomes in the real world are more difficult to measure than in clinical trials. And last, but not least, there are many MS centres where there is still some technological issues to overcome to make it easier to monitor MRI

lesions or brain volume changes. Automatic or semi-automatic software to count lesions or evaluate brain volume changes are already on the market, some are even freeware, but only a minority of MS centres use it in the clinical routine. It is my opinion that in the next 5 or 10 year these methods will become mainstream and most probably will be readily available in the built-in software of the MRI scans – it's unavoidable; it's only a matter of time.

MRI outcomes were reported by 16.8% of the studies reviewed. However these studies combined represent only 4.6% of total patients. A weighted average of 91.6% patients were free of T1Gd+ lesions and 73.6% of them were free of new T2 lesions. These numbers are consistent or slightly better than the results of the clinical trials. In TRANSFORMS, after one year of treatment, 90.1% patients were free of T1Gd+ lesions and 54.8% of them were free of new or enlarged T2 lesions. In FREEDOMS and its extension, after 4 years of fingolimod treatment 79.5% patients were free of T1Gd+ lesions and 80.9% of them were free of new or enlarged T2 lesions.

Worth to mention is a 50 patient's study, from Puerto Rico, where it was evaluated the number of T1 hypointense lesions and brain atrophy. It was reported 86% patients free of new black holes and 90% free of brain volume loss after one year of fingolimod treatment.

Another study, with 30 patients, evaluated the cortical and subcortical volume changes. This study suggests that fingolimod treatment preserves the structural thickness in the majority of the brain areas over one year of treatment.

4.4.6. Composed Outcomes

I think the natural evolution of MS outcome measures is going from evaluating only the clinical outcomes, EDSS and relapses, to focus in the patient as whole and thus considering the all 4 measures of NEDA-4. It's of no use to have a treatment very good at reducing the clinical activity, if it does not do much about the imaging outcomes, T2 and T1Gd+ lesions and brain volume loss, or *vice-versa*. The ultimate goal of each and every MS treatment should be to achieve Non Evidence of Disease Activity in its 4 dimensions. (Figure 19)

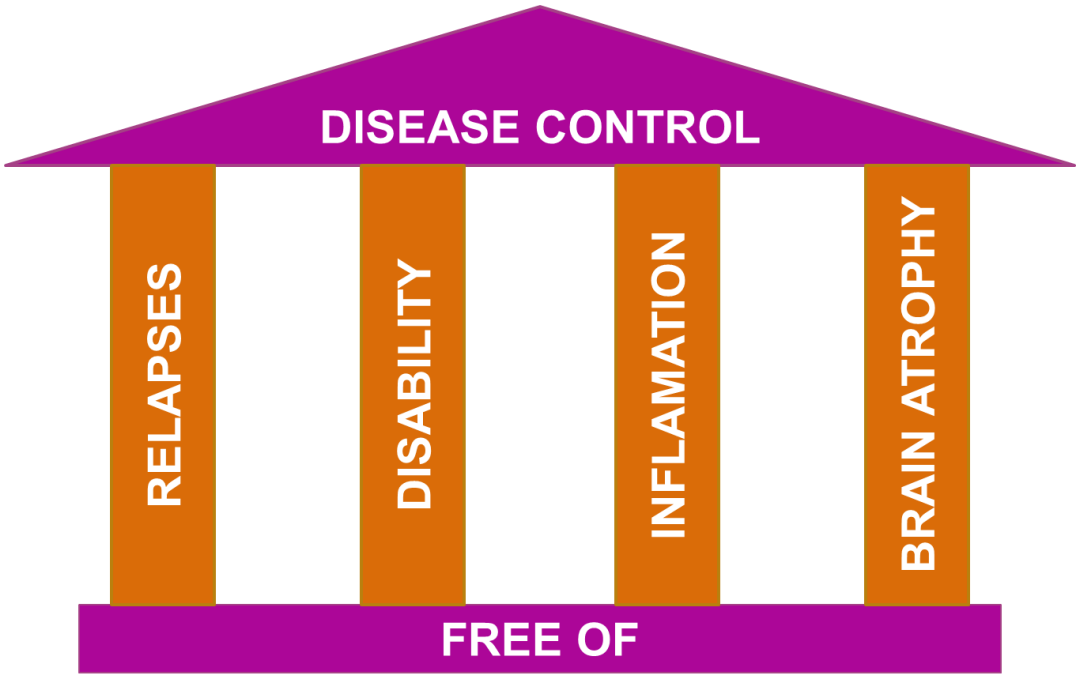

Figure 19 NEDA-4 should be the goal of every DMT

Only 13 studies, representing 4.9% of the patients, have reported composed outcomes. Due to the low number of studies reporting most of the composed outcomes, these results most be interpreted with caution. (Table 17)

Table 17. Weighted average of composed outcomes reported

	WA	Max	Min	Nr	Pts
Studies	117	306	21	13	1515
Patients with NTZ as previous treatment	28,2%	100,0%	15,3%	7	741
Baseline ARR (previous 12 months)	0,64	1,20	0,34	6	606
Baseline MRI activity	78%	78%		1	100
Baseline EDSS	2,4	3,9	2,3	6	572
FU (months)	11,96	16,30	6,00	12	1494
Free of relapse and T1Gd+	93,5%	97,3%	89,1%	2	141
Free of EDDS progression and MRI activity	42%	42%	42%	1	37
Free of relapse and MRI activity	68,8%	87,6%	47,6%	3	504
Free of relapse and EDSS progression	**66,6%**	**79,7%**	**56,3%**	**7**	770
Free of relapse, EDDS progression and MRI activity	**62,5%**	**72,5%**	**50,3%**	**3**	419

WA – Weighted Average; Max – Maximum; Min – Minimum; Nr – Number of studies; PTS – Number of patients

Only two of the composed outcome measures reported can be compared with data from the clinical trials. (Table 18) With the exception of Free of EDSS progression and MRI activity, which was only reported by one study, fingolimod achieved a good proportion of patients free of activity in all the other composed outcomes. This reinforces the effectiveness of fingolimod in the clinical practice.

Table 18. Composed outcomes of pivotal trials

	FREEDOMS 2 years	TRANSFORMS 1 year
Patients	425	429
Mean Follow-up (months)	24	12
Baseline ARR (previous 12 months)	1.5	1.5
Baseline EDSS	2.24	2.2
Free of relapse and EDSS progression	**62%**	**79%**
Free of relapse, EDDS progression and MRI activity	**33%**	**46%**

4.4.7. PRO, FDO and Other outcomes

4.4.7.1. Persistence

Persistence and adherence are very important factors to the effectiveness of any treatment, because even with a very efficacious treatment it is of no use if the patients discontinue treatment, or do not adhere to the recommended posology.

Table 19. Studies excluded from the weighted average calculation

Cohort	Patients	FU (months)	Persistence [Adherence]
Gilenya* Go Program(TM)	1700		85.5%
Tokyo	44		75%
MSBase Registry	148	11	Persistence on FTY was better compared to DMT (hazard ratio 0.39)
Granada	42		98%
Multicenter1 Spain	101		96%
Santiago de Compostela	143		99.4%
Wimbledon	305		84.59%
Boston			Adherence [MPR 75.4% (NTZ 79%)]

FU – Follow-Up; FTY – fingolimod; DMT – Disease Modifying Treatment; MPR – Medication Possession Rate; NTZ – natalizumab

Persistence/adherence was reported by 46.3% of the studies, which observed a total of 18,471 patients. To calculate the weighted average, 8 studies were excluded because

either the persistence was not reported in a way compatible with the calculation, or because the study did not report the follow up period. The weighted average, considering both patient numbers and follow-up duration, was 82.8%, after 11.6 months of follow-up.

4.4.7.2. Patient and neurologist reported outcomes

The impact of a chronic, debilitating disease as MS goes beyond its impact on patient's physiology, and is of utmost importance to give the deserved relevance to other outcomes that are also important, like quality of life, treatment satisfaction, work productivity, cognition, impact on family and care givers, etc. Unfortunately most pivotal trials do not assess this outcomes and that's where real world evidence can give an important contribution to the body of knowledge.

Montalban et al.[264] published one study about the effect of treatment in quality of life and depression in the phase II fingolimod trial. This is the only one of its kind and, being a randomized, double-blind, placebo-controlled clinical trial, it's important to look at its results, even though the lowest dose used was 1.25mg, in contrast with the approved 0.5mg. After 6 months of treatment it was possible to observe an improvement in the reported quality of life in fingolimod treated patients and a deterioration in the placebo arm (1.25mg vs placebo, p= 0.044), particularly in the fatigue/thinking sub-domain of the Hamburg Quality of Life Questionnaire in Multiple Sclerosis (HAQUAMS). This study suggests that with fingolimod a smaller proportion of patients present signs of clinical depression than with placebo (p= 0.0018), even though at baseline there was more fingolimod patients with signs of clinical depression. The 24 month extension of this study suggests that this effect can be observed in the first 6 month of treatment, and after that the depression symptoms are stabilized until month 24.

Generally speaking fingolimod has a positive impact on patients' lives, in the domains reported by these studies: quality of life, cognition, tolerability, depression, treatment satisfaction and fatigue. (Table 20) In these 19 studies, which reported PRO/NRO, a total of 6,905 patients were followed up. Given the great impact this domains have in patients' lives it is desirable that in the future even more studies look into this.

Table 20. Patient and Neurologist Reported Outcomes summary

Dimension	Patients	Patients (%)	Studies (%)	Results
Cognition	711	2,3%	2,1%	2/2 positive
Depression	1609	5,2%	7,4%	2/7 neutral; 5/7 positive
Fatigue	790	2,5%	1,1%	1/1 positive
QoL	6136	19,7%	7,4%	2/7 neutral; 5/7 positive
Tolerability	4021	12,9%	2,1%	2/2 positive
Treatment Satisfaction	1382	4,4%	4,2%	4/4 positive

QoL – Quality of Life

4.4.7.3. First Dose Observation

The transient cardiovascular effects of the first fingolimod dose were reported by 25.3% of the studies, which enrolled 16,683 patients. The first dose of fingolimod was uneventful in a big proportion of patients, usually > 90%, and most of the patients (≈95%) were discharged after the 6 hour observation period. In clinical trials it was reported 4.7% incidence of first degree AV block and <0.2% of second degree AV block.

FIRST[208] was a 2,417 patient open-label multicentre study, sponsored by Novartis, to assess the overall short-term safety and tolerability of fingolimod 0.5mg. This study enrolled 12.2% of patients with cardiac risk and 5% treated with calcium channel blockers and/or beta blockers. A Mobitz type I second-degree atrioventricular block was found in 4.1% patients before the first dose administration. In the group without cardiac risk there was an incidence of new events post-dose of 1.1% and this incidence was 4.0% in the group with cardiac risk.

An Italian study,[199] with 112 patients found that 20.1% patients showed abnormalities in the baseline ECG, including a first-degree atrioventricular block in 0.9% of patients. There was 1.8% of *de novo* first degree AV block and 0.9% of *de novo* second degree AV block.

4.4.7.4. Other outcomes

A 12 month follow up study of 55 Italian patients reported that active patients had more relapses before fingolimod (p= 0.043), a lower mean white blood cell count (p = 0.027) and a lower mean lymphocyte count (p = 0.05) compared to disease-free patients. The authors' hypothesis is that "protective" cell subsets maybe also affected by the excessive lymphopenia.[191]

One Italian study, with 35 patients, focused on fingolimod effect on headache and reported 2.9% of *de novo* headache. Headache was reported in 23-25% of patients in the phase III clinical trials, but there is no reference to the baseline incidence.[203]

Retinal and macular volume effects of fingolimod were the focus of two American studies, with 232 and 126 patients.[241,256] In the Cleveland Clinical study wasn't possible to detect a change in macular volume, but a small increase in central subfoveal thickness and outer retina thickness was detected. The study from Seattle reported a decrease in macular volume (p=0.003). Both studies reported a trend of ganglion cell/internal plexiform layer and retinal nerve fibre layer thinning. It can be speculated that these changes maybe disease and/or treatment related, thus further studies with a control arm would be helpful to clarify how much of these effects can be attributable to fingolimod.

4.4.8. Natalizumab Switch

Given that natalizumab patients have a risk of developing PML, especially after 24 months of treatment;[265] it is common to switch these patients to fingolimod, as a therapy of similar efficacy. Natalizumab patients are usually in a more advanced state of disease, than patients previously treated with first line DMTs. So it is expectable to get results that aren't as good as when fingolimod is used in a DMT switch population.

Around 20% of studies focused on the natalizumab switch strategy. Many strategies have been employed (Table 21) and the review of these studies suggests that the switch to fingolimod is the most successful strategy, even though it is not in this population that we can observe fingolimod's best results.

Table 21. Natalizumab switch strategies used in these studies

Natalizumab switch strategies
Different washout periods from 0 to 52 weeks
Washout period with monthly methylprednisolone
Fingolimod as subsequent treatment
DMTs as subsequent treatment
Other immunomodulating agents as subsequent treatment
No treatment after natalizumab

EDSS values greater than 3, washout period length and ARR (pre-treatment and during natalizumab treatment) have been proposed as predictors for relapses post-natalizumab,

but probably due to the small samples, it has been impossible to achieve significant results across several studies. The washout period length seems to be the most consensual predictor.

The studies reviewed suggest that in a significant number of patients after natalizumab interruption disease activity goes to pre-natalizumab levels in the first 6 months. The majority of studies recommend a maximum washout period of 6 to 12 weeks. In most studies, a shorter washout was associated with a lower risk of disease reactivation. Fingolimod in these studies has shown to be able to control disease reactivation in most patients. It can be speculated that patients previously treated with natalizumab are, for obvious reasons, in a more advanced disease level, when the diffuse damage starts to be relatively more important than the focal damage, even though the two coexists. This hypothesis could explain both the inefficacy of first line DMTs, which only act on the inflammation (focal damage); and the efficacy of fingolimod, which has a double mechanism of action, acting upon the immune system, reducing the focal damage, and directly in the central nervous system reducing the diffuse damage.

In this population, even though it's not easy to reach a conclusion, fingolimod seems to avoid a reactivation of disease in most patients.

4.4.9. Safety Outcomes

Safety issues, even more than efficacy outcomes, suffer from a great variability in the way they are reported. In the 7 cohorts analysed, 145 different AEs were identified, but some appear twice in the list if in one study was classified as AE and in another study was classified as SAE. In 77.9% of the AEs/SAEs, the incidence reported in the clinical trials was superior or similar to the reported incidence in the studies reviewed. Generally, the real world practice confirms the safety profile already established in the clinical trials.

In the first dose of fingolimod a short-term and asymptomatic heart rate decrease can be observed in most cases. The studies reviewed confirm the findings in the clinical trials for the cardiovascular AEs[44,266], with only a small number of events, reported in more than one of these studies, being unheard before. In Table 22 can be found a summary of the cardiovascular AEs not reported or reported with higher incidence than in clinical trials.

Table 22. CV AEs not reported or reported with higher incidence than in clinical trials

AE	Incidence in individual studies	Incidence in all studies
Angina pectoris	0.0% (1)	0.009%
Blood pressure decreased	0.6%	0.04%
Cardiac flutter	0.3%	0.02%
Heart rate decrease	1.4%	0.1%
Palpitations	1.5%; 0.6%	0.24%
QT interval prolongation	0.7%	0.11%
Sinus bradycardia	0.0% (1); 1.0%	0.08%
Tachycardia	0.1%	0.02%
Thromboembolic events	0.9%; 0.5%	0.20%
Ventricular extra systoles	0.2%	0.05%
Pulmonary oedema	0.1%	0.015%

Incidence in all studies was calculated by dividing the number of reports in the study, by the total population enrolled in the reviewed studies for safety outcomes

Asymptomatic ALT and bilirubin increases were reported mostly in the first months of fingolimod treatment in clinical trials. After treatment interruption, the enzymes returned to normal levels within 6 months.[44,267] The reviewed studies reported also other liver function changes, which are summarized in Table 23. Once more, the different ways of reporting these AEs, make it difficult to make a comparison between studies.

Table 23. Liver function AEs not reported in clinical trials with these designations

AE	Incidence in individual studies	Incidence in all studies
Liver disorder	1.5%	0.11%
Liver dysfunction	6.5%	0.48%
Liver enzyme elevation	3.5%	0.45%
γ-glutamyltransferase increased	1.8%; 3.9%; 1.1%	0.70%

Incidence in all studies was calculated by dividing the number of reports in the study, by the total population enrolled in the reviewed studies for safety outcomes

Macular oedema was reported in 0.6% patients in clinical trials[44], mostly in the first 6 months of treatment, and in most cases with recovery after treatment interruption without the need of macular oedema treatment. The macular oedema incidence reported in all studies was 0.32%, and it was calculated by dividing the number of reports in the study, by the total population enrolled in the reviewed studies for safety outcomes.

Fingolimod overall incidence of infections and serious infections was similar to placebo in clinical trials, with lower respiratory tract infections being the only type of infections with slightly higher incidence than placebo[44]. The reviewed studies confirmed this infection

profile. Worth to mention are the incidences in all studies (calculated by dividing the number of reports in the study, by the total population enrolled in the reviewed studies for safety outcomes) of reactivation of chronic viral infections (0.79%) and Herpes zoster infections (0.25% vs 3.1% reported in clinical trials[44]).

Fingolimod mechanism of action involves the selective and reversible retention of lymphocytes in secondary lymphoid organs, thus having an impact in the serum quantification of these cells. Because the different studies may have used different thresholds and ways of reporting, it's useful to present these differences in Table 24. In clinical trials it was reported a 73% average decrease in lymphocyte count compared to baseline and lymphopenia was reported as an AE in 12% patients[44]. Leukopenia was reported in 2.82% patients in FREEDOMS and 1.6% in TRANSFORMS.

Table 24. Leukocyte and lymphocytes count changes

AE	Incidence in individual studies	Incidence in all studies
Leukopenia and lymphopenia	6.9%	1.03%
Lymphocyte count decreased	0.3%; 1.24%; 3.8%; 2.1%; 2.3%	1.21%
Lymphopenia	6.5%	1.23%
White blood cell count decreased	8.3%	0.3%

Incidence in all studies was calculated by dividing the number of reports in the study, by the total population enrolled in the reviewed studies for safety outcomes

Incidence of malignancies in clinical trials was similar in the fingolimod 0.5mg and in the placebo arms. In clinical trials, the Basal Cell Carcinoma (BCC) showed a trend towards a bigger incidence with fingolimod 0.5mg than with placebo[44]. Table 25 summarizes the malignancies findings in these studies, which were not reported in clinical trials. Other malignancies not shown here had an incidence lower than in clinical trials or have not been reported in these studies. Two studies reported only skin cancer, whereas in clinical trials skin cancers were classified as BCC or Squamous Cell Carcinoma (SCC). The ratio BCC/SCC is similar to the observed in general population[268], and different from the observed in immunosuppressed populations[269].

Table 25. Malignancies not reported in clinical trials

AE	Incidence in individual studies	Incidence in all studies
Other malignant neoplasms	0.4%; 0.5%	0.12%
Pancreatic carcinoma	0.1%	0.013%
Skin cancer	0.7%; 0.1%	0.12%

Incidence in all studies was calculated by dividing the number of reports in the study, by the total population enrolled in the reviewed studies for safety outcomes

Other AEs not reported in clinical trials are summarized in Table 26. The very low incidences suggest that it is not probable an association to fingolimod treatment, but time will tell.

Table 26. Other AEs not reported in clinical trials

AE	Incidence in individual studies	Incidence in all studies
Bronchoconstriction	2.1%	0.31%
MS symptoms worsening	0.76%	0.12%
Nephrolithiasis	0.1%	0.01%
Decreased renal function	0.3%	0.04%
Itchiness	0.06%	0.01%
Muscular weakness	1.1%	0.08%
Physical weariness	1.7%	0.12%
Pregnancy	0.3%	0.04%
Reproductive toxicity	0.9%	0.13%

Incidence in all studies was calculated by dividing the number of reports in the study, by the total population enrolled in the reviewed studies for safety outcomes

5. Conclusions

Fingolimod is a small lipophilic molecule, which readily crosses the BBB, and appears to have a dual mechanism of action. Its immunomodulatory effect is exerted through the selective retention of central memory and naïve T cells in secondary lymphoid tissues, preventing their egress to the CNS, where they would cause the focal inflammation characteristic of multiple sclerosis.

The effectiveness of fingolimod in the treatment of Multiple Sclerosis seems to go beyond its immunomodulatory effects. Studies in several *in vitro* and *in vivo* models have shown evidence in favour of a direct neuroprotective function of fingolimod, and results from clinical trials are consistent with a mechanism of action that includes direct effects in CNS cells, reducing the diffuse damage. Moreover, recent reports in different pathologies suggest that fingolimod has neuroprotective effects in several models.

Fingolimod has proven in an extensive clinical development program its effectiveness in reducing relapse rate, disability progression, MRI activity and brain volume loss. In FREEDOMS, after 2 years, the likelihood of achieving Non-Evidence of Disease Activity in its 4 parameters with fingolimod was more than 4-fold higher vs placebo.

In the clinical development program fingolimod showed to avoid a higher number of relapses. In the long-term, early treatment with fingolimod is associated with lower costs per relapse avoided compared with delayed treatment. Under the Portuguese NHS hospital perspective, early treatment with fingolimod is expected to result in better clinical outcomes associated with a more efficient healthcare resources allocation.

In clinical practice, patients aren't as perfect and homogeneous as in clinical trials - they have comorbidities, concomitant medications and other idiosyncrasies. Real world evidence is very important to answer questions for which clinical trials haven't looked for the answers and to assess treatments' effectiveness in clinical practice.

In this review a very broad inclusion criteria was used. This strategy yields a big dataset to work with, but it has the disadvantage of lack of homogeneity that make very hard to reach a quantitative conclusion for most variables analysed.

Regarding the ultimate goal of this review, to have an overview of fingolimod effectiveness in clinical practice, I think the objective was achieved. The results suggest that fingolimod treatment is initiated in a later phase than in clinical trials, in patients with more disease duration and higher EDSS score, even though with a lower ARR. The relapse outcomes achieved with fingolimod are pretty much in line of those obtained in clinical trials, with an ARR reduction usually above 70%. Fingolimod achieved 90% of patients without disability progression after 13.2 months of follow up. In the MRI parameters, 91.6% patients were free of T1Gd+ lesions and 73.6% of them were free of new T2 lesions, which is a result slightly better than what was seen in clinical trials. Unfortunately a very low number of studies reported composed outcomes, but what was reported suggests that fingolimod has better results than in clinical trials, having achieved 62.5% patients free of relapse, EDDS progression and MRI activity in three studies and 66.6% patients free of relapse and EDDS progression in 7 studies.

In these studies, after 11.6 months 82.6% of patients continue on treatment and fingolimod showed a positive impact in patients' quality of life, cognition, tolerability, depression, treatment satisfaction and fatigue. The first dose observation results presented no surprises, just confirming what was seen in clinical trials. It was uneventful in a big proportion of patients, usually > 90%, and most of the patients (≈95%) were discharged after the 6 hour observation period.

In a final word about efficacy, I would like to address fingolimod results in comparative studies with other DMTs and in the natalizumab switch studies. Fingolimod has demonstrated superiority in all studies where interferon and/or glatiramer acetate was the control, and with DMF, even though the study is too short to reach any reliable conclusion, fingolimod was more efficacious. In the studies where natalizumab was used as control, fingolimod seems to be, at least as effective as natalizumab when used in second line therapy. Fingolimod reported similar or superior results in 50% of the studies. It's hard to reach any firm conclusion because only 25% of these studies reported the proportion of patients who had natalizumab as previous treatment, which in two of these studies was as high as 50%.

In the natalizumab switch studies fingolimod seems to avoid a reactivation of disease in most patients. And it seems clear that a short natalizumab wash out is associated with

better outcomes. The majority of studies recommend a maximum washout period of 6 to 12 weeks.

These studies suggest that fingolimod is well tolerated and with a safety profile that is very similar to what was observed on clinical trials. No new safety signals have been identified and the AEs which were not reported in clinical trials have a very low incidence.

To further investigate fingolimod effectiveness in the Portuguese population, I have designed and I am now implementing a national observational study sponsored by Novartis Farma S.A.. POSITIVE is a 12-month, observational, parallel cohort, multicentre study enrolling 152 patients, eligible for treatment with fingolimod or a first line injectable DMT. POSITIVE will evaluate treatment adherence and satisfaction, quality of life, work productivity; annualized relapse rate and persistence on fingolimod versus first line injectable DMTs. Moreover, this study will provide important real-world clinical and patient-reported outcomes of relevance to early fingolimod therapy. Enrolment will start in November 2014; approximately 12 investigational sites across Portugal will recruit patients until December 31, 2015. Study results are expected in 2017.

6. Final remarks

Part of this work is or will be published in peer reviewed journals. The paper “Central effects of fingolimod”, published in *Revista de Neurología*, was the final result of a literature review done by me and Vitor Cruz.[79]

The cost effectiveness analysis was done by me, Daniel Viriato, João Carrasco and Ricardo Pacheco and the preliminary results were presented at the Joint ACTIRIMS-ECTRIMS Meeting, September 10–13, 2014, Boston, USA[136,270] and at ISPOR 17th Annual European Congress, November 8-12, 2014, Amsterdam, The Netherlands.[271] A more in depth analysis and discussion manuscript is being prepared by me, Daniel Viriato, João Carrasco, João Fernandes, João Cerqueira, Sofia Oliveira Martins and José Cabrita. This manuscript will be submitted on March 2015.

The results of the real world evidence review of fingolimod will be summarized in a manuscript and submitted for publication before April 2015.

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