Drugs (2013) 73:625–650
DOI 10.1007/s40265-013-0030-6

REVIEW ARTICLE

Drugs in Development for Relapsing Multiple Sclerosis

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Published online: 23 April 2013

Abstract Drug development for multiple sclerosis (MS), as with any other neurological disease, faces numerous challenges, with many drugs failing at various stages of development. The disease-modifying therapies (DMTs) first introduced for MS are only moderately effective, but given the lack of competition, they have been widely accepted in clinical practice. Although safety and efficacy continue to be the two main metrics by which drugs will be judged, the newer agents in the market also face challenges of a more comparative nature—are they more efficacious than the currently available drugs on the market? Are they safer or better tolerated? Do they offer any practical advantages over current treatments? Fingolimod represented a milestone following its approval as an oral drug for MS in 2010, offering patients a far more convenient administration route. However, association with cardiovascular complications has led to a more cautious approach in its initial prescribing, now requiring cardiac monitoring for the first 6 h as well as subsequent monitoring of blood pressure and for macular oedema. Natalizumab, amongst licensed drugs, represents the current benchmark for efficacy. The risk of progressive multifocal leukoencephalopathy during natalizumab treatment is now more quantifiable. Other monoclonal antibodies are in various phases of development. Marketing authorisation for alemtuzumab has been filed, and whilst trial data suggest that its efficacy outperforms both licensed drugs and others in development, there is a significant risk of secondary autoimmunity. Its once-yearly administration, however, seems particularly advantageous. Rituximab is unlikely to be developed further as its license will expire, but ocrelizumab, another monoclonal antibody directly targeting B cells, is currently in phase 2 development and looks promising. Daclizumab is also moderately efficacious but may struggle to establish itself given its monthly subcutaneous dosing. There are new oral drugs in development, and it is likely that BG-12 will be licensed this year. This has been licensed for psoriasis so there are good safety data in humans that may also hold true in MS; however, its three times daily dosage will probably impact on patient compliance. Laquinimod has lower efficacy than BG-12 but appears safe and could find a place as a first-line agent. Teriflunomide has just been licensed by the US FDA and may challenge the current injectable first-line therapies as it has a similar efficacy but the advantage of being taken orally. However, risk of teratogenicity may caution against its use in some women of child-bearing potential. This review will examine drugs that have been recently approved as well as those that are in late phase 2 or 3 development as treatment for relapsing MS, highlighting their mechanism of action as well as the clinical trial and safety data before discussing their potential for success in an increasingly florid and complex DMT armamentarium.

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1 Introduction

There has been a rapid expansion of drugs licensed for multiple sclerosis (MS) since the first disease-modifying treatment (DMT) interferon (IFN)-β1b was introduced in 1993. These DMTs aim to modulate the underlying damage to the central nervous system (CNS) in MS but their results

have been, to an extent, limited by and have mirrored our developing understanding of the disease process itself. Fig. 1 illustrates key processes in MS pathophysiology that serve as drug targets.

The initial DMTs IFN-β1a, IFN-β1b and glatiramer acetate (GA) reduced the relapse rate by approximately 30 %. The emergence of the more efficacious monoclonal antibody (mAb), natalizumab, able to reduce relapse rates by almost 70 %, gave impetus to further mAb development. However, as this review will discuss, mAbs rarely act as the "magic bullets" envisaged by Ehrlich [1] and have a range of indirect effects impacting their efficacy and safety.

Though potentially more effective than earlier compounds, mAbs need to be given by injection and this has driven the development of therapies with a more convenient mode of administration. Thus, fingolimod, approved as an oral treatment for MS in 2010, offers a significant improvement in route of administration; however, alemtuzumab, a mAb in development, offers yet another approach to convenience by allowing yearly intravenous dosing.

This review will only give an overview of the drugs licensed pre-2012 but will highlight the safety issues that have arisen after licensing a number of these drugs. Safety is the predominant concern of regulatory authorities given the onset of MS at a relatively young age, the long disease course and the existence of a range of licensed options. Our attention will then focus on the recently approved teriflunomide and the most promising drugs currently in late phase 2 or phase 3 development, detailing their mechanism of action, clinical trial efficacy and known safety. Finally, key aspects of the changing landscape in MS therapy and the implications for clinical practice will be discussed.

2 Licensed Treatments Pre-2012

2.1 Interferon-β

Three IFN-β preparations are in widespread use as first-line therapies for relapsing forms of MS, which includes both relapsing remitting MS (RRMS) and secondary progressive MS (SPMS) with relapses: IFN-β1b (Betaseron®, Extavia® both requiring subcutaneous administration) and IFN-β1a (Avonex® given intramuscularly, and Rebif®, a subcutaneous preparation). Avonex® and Betaseron® are also approved by the US FDA for clinically isolated syndrome (CIS) in patients with magnetic resonance imaging (MRI) features consistent with MS, but the situation varies in other countries [2, 3].

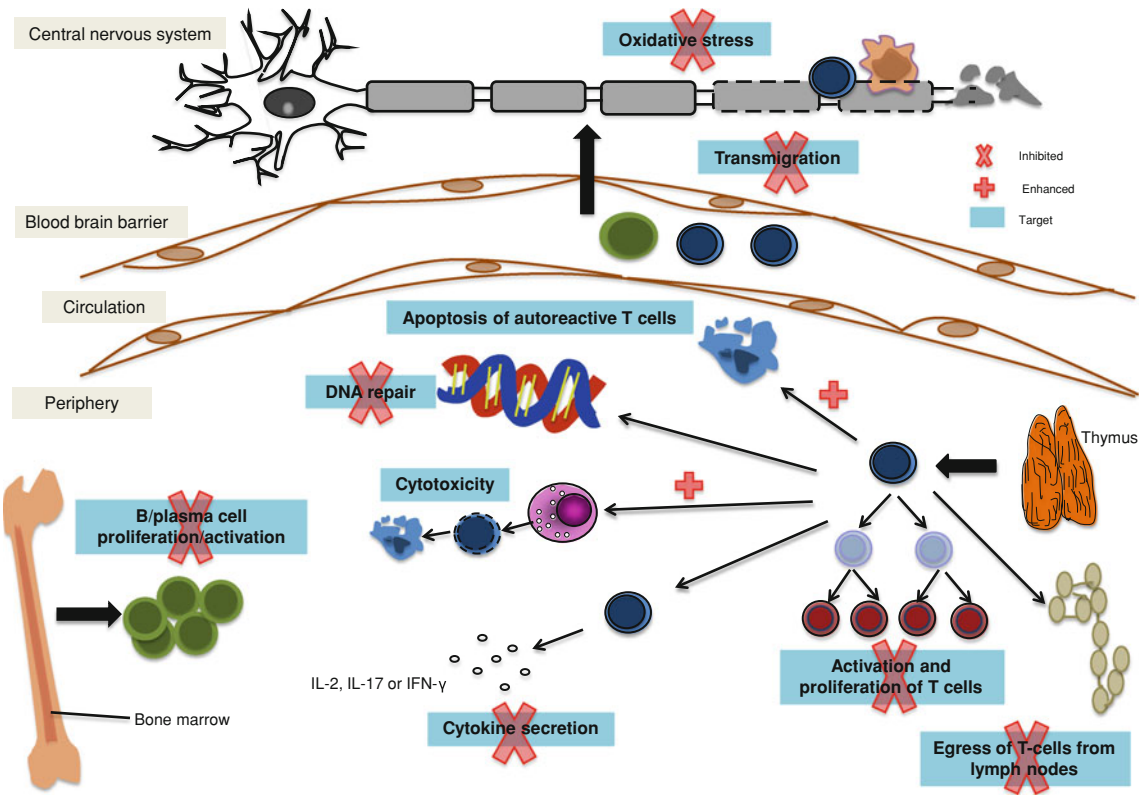

Fig. 1 Processes involved in the pathophysiology of multiple sclerosis and serving as potential drug targets. *IFN* interferon, *IL* interleukin

2.1.1 Mechanism of Action

A number of different mechanisms have been proposed to explain the anti-inflammatory and immunomodulatory effects of IFN-β, but the in vivo relevance is uncertain. IFN-β, by downregulating the level of expression of major histocompatibility complex (MHC) class II on antigen-presenting cells (APCs) and expression of the co-stimulatory molecules e.g. CD80 and CD40 (found on APCs) and CD40L and CD28 (found on T cells), inhibits T cell activation and proliferation [4–6]. IFN-β administration also leads to an upregulation of cytotoxic T lymphocyte antigen 4 (CTLA4, also known as CD152) and Fas surface molecules on $CD4^+$ T cells, promoting apoptosis [7]. Other mechanisms include decreasing the migratory capacity of pathogenic T cells into the CNS by downregulation of the integrin very late antigen 4 (VLA-4) expression and restoring suppressive T cell functions, possibly by upregulation of interleukin (IL)-10 and transforming growth factor (TGF)-β [8, 9].

2.1.2 Licensing Trials

Each of the three IFN-βs were licensed following single multicentre, double-blind, placebo-controlled phase 3 trials. The IFN-β1b (Betaseron®, 1.6 and 8 MIU) trial in 372 patients with RRMS revealed lower exacerbation rates in the two treatment arms and reduced MRI activity compared to placebo [10]. The Multiple Sclerosis Collaborative Research Group recruited 301 relapsing patients and showed that treatment with intramuscular IFN-β1a (Avonex®, 30 μg) led to a reduced proportion of patients with disability progression (21.9 vs. 34.9 % in placebo group; $p = 0.02$), fewer exacerbations ($p = 0.03$) and a reduced number of gadolinium-enhancing lesions (GELs) on MRI compared to placebo [11]. The Prevention of Relapses and Disability by IFN-β1a Subcutaneously in Multiple Sclerosis (PRISMS) study showed that subcutaneous IFN-β1a (either 22 or 44 μg) significantly reduced the relapse rates and MRI disease burden compared to placebo in the 560 MS patients recruited [12].

2.1.3 Post-Marketing Surveillance

Studies show IFN-β to be well tolerated, although influenza-like symptoms are common and abnormalities of liver function tests (LFTs) are recognised, with rare cases of severe hepatic injury described [13]. A global safety database accumulated over 15 years in the post-marketing period for intramuscular IFN-β1a showed no increased malignancy risk [14].

2.2 Glatiramer Acetate (GA)

GA (Copaxone®), a four-amino acid synthetic copolymer based on the composition of myelin basic protein, is approved for relapsing forms of MS and in some countries for CIS patients who have MRI features consistent with demyelination.

2.2.1 Mechanism of Action

GA attenuates the regulatory T cell (T_{reg}) defect described in RRMS patients, inhibits myelin reactive T cells, mediates a T cell shift towards an anti-inflammatory T helper (Th)-2 phenotype by dendritic cells (DCs) and monocytes, enhances the suppressor activity of $CD8^+$ T cells towards $CD4^+$ T cells and can also have neuroprotective effects by stimulating production of brain-derived neurotrophic factor (BDNF) by T cells [15–18]. GA also affects B cells by modulating their cytokine secretion and altering the expression of CD80, CD86 and MHC class 2 expression, which will affect the co-stimulatory signal required by T cells [17].

2.2.2 Licensing Trials

GA (20 mg) was licensed on a single multicentre, randomised, placebo-controlled trial of 251 patients with RRMS followed for 2 years [19]. There was a 29 % reduction in the relapse rate ($p = 0.007$) in the treated group and a higher proportion of people worsened on placebo than on GA. A subsequent multicentre, double-blind, placebo-controlled trial used MRI activity as a primary outcome measure in 239 patients with RRMS, and at 9 months there were reductions in the number of enhancing lesions, lesion volume and number of new T2 lesions [20].

2.2.3 Post-Marketing Surveillance

No major safety issues have arisen. Local injection site reactions are common and post-injection reactions occur in about 15 %, but chronic GA therapy for up to 15 years is not associated with haematological or liver enzyme abnormalities [21–23].

2.3 Mitoxantrone

Mitoxantrone (Novantrone®), an anthracenedione cytotoxic agent, is approved in some countries to reduce neurological disability and relapse frequency in worsening RRMS, SPMS and progressive relapsing MS.

2.3.1 Mechanism of Action

Mitoxantrone inhibits DNA replication, DNA-dependent RNA synthesis and inhibits topoisomerase II activity, thus preventing DNA repair [24]. In the periphery, mitoxantrone inhibits monocyte and lymphocyte migration, induces apoptosis of dendritic cells, decreases secretion of pro-inflammatory cytokines such as tumour necrosis factor (TNF)-α and IL-2, inhibits B cell function and increases T cell suppressor function [25]. Mitoxantrone can cross the disrupted blood–brain barrier (BBB) in MS and in vitro evidence suggests it can induce microglial death [26].

2.3.2 Licensing Trials

Three randomised controlled trials (RCTs) were performed. In 1997, an Italian group published their phase 2 results of 51 patients with RRMS followed for 24 months comparing mitoxantrone (at a dose of 8 mg/m^2 every month for 1 year) to placebo and showed a significant reduction in both the number of exacerbations observed and the proportion of patients experiencing disease progression [27]. The Anglo-French collaboration recruited 42 patients with very active MS and compared a monthly intravenous combination therapy of mitoxantrone (20 mg) and methylprednisolone (1 g) to the same dose of intravenous methylprednisolone alone monthly over 6 months [28]. It found that the combination-treated group had significantly more patients without GELs on MRI (90 vs. 31 %; $p < 0.001$), fewer relapses and greater improvements in final mean Expanded Disability Status Scale (EDSS) scores ($p < 0.001$). The Mitoxantrone in Multiple Sclerosis (MIMS) phase 3 study assigned 194 patients with worsening RRMS or SPMS to receive placebo or mitoxantrone (either 5 g/m^2 or 12 mg/m^2) every 3 months intravenously [29]. Patients receiving 12 mg/m^2 showed significantly reduced disability progression and had fewer clinical exacerbations.

2.3.3 Post-Marketing Surveillance

Mitoxantrone has well-known side effects that include nausea, alopecia, increased risk of infection and infertility, but post-marketing reports of cardiotoxicity [30] resulted in a 2005 FDA "black box" warning and an American Academy of Neurology review found that systolic dysfunction occurred in 12 % of MS patients treated with mitoxantrone, congestive heart failure in 0.4 % and treatment-related acute leukaemia (TRAL) in 0.8 % [31]. A multicentre retrospective analysis of mitoxantrone use in 3,220 patients from 40 centres in Italy identified 30 cases of TRAL, giving a higher than expected incidence of 0.93 % [32]. Following these post-approval safety concerns, a 5-year phase 4 study (the Registry to Evaluate Novantrone Effects in Worsening Multiple Sclerosis or RENEW) of 509 patients in 46 US centres was established in 2000; it reported that ten patients developed congestive heart failure (2 %) and three patients developed TRAL (0.6 %) [33].

2.4 Natalizumab

Natalizumab (Tysabri®) is a humanised monoclonal antibody targeting the α4-integrin molecule, a component of VLA-4, and is approved for highly active MS in most countries.

2.4.1 Mechanism of Action

Natalizumab binds to the α4-integrin molecule, a component of VLA-4, on lymphocytes preventing binding to the ligand vascular cell adhesion molecule (VCAM) found on endothelial surfaces. This blocks the adhesion and subsequent migration of lymphocytes across the BBB, attenuating CNS inflammation.

2.4.2 Licensing Trials

The pivotal phase 3 trial AFFIRM (Natalizumab Safety and Efficacy in Relapsing Remitting Multiple Sclerosis) assigned 942 RRMS patients in a 2:1 ratio to receive either natalizumab (300 mg) or placebo intravenously every 4 weeks for up to 116 weeks. The clinical relapse rate was reduced by 68 % ($p < 0.001$), and the risk of sustained progression of disability was reduced by 42 % over 2 years ($p < 0.001$). MRI activity was reduced by 92 % in the natalizumab-treated group ($p < 0.001$) [34, 35]. This trial led to its fast-track approval.

2.4.3 Post-Marketing Surveillance

Following two cases of progressive multifocal leukoencephalopathy (PML) [36] in the SENTINEL (Safety and Efficacy of Natalizumab in Combination with IFN-β1a in Patients with Relapsing Remitting Multiple Sclerosis) trial, natalizumab was voluntarily suspended in 2005 by the manufacturer but reintroduced in June 2006 with revised labelling [37] and risk management programmes being introduced [38]. The incidence of PML—currently quoted as 1.5 per 1,000 patients—increases with the length of treatment [39] and is 3–4 times more likely in those treated with an immunosuppressant before receiving natalizumab [40]. As of August 2012, there have been 271 cases of PML worldwide, 59 of whom have died [41]. The availability of the JC virus testing has allowed further risk stratification during natalizumab administration.

2.5 Fingolimod

Fingolimod (Gilenya®) derived from myriocin, a metabolite of the fungus *Isaria sinclairii* [42], was approved for RRMS patients experiencing continued relapses despite treatment with first-line DMTs in the European Union and for relapsing forms of MS in the USA.

2.5.1 Mechanism of Action

In vivo, fingolimod is phosphorylated to fingolimod phosphate, which resembles naturally occurring sphingosine-1-phosphate, allowing it to act as a functional antagonist at four of the five S1P receptor subtypes ($S1P_1$, $S1P_3$, $S1P_4$ and $S1P_5$) [43]. Binding to the $S1P_1$ on T cells results in its internalisation, reducing their responsiveness to chemotactic cues and preventing their exit from lymphoid organs and so indirectly preventing infiltration into the CNS. Fingolimod therapy thus causes a functional lymphopenia in the absence of cytotoxicity. S1P receptors are found on virtually all neural cell lineages, and whilst there are in vitro data suggesting that fingolimod could affect oligodendrocyte precursor cell (OPC) survival, recruitment and activation, as well as attenuating astrogliosis, the evidence is not consistent and lacks supporting in vivo data [42].

2.5.2 Licensing Trials

The two double-blind, randomised phase 3 trials (FREEDOMS and TRANSFORMS) supported the results of an earlier phase 2 trial [44].

FTY720 Research Evaluating Effects of Daily Oral Therapy in MS (FREEDOMS) recruited 1,272 RRMS patients for 24 months and compared placebo to either 0.5 or 1.25 mg fingolimod [45]. There was a significant reduction in the annualised relapse rate (ARR)—0.18 in the 0.5-mg fingolimod group, 0.16 in the 1.25-mg group and 0.40 in the placebo group ($p < 0.001$ for both doses). Fingolimod reduced the risk of disability progression over 24 months ($p = 0.02$ vs. placebo for both doses) and both doses had a significant impact on the MRI end points ($p < 0.001$ for all comparisons) [46]. Fingolimod as either 0.5 or 1.25 mg was also superior to IFN-β1a in 1,292 subjects over 12 months in the TRANSFORMS (Trial Assessing Injectable Interferon vs. FTY720 Oral in RRMS) study, reducing the relapse rate by a further 40 % over active treatment [47]. TRANSFORMS reported on two fatal cases of disseminated varicella zoster virus and herpes simplex encephalitis with the 1.25-mg dose, so the lower dose of 0.5 mg was approved.

2.5.3 Post-Marketing Surveillance

Herpes infection and skin cancers were more common in the fingolimod groups, with other adverse events identified in the licensing trials including mild hypertension, elevated liver enzymes and macular oedema [44, 45, 47, 48]. Cardiac adverse events, a consequence of modulation of the $S1P_1$ and $S1P_3$ receptors on atrial myocytes [42], were seen in the phase 3 studies and included bradycardia, maximal within 6 h of first administration, and first- and second-degree atrioventricular conduction block. The license for fingolimod evolved after reviews by the European Medicines Agency (EMA) and FDA [49, 50], triggered by unexpected deaths and serious cardiovascular events in patients started on the drug. Overall, they concluded that the benefits of the drug outweighed the risks but recommended the drug not be prescribed to patients with pre-existing cardiac or cerebrovascular disease or to those taking antiarrhythmics; however, if treatment was deemed necessary, a prior cardiology opinion was advised. Closer monitoring of patients was advised with a baseline electrocardiogram (ECG) becoming a requirement in all patients (previously only those at high risk of bradyarrhythmia) and continuous ECG monitoring for 6 h after the first dose to be extended to at least overnight in any patient developing a cardiac abnormality during the monitoring period being required. The previous requirements of baseline full blood count (FBC) and LFTs and ophthalmic evaluations, the latter on commencing fingolimod and after 3–4 months of therapy, remained unchanged.

2.6 Summary

IFN-β and GA are regarded as safe treatments and this view has been supported by long-term follow-up studies, which is a major advantage but they are only moderately effective, and up to 40 % of patients continue to show disease activity whilst on treatment [51]. It is increasingly evident—as demonstrated with the more efficacious agents mitoxantrone, fingolimod and natalizumab—that phase 3 trials are often insufficient to detect infrequent but serious adverse events (as with PML and natalizumab) or underestimate the risk (TRAL and cardiotoxicity with mitoxantrone or cardiovascular deaths with fingolimod), in part because of their very short duration and the relatively low numbers of patients treated. The emergence of such adverse events has made the use of these drugs more complex.

These drugs are a potent reminder of the demands on any new agents and the challenges that they will face: being sufficiently efficacious to merit their use, having an acceptable side effect profile and a convenient mode of administration. Despite meeting all these criteria in

licensing trials, they will also then need to withstand the rigour of post-marketing surveillance programmes.

3 Monoclonal Antibodies

There has been substantial progress since the original description by Kohler and Milstein [52] of the manufacture of specific antibodies by hybridisation of antibody-producing cells with myeloma cells and their prediction that "such cultures could be valuable for medical and industrial use". In mAbs, the variable domains of the immunoglobulin (Ig) heavy chain (V_H) and light chain (V_L) pair to generate a unique fragment variable (Fv) and it is the degree of the homology of the Fv sequences with human sequences that determines whether the antibody is termed "chimeric" or "humanised". Humanisation aims to reduce the immunogenicity of non-human variable domains [53]. The Fv of a chimeric mAb is entirely non-human in origin and is engrafted to the human constant region, Fc, (e.g. rituximab) but in humanised antibodies, only the complementarity determining regions (CDR)—which determine specificity—are of non-human origin and these are engrafted onto a human framework of gene sequences (e.g. alemtuzumab, daclizumab, natalizumab) [54, 55]. Chimeric mAbs are approximately 66 % human structure, humanised mAbs are greater than 90 % human and fully human mAbs (e.g. ofatumumab) are 100 % human [56]. The nomenclature of mAbs—and in particular, the suffix—reflects these origins [55].

Interactions of the mAb with the target molecule fall into three broad categories:

(a) Binding mAbs. The mAb binds to a specific antigen but does not prevent the physiologic ligand binding to another epitope of that same antigen. It activates effector functions, depending on the Fc portion, leading to the destruction of that immune cell to which it has bound. The mechanisms are complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC) and phagocytosis. Examples include alemtuzumab and rituximab.
(b) Blocking mAbs. These prevent interaction between the target antigen and its innate ligand, interrupting signalling but usually sparing the target cell. Examples include natalizumab and daclizumab.
(c) Signalling mAbs. These mimic the ligand and induce signalling changes within the target cell. Examples include alemtuzumab and rituximab.

However, such 'biological' therapies may have other, and sometimes unexpected, indirect effects mediating their therapeutic action; one example of this is the induction of regulatory natural killer (NK) cells by daclizumab, as described below. Although humanised, mAbs can be immunogenic and their use may be limited by the induction of neutralising antibodies and, uncommonly, systemic inflammatory reactions [57].

3.1 Alemtuzumab

The Campath-1 series, initially manufactured to treat lymphocytic malignancies, were subsequently humanised (Campath-1H) and modified to increase the affinity to the target antigen, CD52 [53]. Campath-1H, recently renamed alemtuzumab, is an IgG1κ mAb to CD52, a glycosylphosphatidylinositol (GPI)-anchored polypeptide, and binds to the proteolytic fragment containing the C-terminal tripeptide and to the GPI anchor [58].

Alemtuzumab peaks 15–30 min after intravenous administration, has a steady state volume of distribution of 0.185 L/kg [59] and a terminal half-life of 15–21 days [60].

3.1.1 Mechanism of Action

CD52 is expressed on the majority (>95 %) of lymphocytes, NK cells, monocytes and some granulocytes (excepting neutrophils) [61]. The function of this abundant glycoprotein—that covers 5 % of lymphocyte cell surfaces—is as yet unknown [62, 63] but a role in T lymphocyte migration and co-stimulation has been suggested [64–67]. It is an excellent target for CDC and ADCC following alemtuzumab binding [65]; however, the relative contribution of each in vivo is unclear. A transgenic mouse expressing human CD52 was used to show that most effects were mediated by neutrophils and NK cells [68], suggesting a predominant role for ADCC. In addition to the direct effect of depletion of CD52-bearing cells, pro-apoptotic mechanisms independent of both CDC and ADCC but also of caspases have been proposed [69, 70].

Alemtuzumab produces a rapid (within 1 h) and profound lymphopenia that lasts for several years [71]. However, as alemtuzumab does not target haematological precursors [72] and only has a short half-life, these factors cannot account for such a prolonged effect. Total lymphocyte counts rarely returned to baseline levels and the rate and degree of lymphocyte repopulation varies between the different subsets [73]. B cell repopulation was fastest, returning to baseline within 3 months, but there remained a significant and persistent depletion of memory ($CD27^+$) B cells, which only reached 25 % of baseline by 12 months. Within T cell subsets, repopulation was more rapid for $CD8^+$ cells compared to $CD4^+$ which took up to 5 years to recover [73–75]. Repeated cycles of alemtuzumab did not alter the kinetics of immune cell reconstitution [74].

3.1.2 Clinical Trial Data

3.1.2.1 Efficacy Alemtuzumab has been used as an experimental treatment for MS in Cambridge since 1991 [62]. Earlier studies in patients with SPMS showed that despite there being a significant reduction of new GELs on MRI by more than 90 % for at least 18 months and a reduction in the number of clinical relapses, patients continued to accrue disability and demonstrate evidence of brain atrophy on MRI in contrast to a cohort of relapsing patients [71, 76, 77]. This supported the notion of a "window of opportunity" with greater potential for benefit from early immunotherapy in MS and served as the basis for an RCT, CAMMS223, comparing alemtuzumab with IFN-β1a in the treatment of early RRMS.

CAMMS223, a phase 2 rater-blinded trial, randomised 334 treatment-naïve patients with RRMS, EDSS scores of 3.0 or less, with disease duration less than 3 years and who had had at least two relapses in the preceding years with at least one GEL on brain MRI to receive either subcutaneous IFN-β1a (44 μg three times a week) or annual intravenous cycles of alemtuzumab, at a dose of either 12 mg or 24 mg per day for five consecutive days at month 0 and for 3 consecutive days at month 12 (and in some cases also at month 24 if the $CD4^+$ T cell count $\geq 100 \times 10^6$ cells/L). At the 36-month follow-up, there were no differences in efficacy between the groups receiving 12 mg or 24 mg of alemtuzumab; both doses reduced the risk of sustained disability by 71 % and rate of relapse by 74 % compared to IFN-β1a and 80 % of patients remained relapse-free at 36 months compared to 52 % for IFN-β1a [78]. Alemtuzumab was also shown to reduce relapse rates and improve clinical scores in patients with active RRMS [79] including those who had been refractory to interferon therapy [80]. Post hoc analysis revealed that a significantly greater number of patients experienced a sustained improvement in disability after alemtuzumab than with IFN-β1a (51.6 vs. 27.2 %) [81], and 5-year follow-up data of 198 of the original 334 patients showed that alemtuzumab remained significantly more efficacious than IFN-β1a over that period—lowering the risk of sustained accumulation of disability by 72 % and the risk of relapse by 69 % compared to IFN-β1a ($p < 0.0001$) [82].

The two completed phase 3 trials were published in November 2012 [83, 84]. Comparison of Alemtuzumab and Rebif Efficacy in Multiple Sclerosis (CARE-MS 1), a 2-year trial, did not replicate the phase 2 study's results of preventing disability progression. It had a similar trial design to CAMMS223, being rater-blinded, comparing alemtuzumab (12 mg/day for 5 days intravenously and then a second 3-day administration 1 year later) to treatment with IFN-β1a (44 μg subcutaneously three times a week) in 581 treatment-naïve MS patients. Key inclusion criteria required disease duration no greater than 5 years, at least two relapses in the previous 2 years, with at least one in the preceding year, an EDSS score of 3.0 or less and MRI brain abnormalities attributable to MS. The co-primary outcomes were relapse rate and time to 6-month sustained accumulation of disability, the latter defined as an increase from baseline of at least one EDSS point or at least 1.5 points if baseline EDSS was 0. At the 2-year time point, 8 % of the alemtuzumab group experienced a sustained increase in the EDSS compared to 11 % of the IFN-β group, but the difference was not significant ($p = 0.22$) and there was no difference in the mean EDSS between the groups. It did confirm, however, alemtuzumab's superiority in reducing relapse frequency—reducing the relapse rate by 55 % compared to Rebif® ($p < 0.0001$); 78 % of alemtuzumab-treated patients were relapse-free at 2 years (a secondary end point) compared to 59 % of the comparator group ($p < 0.0001$). When compared to IFN-β1a, alemtuzumab reduced the proportion of patients with GELs at 24 months (7 vs. 19 % MRI; $p < 0.0001$) and those with new or enlarging T2 lesions (49 vs. 58 %; $p = 0.035$) and reduced brain volume loss by 40 % ($p < 0.0001$) [83].

Results from the second phase 3 trial, CARE-MS 2, provided more encouraging data for the efficacy of alemtuzumab in reducing disability—the design was similar to CARE-MS 1 with the same treatment arms but the 840 RRMS patients recruited had experienced a relapse on previous treatment (which could have included IFN-β). Other inclusion criteria included a disease duration no greater than 10 years, at least two relapses in the previous 2 years with at least one in the previous year, and an EDSS score no greater than 5.0. They were allocated to receive either subcutaneous IFN-β1a 44 μg administered thrice weekly, intravenous alemtuzumab 12 mg per day or alemtuzumab 24 mg per day given as before. The 24-mg dose group was subsequently discontinued but included in the safety analyses. Results were significant for the two primary outcomes: alemtuzumab reduced the relapse rate by 49 % as compared to Rebif® ($p < 0.0001$), and there was a 42 % reduction in the risk of a 6-month sustained accumulation of disability as measured by the EDSS compared to Rebif® ($p = 0.0084$). Other reported clinical secondary outcomes included a decrease in the mean EDSS score for patients treated with alemtuzumab but an increase for Rebif® (−0.17 points vs. 0.24; $p < 0.0001$), 29 % of the alemtuzumab group experienced a sustained reduction in disability compared to 13 % for Rebif® ($p = 0.0002$) and 65 % of patients treated with alemtuzumab were relapse-free at 2 years compared to 47 % with Rebif® ($p < 0.0001$). The MRI data were again statistically significant for alemtuzumab, leading to a reduction in the number of patients with new or enlarging T2 lesions (46 vs. 68 %; $p < 0.0001$) or GELs (9 vs. 23 %; $p < 0.0001$), but

the change in T2 hyperintense lesion volume from baseline to year 2, a secondary end point, was not significant ($p = 0.14$) [84].

3.1.2.2 Safety Profile The CAMMS223 study had already outlined the safety profile of alemtuzumab and this has been confirmed in the two recent CARE-MS phase 3 trials:

i. Infusion reactions: These occurred in 98.6 % of alemtuzumab-treated patients and were serious in 1.4 % in the phase 2 study [78]. In both CARE-MS studies, 90 % had infusion-related reactions with the 12-mg alemtuzumab dose, the figure rising to 97 % with 24-mg dosing in CARE-MS 2, and 3 % of patients in these trials had serious infusion-associated events [83, 84]. Common adverse events associated with the infusion in the alemtuzumab group (12 mg) included a rash (41 % in CARE-MS 1 and 39 % in CARE MS 2), headache (43 %), pyrexia (33 % in CARE-MS 1; 16 % in CARE-MS 2), nausea (14 % in CARE-MS 1; 17 % in CARE-MS 2) and flushing (11 % in CARE-MS 1)—all related to the acute cytokine release syndrome.
ii. Infections: These were more common in the 12-mg alemtuzumab group than in the IFN-β1a group in all three trials (67 vs. 45 % in CARE-MS 1 and 77 vs. 66 % in CARE-MS 2), with the most common infections being those of the upper respiratory tract, urinary tract and herpetic. A total of 16 % of alemtuzumab patients had herpes infections in both CARE-MS 1 and CARE-MS 2 compared to 2 and 4 %, respectively, in the interferon group. The increased frequency of herpetic infections, although noted in earlier studies (8.3 % as opposed to 2.8 % in the IFN-β1a group in CAMMS233 [78]), led the study investigators to request a protocol amendment in 2009 such that alemtuzumab patients received oral acyclovir 200 mg twice daily during the infusion and for 28 days thereafter as prophylaxis against herpes infections. This intervention decreased the frequency of herpetic infections in CARE-MS 1 from 3 % in those untreated with acyclovir to 1 % following the second course of alemtuzumab [83] with similar improvements in CARE-MS 2 (0.5 vs. 2.8 % after the first course and 0.4 vs. 2.1 % after the second course) [84].
iii. Malignancy: Three cancers in the alemtuzumab group were reported in the CAMMS223 study [non-Epstein–Barr virus (EBV)-associated Burkitt's lymphoma, breast cancer and cervical cancer in situ] compared to one case of colon cancer in the IFN-β1a group. A case of malignant transformation of a melanocytic naevus was reported following treatment with alemtuzumab for MS, suggesting impaired tumour immunosurveillance [85]. In CARE-MS 1, two patients developed thyroid papillary cancer (1 %) with no cases of malignancy in the interferon group, whilst in CARE-MS 2 [83], one patient in each of the three treatment groups developed basal cell carcinoma, one patient in the 24-mg alemtuzumab group developed vulval cancer and another patient developed colon cancer [84].
iv. Autoimmunity: This continued to represent the major safety concern—idiopathic thrombocytopenic purpura (ITP) developed in six patients (2.8 %) receiving alemtuzumab and in one patient (0.9 %) in the IFN-β1a group in the phase 2 trial. It caused the death of one patient in the alemtuzumab group following a fatal brain haemorrhage. Remission of ITP occurred in one patient without treatment, two patients required corticosteroids and two others rituximab therapy [78]. In CARE-MS 1, three patients developed serious ITP between 11 and 22 months after commencing alemtuzumab, all of whom recovered with treatment, although one required rituximab. The IFN group had one case of mild ITP not requiring treatment [83]. Similarly, in CARE-MS 2, seven patients developed ITP 3–24 months after receiving alemtuzumab; only one case did not need treatment, with five cases being classified as serious [84]. In CARE-MS 1, one patient developed presumed autoimmune pancytopenia that resolved with treatment, but failure to comply with prescribed corticosteroids on discharge led to sepsis and death. Another patient developed glomerulonephritis following receipt of a third alemtuzumab treatment after the study had ended [84].

Thyroid disorders were common, occurring in 18 versus 6 % of the IFN-β1a group in CARE-MS 1 compared to 16 % (19 % with 24 mg alemtuzumab) versus 5 % in CARE-MS 2 [83, 84].

A follow-up of 248 patients treated with alemtuzumab (between 2001 and 2009) in five UK centres reported that novel autoimmune disorders developed in 22.2 %, with thyroid disorders being most common (15.7 %), and they occurred most frequently 12–18 months after treatment. No new cases were identified 60 months after initial treatment. Individual risk appeared to be modified by smoking and family history (odds ratio 3.05 and 7.31, respectively) [86].

The incidence of secondary autoimmune disease declined in the extension phase of CAMMS223 but onset ranged from 6 to 61 months after the first alemtuzumab exposure. One case of Goodpasture's syndrome occurred in the alemtuzumab group 39 months after the second annual cycle of treatment [82]. Patients from the CARE-MS 1 trial continue follow-up in a 4-year extension study [83].

Autoimmunity is thought to be driven by higher levels of IL-21—raising the possibility that this could serve as a biomarker to identify patients at risk [62].

3.2 B Cell-Targeted Monoclonal Antibodies

Although previously MS was considered to be a T cell-mediated autoimmune disorder, there is an increasing awareness that B cells may play a more fundamental role in the pathogenesis, actually unsurprising given the intrathecally produced immunoglobulins, antibody and complement deposition in most MS lesions, as well as the presence of ectopic lymphoid follicles [87] and B cell-related chemokines in the CNS [88]. However, despite the evident implication of B cells, our understanding of their role in MS remains far from complete. Whilst rituximab has shown efficacy in clinical trials (see below), the phase 2 trial for atacicept, ATAcicept in Multiple Sclerosis (ATAMS), was terminated following an unexpected increase in MS disease activity both in terms of new relapses and MRI lesions (clinical trials.gov NCT00642902). Atacicept was a human recombinant fusion protein that bound to the cytokines, B lymphocyte stimulator (BLyS) and a proliferation-inducing ligand (APRIL), which are involved with B cell maturation, function and survival. Despite having selective effects on mature B cells and plasma cells and showing efficacy in systemic lupus erythematosus and, more variably, in rheumatoid arthritis (RA), atacicept failed as a potential MS therapy [89].

3.2.1 Rituximab

Rituximab was the first B cell mAb trialled in MS. It is licensed for the treatment of non-Hodgkins lymphoma and for RA refractory to anti-TNF therapies. Rituximab is a chimeric mAb of the IgG1κ type that targets CD20—expressed by more than 95 % of B cells, with the exception of plasma cells and haematopoietic stem cells. After intravenous infusion, serum drug concentrations follow a biphasic profile and mean terminal half-life of the drug is 22 days [90]. In the Helping to Evaluate Rituxan in Relapsing Remitting Multiple Sclerosis (HERMES) trial, almost 25 % of the active comparator group developed antibodies but there did not appear to be a correlation with the efficacy of response [91].

3.2.1.1 Mechanism of Action B cell depletion is mediated by binding of rituximab to the CD20, which functions as a Ca^{2+} channel and is involved in B cell proliferation and differentiation [92]. Depletion occurs usually within 2 weeks via CDC and ADCC mechanisms (the latter was suggested to be more relevant), promotion of apoptosis and phagocytosis of the opsonised B cells [92, 93]. Circulating B cell numbers remain depressed for 6–9 months before recovering by 12 months, and there have been suggestions that they are associated with the reactivation of the disease [94, 95]. Consequently, de novo antibody production is affected but antibody production by plasma cells continues—explaining why the existing humoral immunity remains intact. Although this is the primary and direct mode of action, the actions of rituximab do extend beyond this. The "immune complex decoy hypothesis" suggests that the binding of rituximab to CD20 on B cells generates "decoy sacrificial cellular immune complexes" that attract Fcγ receptor (FcγR)-expressing effector cells, diminishing their recruitment to sites of immune complex formation so preventing tissue inflammation and damage [96]. Rituximab therapy has also led to a decrease in the number of T cells in the cerebrospinal fluid (CSF)—reported in one study to be 50 %, which may be an indirect effect, reflecting lack of available B cell help (such as production of chemokines e.g. CXCL13) for T cell trafficking [97]. Rituximab leads to downregulation of CD40 and CD80, co-stimulatory molecules on B cells, involved in T cell activation, and also decreased macrophage activation by producing increased IL-10, an anti-inflammatory cytokine. CD20 is also expressed in much smaller quantities on T and NK cells; their numbers decrease after rituximab therapy and take about 5 months to recover [94].

3.2.1.2 Clinical Trial Data

(a) Efficacy Rituximab treatment was shown to reduce the number of new GELs and T2 lesion volume as well as reduce the relapse rate in a 72-week, open-label phase 1 trial of 26 RRMS patients of whom 80.8 % remained relapse-free over 72 weeks [90]. In HERMES, a 48-week phase 2 trial of 104 RRMS patients assigned in a 2:1 ratio to two different arms, 69 patients received a single course of 1,000 mg of intravenous rituximab on days 1 and 15 whilst the other group received placebo [91]. Rituximab-treated patients had reduced total GELs ($p < 0.001$), and key secondary outcomes included a reduced number of new GELs ($p < 0.001$) and a reduced proportion of patients relapsing following treatment with rituximab—at week 48, it was 20.3 versus 40.0 % for placebo ($p = 0.04$).

The role of rituximab was explored as a potential add-on therapy in patients responding inadequately to standard injectable therapies [98]. Thirty patients with relapsing MS, an EDSS no greater than 6.5, at least one clinical relapse in the prior 18 months, at least one GEL on a pretreatment MRI scan and who had been on an injectable DMT for at least 6 months were treated with weekly rituximab for 4 weeks (375 mg/m^2) and followed-up for 52 weeks with the primary end point being a reduction in the number of GELs on MRIs at 12, 16 and 20 weeks

post-treatment. Add-on therapy led to 74 % of post-treatment scans being free of GELs compared to 26 % at baseline ($p < 0.0001$), whilst EDSS remained stable.

(b) Safety Profile Common adverse events that occurred in more than 10 % in both groups in the HERMES trial included chills, headache, nausea, pyrexia, fatigue, pruritus, and pharyngeal pain. Some of these were related to the infusion and were significantly higher in the rituximab group after the first infusion (78.3 vs. 40.0 % in the placebo). The infection rate was similar in both groups and no opportunistic infections were reported [91]. However, more than 50 cases of PML caused by reactivation of the JC virus have been reported in patients treated with rituximab [99]. Repeated treatment with rituximab at 6- to 9-month intervals has been reported as safe and well tolerated but continued pharmacovigilance is required [95].

Regretfully, as the patent for rituximab expires in 2013 (2016 in the USA), phase 3 trial development is currently not planned. A number of rituximab analogues are in pharmaceutical pipelines as well as other B cell-targeting agents.

3.2.2 Ocrelizumab

Also targeting B cells, ocrelizumab is a recombinant humanised IgG1 anti-CD20 antibody that binds more avidly to CD20, the same target as for rituximab, but to a different but overlapping epitope of the extracellular domain of CD20 and leads to a dose-dependent depletion of B cells. Owing to the human origin, it is expected to be less immunogenic and hence less likely to cause infusion reactions or induce neutralising antibody formation. It has a mean terminal half-life of 23–28 days and a slow systemic clearance of 0.19–0.7 L/day at steady state. The volume of distribution is low, ranging between 5.4 and 6.1 L [100].

3.2.2.1 Mechanism of Action Ocrelizumab has a similar mechanism of action to rituximab but the former is thought to be more dependent on ADCC (by about two- to fivefold) than CDC and the relative activities of these two mechanisms are thought to influence infusion-related safety [101, 102]. B cell depletion occurs immediately after infusion but recovery to baseline is seen within 3 months [100, 101].

3.2.2.2 Clinical Trial Data

(a) Efficacy There has only been one phase 2 study of ocrelizumab in MS and this was a multicentre, randomised, parallel and placebo-controlled study involving 218 RRMS patients who were assigned to either placebo, low dose (600 mg) or high dose (2,000 mg) intravenous ocrelizumab in two doses on days 1 and 15, or intramuscular IFN-β1a (30 μg weekly) for 24 weeks. All groups were double-blind, with the exception of the IFN-β1a group, which was only rater-blinded. At week 24, patients in all groups were given 600 mg ocrelizumab except for the 2,000-mg group, which had their dose reduced to 1,000 mg—these dosages were then repeated for a total of three cycles (being given at weeks 24, 48 and 96). The primary end point examined was the total number of GELs on T1-weighted MRI at 4-weekly intervals from weeks 12 to 24. There were highly significant differences in the primary end point ($p < 0.0001$), the total number of GELs at weeks 12–24, in both ocrelizumab groups compared to placebo and IFN-β1a (the relative risk reductions were 89 % for the 600-mg group and 95 % in the 2,000-mg group). More patients were free of GELs in the first 24 weeks in the ocrelizumab groups (77 % for the 600-mg group and 82 % for the 2,000-mg group) compared to either the placebo or IFN group (35 and 48 %, respectively). Relapse-based end points were also reported in this trial but the more rigorous assessments of a longer-term study are awaited. A phase 3 trial comparing ocrelizumab with IFN-β1a is currently recruiting (NCT01412333)—it is expected to enrol 800 patients, randomising them to receiving either ocrelizumab 600 mg intravenously every 24 weeks plus Rebif® placebo subcutaneously three times per week or Rebif® three times weekly with ocrelizumab placebo every 24 weeks. The primary outcome will be relapse rate at 2 years, with the study expected to complete in August 2015.

(b) Safety Profile Rates of adverse events during the phase 2 trial were similar between the treatment arms but there was one death in the 2,000-mg group, and a contributory effect from ocrelizumab could not be excluded [103]. A phase 3 study in RA showed high rates of serious and opportunistic infections—particularly with 500 mg ocrelizumab, some of which resulted in death [104, 105]. These patients were, however, receiving concomitant immunosuppressive medications, e.g. leflunomide, methotrexate. No such opportunistic infections were reported in this phase 2 trial. Infusion-related events were more frequent with the first cycle with ocrelizumab (35 % with 600 mg and 44 % with 2,000 mg) but there were no significant differences between groups after the second part of that treatment infusion given at day 15.

3.3 Daclizumab

Daclizumab was originally developed to block cell proliferation of virally transformed T cells in human T-cell leukaemia virus (HTLV)-induced T cell leukaemia. It has been used to prevent allograft rejection in transplantation (previously being licensed as Zenapax® for this indication), in the treatment of adult T cell leukaemia and treatment-refractory uveitis [106] and is currently in phase 3

development for MS. It is a humanised IgG1 mAb with the variable domains (10 %) derived from mouse mAb and has a high specificity for the human α-chain of the IL-2R. It has a half-life of 20 days, and a single dose of 2 mg/kg is able to saturate IL-2Rs for 43 days whilst a further dose at 14 days extends this to 59 days [107].

3.3.1 Mechanism of Action

The mechanism of action is incompletely understood. Daclizumab is known to competitively antagonise CD25, the IL-2 binding epitope of the IL-2 receptor alpha chain (IL2RA), which is found at low levels on resting T cells but is upregulated on activated T cells, allowing them to receive the high-affinity IL-2 signal. In vitro, daclizumab decreases T cell activation and proliferation, but T cells from patients treated with daclizumab show normal function ex vivo both in terms of proliferation and cytokine production. The expansion of $CD56^{bright}$ regulatory NK cells is thought to mediate daclizumab's effects by cell-mediated lysis of autoreactive T cells. This expansion occurs because IL-2 recognition is able to occur via the intermediate-affinity IL-2R for NK cells whilst binding is blocked (by anti-CD25) to the high-affinity IL-2R on T cells. $CD56^{bright}$ NK cells isolated from patients treated with daclizumab were cytotoxic towards activated T cells [108]. Other mechanisms of action hypothesised for daclizumab include inhibition of T cell activation by mature dendritic cells [109] and direct inhibition of CD40L expression [110]. Further insights into the mechanism have been gained very recently: this stimulatory effect on NK cells was paired with an inhibitory effect on innate lymphoid cells (ILCs). Daclizumab therapy appeared to decrease numbers of these ILCs—thought to be involved in the regulation of the adaptive immune response—and also modified their phenotype away from lymphoid tissue inducer (LTi) cells (found to be elevated in untreated MS patients) towards immunoregulatory $CD56^{bright}$ NK cells via intermediate-affinity IL-2 signalling [111].

3.3.2 Clinical Trial Data

3.3.2.1 Efficacy Daclizumab has been assessed for MS in six clinical trials to date. The first phase 2, open-label, exploratory proof-of-concept study of daclizumab recruited ten patients (six with RRMS and four with SPMS) who had had an incomplete response to IFN-β treatment, observing them for 4 months before giving seven daclizumab infusions (1 mg/kg/dose at weeks 0 and 2 and then monthly for the next five infusions) in addition to IFN-β. Daclizumab add-on therapy resulted in 78 % reduction in new GELs and a 70 % decrease in total GELs—the decline in GELs emerged over 1.5–2 months of therapy [112]. Other phase 2a open-label studies supported the use of daclizumab and also found significant improvements in the EDSS scores and a role for possible use as monotherapy, but some patients required combination therapy or higher doses of daclizumab therapy to achieve disease stabilisation [113–116].

Two phase 2 RCTs have been completed. The first, CHOICE (Daclizumab in Active Relapsing Multiple Sclerosis), recruited 230 patients with active relapsing MS (at least one relapse), an EDSS score of 0–6.5 and who had been taking IFN-β for at least 6 months. Patients were assigned to receive add-on subcutaneous daclizumab at 2 mg/kg every 2 weeks (high dose), or 1 mg/kg every 4 weeks or placebo for 24 weeks, with a further 48 weeks for safety monitoring [117]. There was a decrease of 72 % in new GELs (the primary end point) between the placebo add-on compared to the high-dose daclizumab group (4.75 lesions vs. 1.32, respectively; $p = 0.004$) but this was not significant with the lower daclizumab dose. The second, SELECT (Study to Evaluate the Safety and Efficacy of Daclizumab HYP Monotherapy in Relapsing Remitting Multiple Sclerosis) [118], enrolled 600 patients with early RRMS (EDSS $\leq$5.0), randomising them to receive either subcutaneous placebo or daclizumab (150 or 300 mg) every month for 1 year. ARR, the primary outcome measure, averaged 0.21 and 0.23 with 150 and 300 mg daclizumab, respectively, compared to 0.46 with placebo ($p < 0.001$ for both doses). There was a higher proportion of patients relapse-free at 1 year on daclizumab (80 % for both doses compared to 63 % placebo, $p < 0.001$). MRI end points, including number of new or enlarging GELs from weeks 8 to 24 and number of new or enlarging T2 lesions at week 52, were also significant. The SELECTION study indicated that the efficacy of daclizumab was sustained in the second year of therapy with a similar risk profile; no rebound activity was seen with the treatment washout period [119].

A double-blind, multicentre phase 3 trial, DECIDE [Daclizumab Compared to IFN-β1a (Avonex®) for Relapsing Remitting MS], is currently underway to compare the efficacy and safety of subcutaneous daclizumab compared to IFN-β1a in RRMS with ARR as the primary end point (clinicaltrials.org NCT01064401) and is due to complete in March 2014.

3.3.2.2 Safety Profile In the CHOICE study, four patients discontinued treatment due to adverse events compared to one in the placebo group. Common adverse events (those with an incidence of at least 15 % in any of the treatment groups) were of similar frequency in all groups. These included nausea, fatigue, headaches, musculoskeletal disorders and infections. Rash was overall more common in the daclizumab-treated group (13 %) than in the placebo

add-on group (8 %)—this was thought to be a consequence of the reduction in peripheral T_{regs} [120]. Transient elevations of liver transaminases or bilirubin and lymphadenopathies have also been reported. There were no opportunistic infections or deaths. A similar safety profile was seen when patients were treated for up to 25 months [113]. However, in the SELECT trial, there was one death in the daclizumab group resulting from a psoas abscess. In addition, elevation of liver enzymes—up to five times the upper limit of normal (ULN)—was seen in 4 % of patients in both daclizumab groups (eight patients in each group). The liver enzymes did return to normal in 15 of the daclizumab patients and seven remained on the treatment without interruption. As a consequence, the phase 3 DECIDE trial has instituted monitoring of the liver enzymes to minimise safety concerns. More recently, CNS vasculitis has been described in a patient who continued on monthly daclizumab infusions following completion of a phase 2 trial of daclizumab monotherapy in MS, and it has been speculated that the 8-week interruption in treatment lowered daclizumab concentrations such that antigen-specific T cells became resistant to cell death; such activation would have normally been prevented by blocking IL-2 trans-presentation by dendritic cells in the lymphatic tissues. This patient was thought to be particularly vulnerable as she lacked the expected expansion of $CD56^{bright}$ NK cells and suggests that the availability of a predictive biomarker for such vulnerable patients would enhance the safety of daclizumab [121]. The SELECTION study did not highlight any complications resulting from washout periods [119] and only emphasises the fact that it will be the larger phase studies and post-marketing surveillance programmes that will highlight the prevalence of this complication.

4 Oral MS Drugs

Injectable drugs are generally regarded as inconvenient and can present even greater difficulty to those with needle phobia. The injection-related side effects can be unpleasant or even intolerable, and can lead to poor compliance and discontinuation of treatment. Oral therapies do offer a step forward in convenience compared to the first-phase injectable DMTs and mAbs dosed every few weeks. The licensing of fingolimod, combining good efficacy and a novel mode of action with increased convenience, has raised the standards for other oral drugs in development.

4.1 Teriflunomide

Leflunomide, the prodrug of teriflunomide, has been approved for mild to moderate RA and so the extension to other autoimmune diseases represented a natural transition [122–125]. Leflunomide is rapidly and non-enzymatically converted into the active open-ring malonitrile metabolite (teriflunomide) either in the intestinal mucosa or plasma [126]. The pharmacokinetics of both drugs is similar, and 11 studies of teriflunomide in healthy volunteers and one study involving MS patients [127] showed that it had linear kinetics over the therapeutic dose range, which was unaffected by age, gender or hepatic impairment [123, 126]. More than 99 % of teriflunomide is protein-bound, predominantly to albumin, but the volume of distribution is only 11 L. Under fasting conditions, it is rapidly absorbed, reaching peak plasma concentrations within 1–2 h post-dose but feeding delayed absorption by almost 6 h [127]. Oral bioavailability of teriflunomide is almost 100 % and it is eliminated as either unchanged drug into bile or as a 4-trifluoromethylaniline oxanilic acid metabolite into urine. As enterohepatic recycling is significant, cholestyramine, which decreases the plasma half-life from 10–12 days to 1–2 days [128], can be used to enhance elimination in cases of overdose. Teriflunomide can be administered as a once-daily oral preparation and this should assist treatment compliance. There are only limited data available on drug interactions: there is weak inhibition of cytochrome oxidase $CYP_{450}3A$ [129], and teriflunomide also inhibits $CYP_{450}2C9$ thereby prolonging the half-life of any drugs eliminated by this route (e.g. phenytoin, warfarin and NSAIDs); the clinical significance of such interactions is unclear [130]. Oral contraceptive use does not appear to affect the pharmacokinetics of leflunomide.

4.1.1 Mechanism of Action

Teriflunomide inhibits dihydro-orotate dehydrogenase (DHODH), the rate-limiting mitochondrial enzyme in de novo pyrimidine synthesis, by non-competitively antagonising the binding of its substrate, dihydro-orotate, and also competing with the binding of ubiquinone [131–133]. Pyrimidine synthesis also arises by a salvage pathway that replenishes the pyrimidine nucleotide pool independent of DHODH. Whilst this salvage pathway suffices for resting lymphocytes, fast-proliferating cells such as activated lymphocytes are dependent on de novo synthesis to meet their increased requirements, and this allows a degree of selective targeting by teriflunomide [126, 133].

Interestingly, although an exogenous supply of uridine (a pyrimidine nucleoside) can overcome this cellular inhibition and allow lymphocyte proliferation [134], the other lymphocyte cell functions remain impaired. The actions of teriflunomide extend beyond simply inhibition of DHODH and include impairing the migratory capacity of T cells, interfering with Ca^{2+} signalling within the T cells which alters their activation upon interaction with APCs,

biasing the differentiation of naive T cells towards a Th2 phenotype, decreasing T cell-dependent antibody production and preventing the IL-4-driven antibody class switch to IgG1 in B cells [135, 136]. Teriflunomide is also thought to inhibit the Janus kinases (JAKs) e.g. Jak1 and Jak3, which are the two major tyrosine kinases involved in intracellular signalling for a number of cytokine receptors including IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21 [136, 137] and suppress astrocytic inducible nitric oxide (NO) synthase-mediated NO production [138] but these effects have been demonstrated in vitro using micromolar concentrations and at least one order of magnitude greater than that required to inhibit DHODH, so the in vivo relevance is uncertain [126, 131]. It also targets neutrophils and macrophages by modulating their expression of adhesion molecules and cytokine secretion [130]. Teriflunomide proved efficient in a rat model of experimental autoimmune encephalomyelitis (EAE), reducing demyelination and axonal loss when administered prophylactically or therapeutically [139].

4.1.2 Clinical Trial Data

4.1.2.1 Efficacy The first phase 2 study examining the safety and efficacy of oral teriflunomide in relapsing MS was published in 2006 [140]. A total of 179 patients with relapsing MS (157 with RRMS and 22 with SPMS) were randomised to receive either placebo, teriflunomide 7 mg/day or teriflunomide 14 mg/day for 36 weeks, with MRI scans being performed every 6 weeks. Treatment with either oral dose resulted in significant suppression of more than 61 % of MRI activity as compared to placebo measured as the number of combined unique (CU) active lesions, a combination score comprising the number of new and persisting GELs and T2 lesions on each MRI scan, which served as the primary end point. The reduction in the mean number of CU lesions became significant at 12 weeks and was maintained for the remainder of the study duration.

Two phase 2 trials have investigated the value of teriflunomide as an adjunctive treatment to either IFN-β (now in phase 3 TERACLES) or GA for 24 weeks. In the IFN-β study, the addition of teriflunomide led to a significant improvement in the number of GELs as compared to the first-line DMT alone, with a relative risk reduction of 84.6 % for the 7-mg dose and 82.8 % with 14 mg teriflunomide as compared to IFN-β alone [141]. The combination of teriflunomide with GA produced less impressive results: the addition of 7 mg/day to GA significantly reduced the number of GELs ($p = 0.03$), but a smaller effect on MRI lesion load was achieved by the 14-mg dose. The reduction in the ARR by 38 % was not statistically significant. A recently published open-label extension of the original phase 2 trial, which included 147 patients with a mean follow-up of 5.6 years but up to 8.5 years (the placebo patients were given either 7 or 14 mg teriflunomide), showed that ARR, disability progression and MRI activity remained low and there was a trend towards a dose-dependent advantage with 14 mg teriflunomide [142].

The Teriflunomide Multiple Sclerosis Oral (TEMSO) trial was a large phase 3 study that randomised 1,088 relapsing MS patients with an EDSS score of less than 6.0 and either one relapse in the preceding year or two relapses in the previous 2 years to receive either placebo, 7 or 14 mg teriflunomide once daily for 108 weeks. The primary end point, ARR, was significantly reduced by teriflunomide (0.54 for placebo vs. 0.37 for either 7 or 14 mg teriflunomide, representing a 31 % relative risk reduction). The time to a first relapse was longer and the relative risk of sustained progression was significantly reduced (by almost 30 %) only in the 14-mg teriflunomide group. The MRI results of the phase 2 study showing suppression of active inflammatory lesions were replicated—patients in both teriflunomide groups had significantly fewer GELs and fewer unique active lesions than placebo ($p < 0.001$). The magnitude of these benefits was similar to the currently available first-line standard DMTs [140, 143].

Early results from two other phase 3 studies, TENERE and TOWER, have been released. TENERE was a rater-blinded study comparing two doses (7 mg and 14 mg) to IFN-β1a (44 μg thrice weekly) in 324 patients with relapsing MS. The main outcome measure, time to treatment failure—defined as either a clinical relapse or trial withdrawal—showed no statistical difference between the three main groups. TOWER (Teriflunomide Oral in People With Relapsing Remitting Multiple Sclerosis) was a double-blind study in 1,169 patients with relapsing MS that compared the same two doses of teriflunomide to placebo, with an average 18-month teriflunomide exposure. Patients receiving 14 mg teriflunomide had a 36.3 % reduction in ARR and a 31.5 % reduction in the risk of 12-week sustained accumulation of disability compared with placebo. Whilst the 7-mg dose led to 22.3 % reduction in ARR compared to placebo, there was no difference in preventing the accrual of disability. TOPIC is an ongoing phase 3 study evaluating the efficacy and safety of 2-year treatment with teriflunomide (7 and 14 mg) compared to placebo in 780 patients with CIS, with a primary end point of conversion to clinically definite MS and is expected to complete in 2015.

Teriflunomide, as Aubagio®, has just been approved by the FDA (September 2012) and is currently under review by the EMA, with approval expected in 2013.

4.1.2.2 Safety Profile As leflunomide has been licensed for RA, there are significant safety data available that is also applicable to teriflunomide.

Common adverse events are predominantly gastrointestinal (and include abdominal pain, diarrhoea, dyspepsia, nausea, vomiting and oral ulcers), elevated liver enzymes, alopecia, skin rashes and hypertension [122, 123, 140]. The incidence of diarrhoea, nausea, alopecia and elevated liver enzymes were dose-related [143].

Serious adverse events included elevated liver enzymes and neutropenia. Interstitial lung disease has been reported in patients treated with leflunomide with pre-existing lung disease or methotrexate use [144]. There have been two cases of PML described in patients treated with leflunomide—but both with a previous history of immunosuppression [145, 146]. No serious opportunistic infections were reported in the TEMSO trial [143]. The safety profile characteristics were supported in the phase 2 extension study; however, the discontinuation rate was 42 %, and 19 % were linked to treatment-related adverse events [142]. TOWER reported a similar adverse event profile.

Liver function tests are mandatory before commencing treatment and then need to be performed monthly for 6 months and every 2 months thereafter. Blood pressure will also need to be monitored whilst on teriflunomide.

Although teratogenicity has been described in rat and rabbit models, reproductive toxicity data in humans are limited. It is a requirement that pregnancy is excluded and that women are using effective contraception prior to therapy initiation; males are similarly cautioned to avoid fathering a child whilst on therapy [147]. In women becoming pregnant during treatment, it is advised that drug levels are reduced substantially with a washout procedure before fetal organogenesis begins—ideally as soon as pregnancy is diagnosed after the first missed menstrual period; hence, women need to be made aware of the theoretical risk of teratogenicity based on animal data [147]. Breast-feeding is not recommended whilst on teriflunomide.

4.2 BG-12

BG-12, a dimethyl fumaric acid (DMF) ester (FAE) compound, is already licensed in some countries as a second-line agent in severe psoriasis—a Th1-mediated skin disease—and has proven to be a safe and effective therapy [148]. BG-12 has now been submitted to the FDA and EMA for market authorisation in relapsing MS.

Fumaric acid is poorly absorbed after oral intake, so esters are used in therapeutic formulations [149] but these can cause ulcers; this problem is circumvented by using monomethylfumarate (MMF) and dimethylfumarate (DMF) compounds in enteric-coated tablets [150]. BG-12 or DMF is almost completely absorbed in the small intestine and is rapidly hydrolysed to MMF, the active metabolite. This is further metabolised via the citrate cycle into water and carbon dioxide, and is wholly independent of cytochrome P450-dependent metabolism [149]. The pharmacological half-life of BG-12 is 12 min and that of MMF 36 h [149, 151]. After ingestion, levels of MMF peak between 5 and 6 h, but food intake decreases the rate of absorption so administration before meals is advised [150]. BG-12 does not bind to serum proteins, which may contribute to its rapid turnover in the circulation, whilst only approximately 50 % of MMF binds to serum proteins [149, 151].

4.2.1 Mechanisms of Action

The exact mode of action of BG-12 is poorly understood but a number of anti-inflammatory and neuroprotective mechanisms have been proposed following in vitro experiments; the former appear more dominant from the clinical trial data.

(a) Anti-inflammatory effects on immune system: FAEs have been shown to be effective in EAE [152]. This is achieved as follows. (1) Polarising the immune system towards a Th2 phenotype of $CD4^+$ T cells [153]. (2) Increasing the production of the anti-inflammatory cytokines IL-10 and IL-1 receptor antagonist (IL-1RA) [154, 155]. Prolonged MMF exposure also leads to a diminution of the pro-inflammatory TNF-α production following the initial elevation [154]. (3) Attenuation of lipopolysaccharide-induced production of pro-inflammatory mediators including TNF-α, IL-1β, IL-6 and NO from astrocytes and microglia [151]. (4) Preventing the nuclear translocation of cytoplasmic nuclear factor kappa B protein (NF-κB), and hence the NF-κB-driven transcription of pro-inflammatory cytokines, usually induced by the inflammatory milieu [156].

(b) Neuroprotection via activation of the Nrf-2 (NF-E2-related factor 2) antioxidant pathway. DMF rescues neurons and glia in culture from oxidative stress-induced cell death by induction of Nrf2-mediated dependent pathways, which induces phase 2 detoxifying enzymes e.g. NAD(P)H:quinone oxidoreductase-1 (NQO-1) resulting in a subsequent increase in cellular glutathione—the latter has also been demonstrated in animal models [157, 158].

(c) Other effects: (1) Inducing apoptosis in stimulated T cells with a decrease in expression of the anti-apoptotic protein Bcl-2 [159]. (2) DHF-inhibiting cytokine-induced intercellular adhesion molecule-1 (ICAM-1), VCAM-1 and E-selectin expression on endothelial cells, which are required for the transmigration of leukocytes [160].

4.2.2 Clinical Trial Data

4.2.2.1 Efficacy Schimrigk et al. [161] reported on the first exploratory open-label, prospective study of FAE in ten patients with RRMS. The study design had four phases—a 6-week pretreatment phase, an 18-week treatment phase (with 720 mg FAE per day), a 4-week washout phase followed by a 48-week second treatment phase but with a lower 360-mg dose. There was a significant reduction in the number of GELs following 18 weeks of FAE treatment, with a further reduction at the end of the study indicating that the effect persisted despite the lower drug target dose in the treatment phase.

A 24-week double-blind, multicentre phase 2b study randomised 257 RRMS patients into four groups to receive BG-12 either 120 mg daily, 120 mg three times daily (total 360 mg), 240 mg three times daily (720 mg daily) or placebo. The higher dose of 720 mg daily resulted in approximately 70 % reduction of GELs and 50 % reduction of new or enlarging T2 lesions but no significant differences were found with the other lower BG-12 doses [162, 163].

The results of the two phase 3 studies of BG-12 in RRMS have been published. DEFINE (Determination of the Efficacy and Safety of Oral Fumarate in Relapsing Remitting MS) randomised 1,237 RRMS patients with an EDSS score no greater than 5.0 and at least one relapse in the prior 12 months to three treatment arms—either 240 mg twice daily or 240 mg three times a day or placebo—for 96 weeks [164]. Patients who either relapsed after 24 weeks or showed sustained progression of disability over 3 months of at least 1 EDSS point (or 1.5 points if baseline EDSS was 0) could be switched to an open-label rescue therapy. Results have shown that both doses were superior to placebo in reducing the proportion of patients who relapsed within 2 years ($p < 0.0001$) and they also reduced the ARR (53 and 48 % for the 480-mg and 720-mg daily dose, respectively), number of new or newly enlarging T2 lesions and number of new GELs. BG-12 reduced disability progression at 12 weeks by 38 and 34 % for the 480- and 720-mg daily dose respectively ($p < 0.05$). It was found that 93 % of the 480-mg and 86 % of the 720-mg dosage groups were free of GELs as compared to 62 % of placebo patients [164].

The results of the second phase 3 study, CONFIRM (Comparator and an Oral Fumarate in Relapsing Remitting MS), were consistent with the DECIDE trial. In CONFIRM, 1,430 patients were randomised to receive either of two different dosages of BG-12 (480 mg or 720 mg daily), GA (20 mg/day by subcutaneous injection) or placebo for 100 weeks [165]. Eligibility criteria were similar to those of DECIDE, requiring an EDSS score no greater than 5.0 and at least one clinically documented relapse in the previous 12 months or at least one GEL within 6 weeks prior to randomisation. The study was not sufficiently powered to detect a difference between BG-12 and GA, which prevented a direct comparison; furthermore, there was no blinding to GA treatment. The primary outcomes were met, there being a significant reduction in ARR in all active arms of the trial at 2 years but more pronounced with both doses of BG-12: 51 % with 720 mg BG-12, 44 % with 480 mg BG-12 and 29 % with GA. There was also a statistically significant reduction in the number of new or enlarging T2 lesions (by 73, 71 and 54 %) and proportion of relapsing patients (45, 34 and 29 %). The reduction in disability progression with BG-12 at 12 weeks, however, was not statistically significant.

A further phase 2 open-label study evaluating the safety and efficacy of 720 mg daily of BG-12 as an add-on therapy to either IFN-β or GA was completed in March 2012 (clinicaltrials.gov NCT01156311).

4.2.2.2 Safety Profile In both phase 3 trials, the incidence of adverse events was similar across all study groups [164, 165]. Adverse events occurring more frequently in BG-12-treated patients included gastrointestinal symptoms (including nausea, diarrhoea and abdominal pain) and flushing which would typically occur within 30 min of administration and subside by 90 min. The adverse events and drug discontinuations were more frequent on treatment initiation and then substantially subsided with treatment maintenance [162, 164]. The initial effect of MMF in enhancing TNF-α would account for some of the adverse events experienced in the initial period of BG-12 administration especially flushing, diarrhoea and abdominal cramps [154]. During the first 24 weeks, the proportion of BG-12 patients who developed elevated transaminase levels increased in a dose-related manner compared to placebo. There were no differences in infection rates.

These adverse events have been reported in previous psoriasis trials, which also noted lymphopenia–lymphocyte counts could decrease to 50 % of baseline values after 4 months of FAE treatment in 10 % of patients [149]. In both the DEFINE and the CONFIRM trials, the mean lymphocyte count decreased over the first year, before plateauing, with mean values remaining within the normal range [164, 165]. Renal toxicity has rarely been reported but Ogilvie et al. [166] reported three patients developing proteinuria during FAE treatment that was reversible with treatment cessation. Proteinuria was the most commonly reported renal event in DEFINE, occurring in 8 % of the placebo group, 9 % of the 480 mg daily BG-12 group, but in 12 % of the 720-mg daily BG-12 group. There were no cases of renal failure—most of these renal events were mild, reversible and did not result in drug discontinuation [164].

4.3 Laquinimod

Laquinimod is a novel, small and orally active immunomodulator that was derived from roquinimex [167] but is pharmacologically and chemically distinct from it, resulting in approximately 20 times greater potency and a far more favourable safety profile than roquinimex, whose phase 3 trial was terminated 1 month after commencement following unacceptable cardiopulmonary toxicity [168].

Laquinimod has high oral availability, a small distribution volume of 10 L and is rapidly absorbed. Rat autoradiography studies show that it distributes to the CNS. In healthy rats, with an intact BBB, the peak concentration in the CNS is reached 2 h post-dose and is approximately 7–8 % of the blood concentration at that time point but in EAE mice, with a disrupted BBB, drug levels peak simultaneously (1 h post-dose), with CNS levels reaching 13 % of the concentration in the blood. The termination half-life ($t_{½}$) is similar in both compartments—approximately 6.5 h in the CNS and 7.9 h in peripheral blood [169]. Laquinimod is metabolised by the cytochrome P450 enzyme and is inhibited by other substrates such as ketoconazole, prednisolone and erythromycin but it is probably only ketoconazole that is relevant, with a half maximal inhibitory concentration (IC_{50}) value of 0.2 μM. As laquinimod is a low-affinity substrate for CYP3A4, there is no significant metabolic inhibition of other CYP3A4 substrates [170].

4.3.1 Mechanism of Action

Laquinimod has both anti-inflammatory and neuroprotective effects but is not immunosuppressive [169, 171]. Efficacy has been demonstrated in both acute and chronically relapsing EAE mouse models [169, 171–173].

(a) Anti-inflammatory effects: (1) Inhibition of leukocyte migration into the CNS by reducing VLA-4 responsiveness to the chemokine CCL21, produced by T cells and endothelial cells in the inflamed CNS [174]. (2) Alteration of the cytokine profile to a Th2/Th3 phenotype by inhibiting pro-inflammatory cytokines such as TNF-α, IFN-γ, IL-12, IL-17 and increasing the anti-inflammatory cytokines including IL-10, TGF-β [174, 175]. (3) Suppression of the NFκB pathway leading to downregulation of chemotactic and adhesion molecules (e.g. CCL12, CCR2 and CXCL9), resulting in decreased entry of autoreactive T cells into the CNS. It also led to an increased expression of apoptotic genes in immunocompetent cells, further diminishing the inflammatory response [176]. (4) Suppression of MHC class 2 antigen presentation [176].

(b) Neuroprotective effects: In EAE models, axonal damage was reduced and the protective effect could be mediated by secretion of BDNF. Serum levels of BDNF have been shown to be elevated in RRMS patients treated with laquinimod [177].

4.3.2 Clinical Trial Data

4.3.2.1 Efficacy A phase 2 multicentre, double-blind RCT and proof-of-concept study comparing 0.1 and 0.3 mg oral laquinimod to placebo in 209 patients with relapsing MS, conducted over 24 weeks, showed that there was a significant reduction of 44 % in the cumulative number of active lesions between patients treated with 0.3 mg laquinimod compared to placebo but no differences were found in relapses or disability [178]. A second phase 2b study randomised 306 RRMS patients to receive either 0.3 or 0.6 mg laquinimod per day or placebo [179]. Inclusion criteria required that patients needed to have had at least one relapse in the year prior to recruitment and at least one GEL on MRI. MRI scans were performed at baseline and then monthly from weeks 12 to 36 with the primary outcome being the number of GELs at weeks 24, 28, 32 and 36. Treatment with 0.6 mg laquinimod led to a 40 % reduction compared to placebo of such lesions on the last four MRI scans but by comparison, treatment with 0.3 mg had no significant effect. Other MRI-monitored markers of disease activity such as cumulative number of T2 lesions and new T1 hypointense lesions were also significantly reduced in favour of 0.6 mg laquinimod (by 44 and 51 %, respectively) but there was no significant change in the ARR or disability progression.

The results of the phase 3 ALLEGRO (Assessment of Oral Laquinimod in Preventing Progression in Multiple Sclerosis) study were published in March 2012 [180] and showed, as had the phase 2 studies, that laquinimod reduced disease activity in patients with RRMS [178, 179]. A total of 1,106 patients, with an EDSS score no greater than 5.5 and either two relapses in the previous 2 years or one clinical relapse in the preceding 12 months with a concomitant GEL, had been assigned to receive either oral laquinimod at a dose of 0.6 mg/day or placebo for 24 months. There was a modest (23 %) reduction in the ARR, used as the primary end point, compared to placebo (0.30 for laquinimod vs. 0.39 for placebo; $p = 0.002$). The proportion of patients who remained relapse-free was 62.9 % in the laquinimod group and 52.2 % in the placebo group. The risk of disability progression (defined as a 3-month sustained increase in EDSS score) was reduced for laquinimod group (11.1 vs. 15.7 %, representing a 36 % reduction). Laquinimod also reduced the mean cumulative numbers of GELs (1.33 vs. 2.12 for placebo) and the number of new or enlarging T2 lesions (5.03 vs. 7.14); $p < 0.001$ for both comparisons. Treated patients also had

a lower mean percentage of brain volume loss from baseline to 24 months as compared to the placebo group (−0.87 vs. −1.30 %; $p < 0.001$, and representing a 33 % reduction).

However, in the second phase 3 trial, BRAVO [laquinimod double-blind, placebo-controlled study in RRMS patients with a rater-blinded reference arm of IFN-β1a (Avonex®)], which compared 0.6 mg laquinimod with an oral placebo and IFN-β1a (30 μg/week intramuscular injection) in 1,331 RRMS patients with an EDSS score no greater than 5.5, laquinimod failed to reach its primary end point and showed no reduction in ARR compared to placebo on unadjusted statistical comparison [181].

The failure to meet its primary end point led the manufacturer, Teva Pharmaceuticals, to delay requesting FDA approval whilst conducting more studies; however, it is currently being reviewed by the EMA.

4.3.2.2 Safety Profile In the phase 2 study, one patient with an underlying hypercoagulability disorder developed Budd–Chiari syndrome whilst on 0.6 mg laquinimod, highlighting a possible increased risk of thrombosis following treatment with laquinimod in patients with pre-existing thrombophilia. It was also noted that herpes infections were more common in the 0.3-mg laquinimod group—but no such pattern, either of increased thrombotic risk or herpes infections, was reported in the phase 3 studies [179, 180].

In the ALLEGRO study, the three most common adverse events in the laquinimod group were abdominal pain (5.8 vs. 2.9 %), back pain (16.4 vs. 9.0 %) and cough (7.5 vs. 4.5 %). Twice as many laquinimod-treated patients had elevated liver enzymes as compared to the placebo group—alanine aminotransferase (ALT) levels that were greater than three times the ULN occurred in 3.6 % of the laquinimod group versus 0.4 % in the placebo group, but ALT levels more than five times the ULN occurred equally often in both groups. The liver abnormalities would typically occur in the first 6 months and were reversible.

Serious adverse events were reported in 11.1 % of patients receiving laquinimod as compared to 9.5 % of those receiving placebo. A higher incidence of appendicitis was reported in the laquinimod group than in the placebo group (five cases vs. one) [180].

In the BRAVO study, similar rates of most of the major and minor adverse events were found in all three treatment groups and the ALT levels were more often mildly or moderately raised with laquinimod therapy. Back pain was, as in previous studies, more common with laquinimod (10 vs. 7 % in placebo and 3 % in the IFN-β group), but the reason for this remains unclear.

The ongoing open-label extension of ALLEGRO involving 844 patients will provide further useful safety data.

5 Discussion

5.1 Current Drug Armamentarium for RRMS

The last two decades have seen significant progress in MS drug development, which has been translated—with reasonable success—into clinical practice. The only DMT licensed in 1993 was betaferon, but by 2012 this number had increased to up to seven licensed drugs in some countries to include Betaseron®, Avonex®, Copaxone®, Rebif®, Novantrone®, Tysabri® and Gilenya® and looks set to increase further with a possible license for teriflunomide (recently approved by the FDA as Aubagio®), alemtuzumab (to be marketed as Lemtrada™), BG-12 and possibly laquinimod over the next year.

The early injectable treatments offered little in the way of choice to patients and were only moderately effective and therefore failed to meet the needs of a significant proportion of patients both in terms of efficacy or convenience of administration—both factors impacting on compliance with therapy. However, these earlier treatments have the advantage of a good long-term safety profile—which supports their continued use as first-line DMTs in relapsing MS for now. Drugs that were more efficacious then emerged on the market; natalizumab represented a significant improvement, and whilst it does have a first-line license for highly active RRMS in some countries, its use is somewhat tempered by the risk of PML, although efforts have been made to manage that risk. Prescription of natalizumab therefore involves careful consideration of the risk–benefit profile—more aggressive disease defined as either increased frequency or severity of relapses in a young adult without a 'chequered' history of prior immunosuppressive therapy would more likely prompt treatment with natalizumab. The availability of natalizumab—despite this limitation—has therefore almost made mitoxantrone redundant in some countries because whilst this cytotoxic agent reduced relapses by almost 60 %, it was associated with side effects such as alopecia and infertility (relevant considerations in a disease that predominantly affects the young) but also had more serious adverse events of cardiotoxicity and risk of leukaemia; the former requires regular echocardiograms and limits long-term treatment.

The advent of fingolimod heralded yet another milestone by being the first widely available oral drug for MS patients and offering patients a convenient mode of administration and freedom from the injection-related side effects of the interferons and Copaxone®. However, fingolimod was found to be associated with cardiovascular complications of bradyarrhythmia and atrioventricular conduction block that could arise on initiation of the drug, and patients therefore require monitoring after the first dose. Risks of hypertension and macular oedema associated with the drug

Table 1 Other promising drugs in development for relapsing multiple sclerosis

Drug	Route	Phase	Mode of action	Trial results	Side effects	References
Tovaxin	S/C	Phase 2b Phase 3 planned	Autologous T cell immunotherapy. Principle is to use ex vivo expanded MRTCs, attenuated by irradiation to sensitise immune system to deplete and/or regulate aberrant MRTCs. Tovaxin consists of up to 6 T cell lines raised against immunodominant peptides derived from MBP, MOG and PLP—exact selection depending on the particular myelin T cell repertoires of individuals	Phase 2b trial, TERMS, in 150 patients with CIS or RRMS compared tovaxin to placebo. No statistically significant clinical or radiological differences in the mITT population but analysis of subgroups of patients with ARR >1 and in treatment-naïve patients showed improvement in ARR but not EDSS or radiological measures: 56 % reduction compared to placebo in the former group and 56–73 % reduction in latter with most benefit being in those that were treatment-naïve and had ARR >1	Injection reactions but generally well tolerated	[182]
Ibudilast	Oral	Phase 2	Inhibits phosphodiesterases 3, 4, 10 and 11 but also inhibits leukotrienes, NO synthesis and TNF-α release from astrocytes and microglia	Pilot studies had showed a 48 % relapse reduction but phase 2 study of two doses of ibudilast compared to placebo in 297 randomised RRMS patients showed no relapse reduction but suggested a neuroprotective effect—with a reduction in the proportion of active lesions evolving to black holes ($p = 0.004$ for 60-mg dose) and over 2 years, fewer patients had EDSS progression ($p = 0.026$)	Safe and well tolerated	[183]
Estrogen compounds	Oral	Phase 2	In EAE models, estrogen compounds downregulate TNF-α production, inhibit recruitment of inflammatory cells into the CNS, induce tolerogenic dendritic cells and induce $CD4^+$ $CD25^+$ regulatory T cells. Neuroprotection possibly mediated by protection of neurons from excitotoxic damage by promoting glutamate uptake by astrocytes, decreasing glutamate-induced apoptosis, and protect oligodendrocytes from cytotoxicity	Phase 1 study showed that estriol decreased GELs in RRMS but not SPMS. Two ongoing combination phase 2 trials—one comparing estriol and GA versus GA and placebo (NCT00451204) and the other comparing estroprogestin or placebo in combination with IFN-β1a (NCT00151801)	Possible risk of vascular events	[184]
Ofatumumab	Intravenous	Phase 2	Fully human recombinant anti-CD20 IgG1κ antibody acts predominantly via complement dependent cytotoxicity	Phase 2 trial in 38 RRMS patients showed that 3 different doses (100, 300, 700 mg) all reduced cumulative number of new GELs, total number of GELs and new or enlarging T2 lesions	Infusion reactions	[185]
Firategrast	Oral	Phase 2	A small anti-α4β integrin molecule that—similarly to natalizumab—reduces trafficking of leukocytes across the blood brain barrier. Has a much shorter half-life of 2.5–4.5 h compared to 11 days for natalizumab	In phase 2 study, 343 RRMS patients were assigned to either 150, 600, 900 or 1,200 mg of firategrast or placebo. There was a significant (49 %) reduction in the cumulative number of new GELs in the 900- and 1,200-mg groups	Well tolerated No cases of PML	[186]
ONO-4641	Oral	Phase 2	A S1P receptor agonist, stimulating primarily $S1P_1$ and $S1P_5$ receptors. Preclinical data in rats have shown that ONO-4641 decreases peripheral blood lymphocyte counts by inhibiting lymphocyte egress from secondary lymphoid tissues, suppresses the onset of disease and inhibits lymphocyte infiltration into the spinal cord in a dose-dependent manner. It also prevented relapses in a mouse model of RR EAE	Phase 2b trial (DreaMS) in 407 randomised RRMS patients, comparing placebo with 0.05, 0.10 or 0.15 mg of ONO-4641 once daily for 26 weeks. Active groups showed a significant reduction ($p < 0.0001$) in cumulative number of T1 GELs and new or enlarged T2 lesions	Asymptomatic AV block, transient bradycardia at initiation Lymphopenia Non-disseminated herpes infections	[187, 188]

Table 1 continued

Drug	Route	Phase	Mode of action	Trial results	Side effects	References
BAF312/ siponimod	Oral	Phase 2	A $S1P_1$ and $S1P_5$ modulator that inhibits lymphocyte migration into the CNS	Phase 2 study, BOLD (BAF312 on MRI lesions given once daily) involved 188 patients with RRMS randomised to receive either placebo or siponimod at 0.5, 2 or 10 mg for 6 months. After a pre-planned month 3 interim analysis, 109 RRMS patients were randomised to either placebo or the intermediate doses of 0.25 mg or 1.25 mg. The study found that there was a dose-related decrease in the monthly mean CUAL count (defined as either GELs or new or enlarging T2 lesions), with maintained levels <0.5 starting from month 2 of treatment compared to between 1.5 and 2.1 for placebo	Appeared well tolerated	[189]
Secukinumab/ AIN457	Intravenous	Phase 2a	A monoclonal antibody against IL-17A, a pro-inflammatory cytokine implicated in MS pathogenesis	Proof-of-concept study involving 73 patients with RRMS, not taking a DMT, randomised to receive either AIN457 10 mg/kg or placebo at baseline and then weeks 0, 2, 4, 8, 12, 16 and 20. There was a 49 % reduction in the cumulative number of combined unique active lesions ($p = 0.087$) and a 67 % reduction in cumulative new GELs on MRI at weeks 4–24 ($p = 0.003$). There was a trend to a decrease in the number of relapses (ARR 0.4 with AIN457 vs. 0.7 with placebo) although the study was not powered for this	Infections—mild or moderate	[190]

ARR annualised relapse rate, *AV* atrioventricular, *CIS* clinically isolated syndrome, *CNS* central nervous system, *CUAL* combined unique active MRI lesion, *DMT* disease-modifying therapy, *EAE* experimental autoimmune encephalomyelitis, *EDSS* expanded disability status scale, *GA* glatiramer acetate, *GELs* gadolinium-enhancing lesions, *IFN* interferon, *IL* interleukin, *MBP* myelin basic protein, *mITT* modified intent-to-treat, *MOG* myelin oligodendrocyte glycoprotein, *MRTCs* myelin reactive T cells, *NO* nitric oxide, *PLP* proteolipid protein, *RR* relapsing remitting, *RRMS* relapsing remitting multiple sclerosis, *S/C* subcutaneous, *SPMS* secondary progressive MS, *S1P* sphingosine-1-phosphate, *TERMS* Tovaxin for Early Relapsing Multiple Sclerosis, *TNF* tumour necrosis factor

also require continued observation. Both fingolimod and natalizumab mark important steps towards—yet do not fulfil—all the qualities wished in an "ideal drug" for MS, i.e. one that is highly efficacious, very safe and conveniently administered. Regulatory authorities have yet another important criterion to add to this "wish list"—that of cost or at least of the drug being cost-effective to justify the expense to the public purse.

5.2 Perspective on Drugs Being Developed

Safety and cost-effectiveness remain an unmet need in the treatment of MS and the drugs currently in development, whilst increasing the complexity of decision-making, will provide further choice, which can only be welcomed. Table 1 highlights other promising drugs in earlier phases of development.

Alemtuzumab appears promising and will certainly appeal to some patients as it is able to reduce relapses by at least 70–80 % compared to placebo; the strength of its phase 3 trials, however, lies in the fact that they had an active comparator arm—alemtuzumab retained superiority by being able to reduce risk of relapses by almost 50 % compared to Rebif®. An active comparator is much more reflective of today's reality than a placebo control arm. CARE-MS 2 showed that the risk of sustained disability was reduced, but as with most trials, it will require much longer follow-up to determine the durability of such an effect. Alemtuzumab also then offers another interpretation of convenience—the once-yearly administration may offset the perceived disadvantage of the intravenous route and coupled with its superior efficacy, represents an attractive therapeutic option. However, the cost of alemtuzumab under its new label for MS may well be prohibitive in most health-care systems and so some form of rationing will inevitably occur by way of strict prescribing criteria. The risk of autoimmunity represents another concern, with almost 20 % of patients developing autoimmune complications, most commonly thyroid-related but almost 1 % developed ITP and indeed one patient died in the phase 2 trial from a brain haemorrhage. But as with natalizumab, further work is ongoing to attempt to identify those at risk

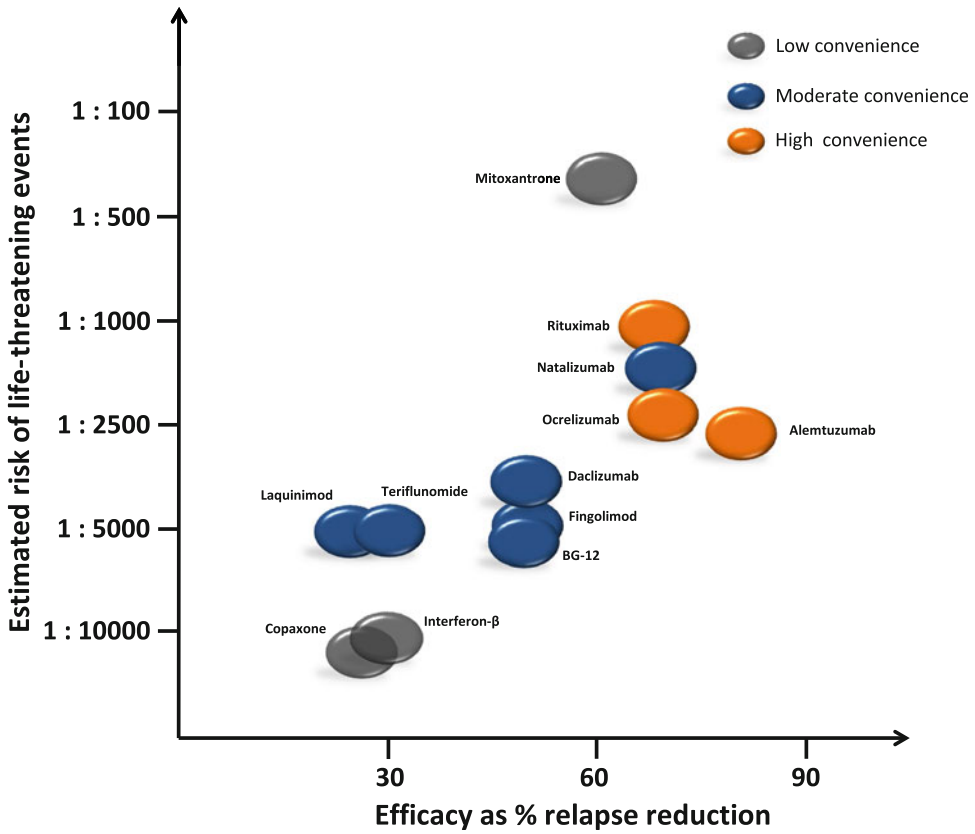

Fig. 2 Efficacy of relapse reduction versus estimated risk of life-threatening adverse events of drugs, both licensed and in phase 2/3 development for multiple sclerosis, indicating the degree of convenience of administration

of secondary autoimmunity and therefore allowing stratification of patients as to their risk and appropriate counselling.

Rituximab, whilst being very efficacious, has been hampered by PML and is unlikely to be developed further given the imminent expiry of its license. The trials with atacicept were prematurely terminated following an unexpected increase in disease activity despite more selective targeting of the B cell population and simply highlight that our knowledge of MS immunobiology is far from complete. Ocrelizumab, another mAb targeting B cells, has a similar mechanism as rituximab but it has only been tested in phase 2 trials, although there is an ongoing phase 3 study; and as the currently licensed drugs discussed demonstrate, these early trials can miss relevant adverse events.

Daclizumab has a good safety profile but it is not as efficacious as alemtuzumab or natalizumab at reducing the relapse rate. This combined with the fact that it still requires monthly injection makes it more difficult to envisage its success, particularly given the emergence of the oral drugs on the market with similar efficacy. Laquinimod and teriflunomide have a similar efficacy to the currently available injectable treatments, but the oral route could mean that they be introduced as first-line DMTs. Teriflunomide has already received FDA approval but it may not be the treatment of choice in women of childbearing age given its teratogenic risk. BG-12 is well tolerated and safe but its more frequent dosing may affect treatment compliance. Fig. 2 provides a simplified illustration of how the drugs discussed in this review compare when considering their efficacy, risk of life-threatening events and convenience of administration—the last of these ranks based on mode and frequency of administration. The parameters used in this figure reflect the practical factors most often considered by patients and their clinicians when choosing a DMT, but despite this there remain inherent limitations to such parameters. Relapse reduction is the most consistent study outcome being measured across the clinical trials and has therefore been used as a comparator of efficacy. However, the authors note that there are significant differences in placebo group behaviour across the studies and whilst more recent studies indicate improved relapse reductions for the older drugs, such reanalysed data have to be interpreted with some caution, given that poor responders will often have switched therapy, leaving a more "benign" population seemingly doing well on therapy. For that reason, Fig. 2 only considers the original licensing trials.

6 Conclusion

The drugs currently in development, whilst offering greater choice in the clinical setting, still focus on relapse and MRI lesion suppression. With a number of similar agents in the marketplace, however, there is hope that this will drive the research into finding a drug to stand out from the competitors. "Setting the bar" higher in treating relapsing MS will mean not solely suppressing the more overt manifestations (relapses and MRI lesions) of the inflammatory disease process, but also arresting any underlying form of inflammatory and/or neurodegenerative process that constitutes the biological basis for subsequent progressive neurological deterioration. Treating RRMS to prevent SPMS, therefore, could be seen as the next frontier in drug development for this disease.

Funding No sources of funding were used to assist in the preparation of this manuscript.

Conflicts of interest Dr Ali has received a travel grant from Merck Serono and from Novartis; these are not relevant to this manuscript. Dr Nicholas has received a clinical grant from Biogen, consultancy fees for participation in advisory boards and lecture fees from Biogen, Novartis and Merck Serono. He has received travel grants from Biogen, Novartis and Teva Pharmaceuticals. Dr Nicholas is grateful for support from the NIHR Biomedical Research Centre. These disclosures are not considered relevant to this manuscript. Dr Muraro has received travel grants and speaker honoraria from Bayer Healthcare, Bayer Pharma and Merck Serono. He is supported by the Medical Research Council (ref. no. G0800679), the UK MS Society (ref. nos. 938/10 and 944/10) and the Italian MS Society (ref. no. 2010/R/24). These disclosures are not considered relevant to this manuscript.

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