Measuring pharmacodynamics in early clinical drug studies in multiple sclerosis

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I

INTRODUCTION

Multiple sclerosis (MS) is the most common autoimmune disorder of the central nervous system, estimated to affect 2-3 million individuals worldwide[1,2]. It is primarily an inflammatory disorder of the brain and spinal cord in which focal lymphocytic infiltration leads to damage of myelin (demyelination) and axons[3]. Direct binding of T lymphocytes to myelin epitopes can lead to activation of macrophages and a subsequent attack of the myelin sheath leading to its phagocytosis[4]. This damage impairs the conduction of signals in the affected nerves. In turn, the reduction in conduction ability causes deficiency in sensation, movement, cognition, or other functions depending on which nerves are involved[5]. Several phenotypes (commonly named types), or patterns of progression, of MS have been described. These phenotypes use the past course of the disease in an attempt to predict the future course. Subtypes that describe the majority of the patients are relapsing-remitting (RRMS), secondary progressive (SPMS), primary progressive (PPMS) and progressive relapsing (PRMS)[6].

DEMYELINATION In demyelination the myelin sheath of neurons is damaged. Demyelinated axons cannot transmit fast trains of impulse, explaining symptoms resulting from physiological fatigue. Depolarisation might traverse the lesion but at reduced velocity, accounting for the characteristic delay of evoked potentials[3]. Partially demyelinated axons can discharge spontaneously, producing unpleasant distortions of sensation. Symptom recovery might suggest resolution of conduction block in structurally intact nerve fibres as the episode of inflammation wanes[7]. When structural damage has occurred, sodium channels are redistributed across the demyelinated axonal membrane[8]. Electrical activity is restored, but alterations in sodium and calcium exchange can prove hazardous until normal nodal arrangements are re-established by remyelination[9]. There are many possible biochemical and cellular mechanisms whereby activated immune cells may destroy myelin and oligodendrocytes (table 1)[4].

REMYELINATION Following acute inflammatory episodes, the resolution of the inflammatory process is probably an important factor contributing to the neurological recovery often observed after clinical relapses. Remyelination can occur to a significant extent in lesion areas, and this probably also contributes to functional recovery. Up to 40% of sclerotic plaques show signs of remyelination[4,10], and it is unknown what determines the fate of a given lesion[11]. However, remyelination is generally incomplete, and the myelin is of lower quality, which might be the reason why the original conduction properties are not fully restored[12]. Although remyelination is most active during the acute inflammatory process, it also occurs in the progressive phase of MS[3,13].

AXONAL LOSS In addition to damage to myelin and oligodendrocytes, axonal loss and injury are characteristic features of sclerotic plaques. In end-state multiple sclerosis, up to 60% of the axons present in sclerotic plaques may have disappeared[14,15]. As with loss of myelin, neuronal axonal damage is likely to be a multifactorial process. A number of cellular and humoral mediators of the immune response have been shown to be capable of damaging axons, including T lymphocyte, macrophages, antibodies, nitric oxide, glutamate and matrix metalloproteases[4,16,17].

PHARMACOLOGICAL TREATMENT OF MS

Current treatment of MS can be divided into disease-modifying treatments and symptomatic treatments. The most common symptoms in MS, such as fatigue, sleep disorders, pain, vestibular symptoms, speech and swallowing, tremors and ataxia, weakness, spasticity, gait, bladder and bowel dysfunction and psychiatric symptoms, are often treated symptomatically with pharmaceutical compounds, physiotherapy and/or psychological help[18]. A new symptomatic treatment is discussed in **chapter 2**, where the effects of a new oral formulation of Δ9-tetrahydrocannabinol (Δ9-THC) on spasticity and pain in 24 patients with progressive MS is described.

Most registered disease-modifying drugs (DMDS) target the immune system, preventing it from destructing myelin. However, with increasing efficacy and discovery of these compounds over the years[2], burden due to side effect of these compounds also increases. Figure 1 shows the different DMDS, of first line treatment (interferons, glatiramer acetate, teriflunomide, dimethylfumarate), second line treatment (natalizumab, fingolimod) and third line treatment (alemtuzumab, mitoxantrone)[3,19]. A short description of the different pharmacological treatments is listed in table 2.

INTERFERONS Interferons belong to the large class of proteins known as cytokines, molecules used for communication between cells to trigger the protective defences of the immune system that help eradicate pathogens and are divided into type 1 (α and β interferons) and type 2 (γ or immune interferon)[20]. Studies show administration of interferon γ increases the exacerbation rate and interferon γ may be involved in the pathogenesis of MS lesions. In contrast, interferon β tends to inhibit the activity of interferon γ and appears to prevent disease activity. Interferon β-1a and interferon β-1b are currently used to treat and control the autoimmune process in MS. The efficacy of subcutaneous interferon β-1b was demonstrated in a double-blind, placebo-controlled trial of 372 patients with RRMS who were randomly assigned to treatment with either interferon β-1b 50 mcg every other day, interferon β-1b 250 mcg every other day, or placebo[21,22]. A study in which safety and efficacy of PEGylated interferon β-1a in 1512 patients with RRMS assessed reported PEG interferon β-1a significantly reduced relapse rate compared with placebo[23]. Injection site reactions are common with IFN-β therapy and can include injection site necrosis.[24]. There is a high prevalence of mainly asymptomatic liver dysfunction associated with IFN-β therapy. However, serious hepatotoxicity associated with IFN-β is rare[25].

GLATIRAMER ACETATE Glatiramer acetate (copolymer 1) is a mixture of random polymers of four amino acids. The mixture is antigenically similar to myelin basic protein, a component of the myelin sheath of nerves. In experimental models, the immunomodulatory mechanism of action for glatiramer involves binding to major histocompatibility complex molecules and consequent competition with various myelin antigens for their presentation to the T lymphocyte. In addition, glatiramer is a potent inducer of specific T helper 2 type suppressor cells that migrate to the brain and lead to bystander suppression; these cells also express anti-inflammatory cytokines[26]. In 1995 a double-blind trial with

glatiramer acetate in 251 patients with RRMS was published. Patients treated with glatiramer acetate (20 mg subcutaneously daily) had a significantly lower relapse rate than those receiving placebo (1.19 versus 1.68)[27]. Furthermore, over 140 weeks, a significantly larger proportion of patients in the placebo group experienced increased disability by ≥1.5 steps on the Expanded Disability Status Scale (EDSS) compared with the treatment group (41 versus 22 percent)[28]. In the clinical trials for glatiramer, the most common adverse reactions reported by those taking glatiramer were skin problems at the injection site (redness, pain, swelling and itching), flushing (vasodilation), rash, shortness of breath and chest pain[29].

TERIFLUNOMIDE The immunomodulator teriflunomide is the active metabolite of leflunomide that inhibits pyrimidine biosynthesis what leads to impaired proliferation of T lymphocyte[30]. The effectiveness of teriflunomide for the treatment of RRMS was demonstrated in several randomized controlled trials. A trial with 1088 adults with relapsing MS found that teriflunomide significantly reduced the relapse rate by approximately 31 percent compared with placebo[31]. In 2014 a phase 3 trial with over 1100 adults with relapsing forms of MS, showed teriflunomide was superior to placebo for reducing the relapse rate[32]. The most common adverse effects of teriflunomide were diarrhoea, nausea, hair thinning, and elevated alanine aminotransferase (ALT) levels[31].

DIMETHYL FUMARATE Dimethyl fumarate and its primary metabolite, monomethyl fumarate, are cytoprotective of neurons and astrocytes against oxidative stress-induced cellular injury and loss, potentially by up-regulation of a Nrf2-dependent antioxidant response[33]. The Nrf2-mediated oxidative stress response mechanisms previously implicated as important for protection of the Central Nervous System (CNS) in a variety of pathological conditions, which can be beneficial for MS patients[34]. Two phase 3 studies reported in 2012 significantly reduced relapse rates and the less development of new brain lesions on MRI in patients with active MS, what suggests it might reduce the rate of disability progression. A first study enrolled 1234 patients and compared dimethyl fumarate to placebo[35]. A second study enrolled 1430 patients and also included glatiramer as treatment[36]. The most common side effects of dimethyl fumarate are flushing and gastrointestinal symptoms, including diarrhoea, nausea, and abdominal pain. Treatment with dimethyl fumarate may decrease lymphocyte counts, so patients should have a complete blood count checked frequently during treatment and discontinue if lymphocytopenia develops[35,36].

NATALIZUMAB Natalizumab is a recombinant monoclonal antibody directed against alpha-4 integrins. Alpha-4 integrin is expressed on the surface of inflammatory lymphocytes and monocytes and may play a critical role in their adhesion to the vascular endothelium and the migration of these cells to the brain[37]. A decrease in migration of immune cells to the brain will leave less immune cells in the brain to destruct myelin. In 2011 a systematic review of trials evaluating natalizumab for RRMS, pooled efficacy data from two randomized controlled trials, and showed that natalizumab significantly reduced the risk for a relapse during two years of treatment. In addition, natalizumab significantly reduced the risk for experiencing progression at two years[38-40]. The JC viral titre is used in the risk assessment in patients using natalizumab. Natalizumab is one of the most effective treatments for MS currently available[41]. However, progressive multifocal leukoencephalopathy (PML) emerged as a rare adverse event from its treatment, generally occurring late (> 24 months) after initiating treatment[42]. PML is caused by reactivation of a latent JC virus in immunocompromised individuals and leads to a debilitating encephalopathy that is fatal in up to 20-50%[43]. Determining a JC viral titre is indicated when patients use immunesuppressive medication or use natalizumab for a period of more than 2 years. However, while 50% of MS patients[44,45] are JCV Ab seropositive, less than 1% will develop PML[46], emphasizing the limitations of this biomarker. The risk in sero-negative patients is 6-18 times lower (chances in sero-positive patients increases after two years)[47]. Also, seroconversion rate was reported to be 26.67%[44].

FINGOLIMOD Fingolimod is sphingosine analogue that modulates the sphingosine-1-phosphate (SIP) receptor, causing internalization of SIP receptors, which sequesters lymphocytes in lymph nodes, preventing them from moving to the central nervous system and cause a relapse in multiple sclerosis.[48]. A 2016 review combined 6 RCTS with a total of 5152 patients, and concluded treatment with fingolimod compared to placebo in RRMS patients is effective in reducing inflammatory disease activity, but it may lead to little or no difference in preventing disability worsening. Also, this benefit is associated with a small increased risk of infection, atrioventricular block, and possibly basal cell carcinoma[49].

ALEMTUZUMAB Alemtuzumab is a monoclonal antibody that binds to CD52, a protein present on the surface of mature lymphocytes, but not on the stem cells from which these lymphocytes are derived. After treatment with alemtuzumab, these CD52-bearing lymphocytes are targeted for destruction. CD52 has been implicated in the activation and migration of T lymphocytes. Alemtuzumab mediates lysis of these cells, suppressing the neuroinflammatory responses in MS[50]. Combined data from 3 trials, involving 1694 patients, concludes there is evidence that annual intravenous cycles of alemtuzumab reduces the proportion of patients with relapses, disease progression, change of EDSS score and developing new T2 lesions on MRI.[100] The main side effects of alemtuzumab were infusion reactions, infections, and autoimmune disorders. Immune thrombocytopenia (ITP) developed in 1 percent of patients at two years, and in 3 percent at three years. [101]

MITOXANTRONE Mitoxantrone has immunosuppressive properties by reducing the number of T cells, inhibiting T helper cell function, and augmenting T cell suppressor activity.[102] The largest trial of mitoxantrone in MS was a single multicenter, double-blind trial of 194 patients with worsening RRMS or SPMS.[103] Treatment with mitoxantrone was associated with significant clinical benefits compared with placebo on multivariate analysis, reducing progression of disability and clinical exacerbations. Because of cardiac toxicity and possible association with a low risk of developing therapy-related acute leukemia, mitoxantrone should be reserved for patients with rapidly advancing disease who have failed other therapies[51].

OCRELIZUMAB Ocrelizumab is a chimeric anti-CD20 monoclonal antibody. It selectively depletes CD20-expressing B lymphocytes while preserving the capacity for B lymphocyte reconstitution and pre-existing humoral immunity[52,53]. A phase 3 trial in 732 patients with primary progressive multiple sclerosis showed that ocrelizumab was associated with lower rates of clinical and MRI progression than placebo. Improvement was reported 12 weeks after the first treatment. Most report adverse events were infusion-related reactions, upper respiratory tract infections, and oral herpes infections[54].

CLADRIBINE Cladribine is a prodrug, but its active metabolite, cladribine triphosphate, accumulates within the cell, resulting in disruption of cellular metabolism, DNA damage and subsequent apoptosis. Cladribine preferentially targets lymphocytes owing to their relatively high ratio of DCK to 5'-nucleotidase, producing rapid and sustained reductions in CD4+ and CD8+ cells and rapid, though more transient, effects on CD19+ B lymphocyte. Cladribine is relatively sparing of other immune cells[55]. A study in 1326 patients with RRMS, treatment with cladribine tablets significantly reduced relapse rates. Adverse events that were more frequent in the cladribine groups included lymphocytopenia (21.6% in the 3.5-mg group and 31.5% in the 5.25-mg group, vs. 1.8%) and herpes zoster (8 patients and 12 patients, respectively, vs. no patients)[56].

METHYLPREDNISOLONE Methylprednisolone (MP), an immunosuppressive drug, is prescribed to MS patients as an acute treatment of a relapse, and has been shown to accelerate neurological recovery[57]. **Chapter 3** describes a first in human study with a new formulation of MP, 2B3-201. This compound consists of liposomes containing methylprednisolone. The liposomes have PEG (polyethylene glycol, also known as macrogol) -molecules attached, ensuring a long residence time in the body,[58,59] and glutathione molecules attached to PEG molecules, improving blood-brain-barrier passage[60,61]. These characteristics in effect change regular methylprednisolone to a slow-release methylprednisolone, with benefits such as lower dosing frequency because of long residue time and lower peak concentrations and peak concentration related side effects.

Figure 1 displays the challenge in treatment development: the search for compounds with a high efficacy and limited burden. Remyelinating and neuroprotective therapies are a new class of medication in MS, for which little data is available on efficacy and burden.

FUTURE OF TREATMENTS IN MS

Although a number of DMDS for the treatment of the inflammatory phase are available, the need for treating neurodegeneration and halting the progression of disability remyelinating and neuroprotective therapies is still unmet[62]. Below eight promising drug candidates are discussed that may have the potential to enhance remyelination or positively affect the CNS (table 3).

ANTI-LINGO-1 ANTIBODIES LINGO-1, a membrane protein, originally attained prominence as an essential subunit of a tripartite receptor complex containing LINGO-1, Nogo-66 receptor (NGR1), and TNF receptor superfamily members P75NTR or Troy/Taj[63]. This complex mediates inhibitory effects of CNS myelin proteins on axon growth[64]. Exposure of mice to an inhibitory monoclonal antibody to LINGO-1 was found to promote spinal cord remyelination in an experimental model of MS, the experimental autoimmune encephalitis (EAE) model[65]. This and similar results of subsequent studies motivated development of opicinumab (BIIB033, Biogen), a LINGO-1 monoclonal antibody suitable for use in humans. Although a phase 2 study with this compound did demonstrate a difference in remyelination between opicinumab and placebo, this effect was only significant in a subgroup of the study population[66].

RHIGM22 ANTIBODIES RHIGM22 is a monoclonal IgM antibody that binds myelin tracts and mature oligodendrocytes, induces calcium influx in astrocytes, oligodendrocyte progenitor cells (OPCS) and pre-mature oligodendrocytes in culture. The antibody promotes remyelination in animal studies[67,68]. One of these animal studies used the cuprizone model, in which the toxin cuprizone leads to demyelination of the central nervous system, demonstrated that treatment with RHIGM22 accelerated remyelination of the demyelinated corpus callosum, and enhancing effects were also accompanied by increased differentiation of OPCS into mature oligodendrocytes[69]. The first clinical studies in humans are ongoing and results are not yet available.

OLESOXIME Olesoxime is an experimental neuroprotective compound that was being developed for amyotrophic lateral sclerosis (ALS)[70] and spinal muscular atrophy (SMA)[71]. It has a number of potentially neuroprotective and neuroregenerative properties, including accelerated maturation of oligodendrocytes and promoting remyelination in cuprizone mouse models of demyelination[72]. A first study in MS patients has started, and preliminary results report olesoxime is safe and well tolerated in patients with MS.

ASIC1 BLOCKERS Blockade of the neuronal proton-gated acid-sensing ion channel 1 (ASIC1) can support cellular protection, which is increased within axons and oligodendrocytes in acute multiple sclerosis lesions[73]. Blocking ASIC1 with amiloride exerts neuroprotective and myeloprotective effects in experimental models of multiple sclerosis[74]. A pilot study suggest that amiloride may exert neuroprotective effects in patients with progressive multiple sclerosis[75].

BENZTROPINE Benztropine functions by a mechanism that involves direct antagonism of M1 and/or M3 muscarinic receptors with subsequent oligodendrocyte maturation. Benztropine has shown to significantly decreases clinical severity in the EAE model[76]. Clinical trials with benztropine have not been announced yet.

GUANABENZ Guanabenz is a α2 adrenergic receptor agonist. It protects oligodendrocytes by preventing dephosphorylation of eIF2, thereby increasing oligodendrocyte survival, and leading to prevention of myelin loss. Preclinical studies demonstrated improvement of deficits in the EAE model[77]. A clinical study has been initiated, but no results have been reported yet.

QUETIAPINE FUMARATE Quetiapine fumarate stimulates proliferation and maturation of oligodendrocytes, increases neurotrophic factors, and inhibits activated microglia, astrocytes, and T lymphocytes. It was shown to have remyelinating and neuroprotective properties in the EAE mouse model[78]. A clinical study has not been initiated yet.

CLEAN SURFACE GOLD NANOPARTICLES Animal studies with clean-surface gold nanoparticles showed remyelination in the cuprizone model. In **chapter 4** we discuss the first-in-human study with these gold nanoparticles (CNM-AU8), performed in 86 healthy male and female subjects to evaluate safety and pharmacokinetics.

BIOMARKERS IN MULTIPLE SCLEROSIS

Definition

The World Health Organization defined a biomarker as 'any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease'[79,80]. Biomarkers in multiple sclerosis can be used to assess disease susceptibility, disease progression and response to treatment[81]. Biomarkers in response to treatment can be used to assess target engagement, pharmacodynamics, safety and proof-of-concept[82].

Current biomarkers

Current frequently used biomarkers in MS diagnosis and treatment management are oligoclonal bands (OCB) in the CSF, neurofilament light, white matter lesions on MRI and JC viral titer[81]. While oligoclonal bands may be considered a diagnostic biomarker, white matter lesions on MRI are diagnostic and used as biomarkers for treatment response, and JC viral titer is a biomarker related primarily to risk of side effects. An immunologic abnormality in MS patients is the presence of intrathecal synthesis of immunoglobulin G (IgG) in an oligoclonal pattern, measured in the CSF[83,84] and it was the first biomarker in the diagnostic criteria of MS in 1983[85]. Although OCBS were removed from the 2010 MacDonald criteria for RRMS, they still are a criterion for the diagnosis of primary progressive MS[86]. Results of a recent biomarker study support the value of serum neurofilament light as a sensitive and clinically meaningful blood biomarker to monitor tissue damage and the effects of therapies in MS[87].

MRI provides a substantial variety of neuroinflammation biomarkers. T1 lesions with contrast enhancement can be considered a biomarker of acute neuroinflammation and Blood-Brain-Barrier (BBB) disruption[88,89]. Hyperintense T2-weighted lesions reflect a combination of mechanisms like inflammation, demyelination, axonal damage and oedema and has a high diagnostic value in MS[90]. Hypotense T1-weighted lesions (black holes) are considered satisfactory biomarkers of axonal damage[91]. Both whole brain atrophy and grey matter atrophy are used as prognostic biomarkers in MS[92,93]. However, MRI techniques lack in adequate correlation with neurodegeneration and disability progression, the so-called 'clinic-radiological paradox'.[94-96]

Potential biomarkers

As mentioned earlier, more pharmaceutical compounds will target remyelination and trials with potential neuroreparative or neuroregenerative agents will need appropriate biomarkers. One of the biggest difficulties in the development of remyelination therapies for MS is the demonstration of remyelination in living patients[97,98]. Conventional MRI sequences have limited specificity for myelination. Imaging modalities which are potentially more specific to myelin content in vivo are magnetisation transfer ratio (MTR), restricted proton fraction f (from quantitative magnetisation transfer measurements), myelin water fraction and diffusion tensor imaging (DTI) metrics, and positron emission tomography (PET) imaging. Although MTR and DTI measures probably offer the most realistic and feasible outcome measures for such trials, they don't have sufficiently high sensitivity or specificity to myelin, or correlation with clinical features[94,99]. The inadequacy of imaging biomarkers to quantify the process of myelin formation, and the need for a pharmacodynamic measure that could be used for studies with compounds that enhance remyelination, led us to set up and validate a more accurate method to measure (re)myelination.

In **chapter 5** we describe the development of a method to determine myelin turnover by labelling myelin with deuterium. First, the method was designed with the help of preclinical analyses and modelling. Subsequently the feasibility of the method was assessed in a clinical study subjects. Healthy subjects drank heavy (70% deuterated) water for a period of 10 weeks, and deuterium incorporation of myelin breakdown products (beta-galactosylceramide) in the cerebrospinal fluid (CSF) was measured with mass spectrometry, after which the level of incorporation was quantified using modelling techniques.
In a second study this labelling experiment was repeated in patients with MS with the goal to quantify myelin turnover and assess the feasibility of this method to be used in patients with MS. The results of this study are described in **chapter 6**.

Demyelination of nerves leads to conduction abnormalities and hence to neurological symptoms. Demyelination of the fasciculus longitudinalis medialis in the brainstem leads to slowing of the adducting eye in horizontal eye movements, which, when it leads to clinical symptoms is called an internuclear ophthalmoplegia (INO). We validated a method to accurately measure eye movements in patients with MS and an INO to be used to demonstrate pharmacological effects of compounds that influence nerve conduction (**chapter 7**). We accurately tracked eye movement in patients with MS and an internuclear ophthalmoplegia. As validation of a method is best done with a compound that has proven to be effective, we used fampridine (4-Aminopyridine) in this study as treatment. Fampridine caused a significant improvement in eye movements, as measured using a highly sensitive method to use eye movements. This method may also be used to quantify the effects of a compounds that enhance remyelination and thereby improve nerve conduction.

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TABLE 1 – Mechanisms of demyelination in multiple sclerosis, derived from Bruck, 2005[4].

Primary immune mechanisms	Secondary mechanisms
T lymphocytes	Excitotoxicity
Cytotoxins	Free radicals
Nitric oxide	Death ligands and receptors
Antibodies	Toxins
Complement	Viruses

TABLE 2 – Disease Modifying Drugs, registered in the Netherlands.

Compound	Target	Development phase
Interferons	Immunomodulatory cytokine	Registered compound
Glatiramer acetate	Immunomodulatory: competition with various myelin antigens presentation to T lymphocyte	Registered compound
Teriflunomide	Inhibits pyrimidine biosynthesis what leads to impaired proliferation of T lymphocyte	Registered compound
Dimethyl fumarate	Neuroprotective effect through Nrf2-mediated oxidative stress response	Registered compound
Natalizumab	Antibody against alpha-4 integrins, decreases migration of immune cells	Registered compound
Fingolimod	Prevents lymphocytes from moving to the CNS	Registered compound
Alemtuzumab	Mediates lysis of immune cells, suppressing the neuroinflammatory responses in MS	Registered compound
Ocrelizumab	Selectively depletes CD20-expressing B lymphocytes	Registered compound
Cladribine	Causes apoptosis of T lymphocyte	Registered compound
Mitoxantrone	Immunosuppressive properties: reducing the number of B lymphocyte, inhibiting T helper cell function, and augmenting T lymphocyte suppressor activity	Registered compound

TABLE 3 – Compounds in developmental phase, selected on those that influence CNS.

Compound	Target	Development phase
Anti-LINGO-1 antibodies	Promotes remyelination	Phase II
RHIGM22 antibodies	Promotes remyelination	Phase I-II
Olesoxime	Neuroprotective and neuroregenerative properties	Phase I
ASIC1 blockers	Neuroprotective and myeloprotective effects	Pilot study
Benztropine	Oligodendrocyte maturation	Animal studies
Guanabenz	Increasing oligodendrocyte survival	Phase I
Quetiapine fumarate	Remyelinating and neuroprotective properties	Animal studies
Clean surface gold nanoparticles	Enhance remyelination	Phase I

FIGURE 1 – Frequently used medication in multiple sclerosis, modified from Coles 2015[19]. Ocrelizumab and cladribine are not in this figure.