Coclitu, Catalina and Constantinescu, Cris S. and Tanasescu, Radu (2016) The future of multiple sclerosis treatments. Expert Review of Neurotherapeutics, 16 (12). ISSN 1744-8360

The future of MS Treatments

Abstract

Introduction. There are not many conditions in which the last few decades have brought such a major change in the landscape of treatments as is the case of multiple sclerosis (MS). A number of disease modifying treatments (DMTs) are presently available for the treatment of the inflammatory phase of this disabling disease; however, the need for treating neurodegeneration and halting the progression of disability is still unmet.

Areas covered: In this paper we review the available information on existing and emerging DMTs and we discuss their place within the context of different treatment strategies in MS,

Expert Commentary: The future of MS treatments should include the development of new treatment strategies tackling disease progression, together with a better understanding of the side-effects and the best sequential strategy of implementation of available and emerging drugs.

Keywords

Multiple sclerosis, immunomodulation, monoclonal antibodies, remyelination, repurposing

Introduction

Multiple sclerosis (MS) is a chronic, immune-mediated condition causing inflammation and neurodegeneration in the brain and spinal cord and the most common non-traumatic cause of neurological disability in young people in the western world [1]. In most patients, MS initially has a relapsing–remitting course (RRMS) with bouts of inflammation (relapses) and periods of remission. In the majority of people with MS, the relapsing course will later be followed by a secondary progressive phase (SPMS) [2]. In around 15% of cases, the disease progresses from the beginning with a primary progressive course without superimposed relapses (PPMS) [2]. More recent classifications put emphasis on the inflammatory activity which can be present at all stages of the disease and can be targeted with disease-modifying treatments (DMTs) [3].

A number of DMTs are currently available for the treatment of RRMS and their aim is to decrease the relapse rate and the inflammation within the central nervous system (CNS) [4]. The past 25 years have brought important changes in the treatment for MS. After several years in which first-line injectable DMTs interferon beta (IFNβ) and glatiramer acetate (GA) were the main treatment options, new medications with different regimens became available. The first oral DMT, fingolimod, was approved in 2010 in the US and soon after that in Europe. A number of other oral agents have been approved since or are currently in phase III trials or are due to be submitted to the regulatory agencies for approval [5]. Three monoclonal antibodies are now approved for MS treatment, and others are also in late stage development. Nevertheless, the challenges raised by the protracted course of the disease, the presence of neurodegeneration in all MS stages and the pathological burden (demyelination and axonal loss) inflicted over time by both inflammation and neurodegeneration raise challenges that are not yet completely tackled by the current therapies. In other words, despite a real breakthrough in treating MS, the available therapies are far from having sorted out the current unmet needs raised by the complexity of MS. Here we provide an outline of the actual treatment landscape in MS and some prospects on its future development.

Expert commentary

Approved treatments in MS

Injectable drugs

Three IFNβ preparations are in widespread use as first-line DMTs for relapsing MS (RRMS and SPMS with relapses) and in some countries for clinically isolated syndrome (CIS). Each of them was licensed following single multicenter, double-blind, placebo-controlled, phase III trials [6]. Two of these medications require subcutaneous administration and one is given intramuscularly. GA (Copaxone®) is a four amino acid synthetic copolymer based on the composition of myelin basic protein [7]. GA was approved after a single phase III multicenter randomized placebo-controlled clinical trial [8]. GA is approved for RRMS and, in some countries, for patients with CIS. IFNβ and GA have different immunomodulatory effects but relatively comparable efficacy, reducing the relapse rate (RR) by approximately 30% [9]. Data from a large observational cohort study recently showed that treatment with IFNβ and GA reduces disability progression measured by EDSS scores over 6 years of treatment [10]. Both IFNB and GA are generally safe and well tolerated. Nevertheless, both IFNβ and GA require regular, long-term, self-injections. Side effects of IFNβ preparations include flu-like symptoms, an increase in liver enzymes, and injection-site reactions. Side effects of GA include local injection site reactions and post-injection reactions which occur in about 15% of people [11]. Issues of adherence and tolerance may therefore reduce the likelihood of achieving durable treatment efficacy [12].

Approved therapies such as the humanized antibodies natalizumab, alemtuzumab [13] and daclizumab, and the currently less used, cytostatic agent mitoxantrone are more effective but their use is associated with safety concerns. These drugs are administered parenterally and can have potentially severe side effects [e.g. progressive multifocal leukoencephalopathy (PML) for natalizumab; autoimmune-associated conditions for alemtuzumab; liver injury, colitis and skin reactions for daclizumab; cardiotoxicity and acute leukemia for mitoxantrone]. They are, therefore, reserved for the treatment of highly active MS.

Natalizumab is a humanized recombinant monoclonal antibody directed against α4-integrin [13]. Natalizumab interferes with leukocyte migration from the peripheral blood into the CNS by preventing its binding via α4-integrin to the vascular cell adhesion molecule (VCAM) on endothelial cells [13]. This step has an impact on CNS inflammation as it blocks the adhesion and subsequent migration of lymphocytes across the blood–brain barrier (BBB). The drug was suspended in 2005 by the manufacturer [15] following two cases of PML in the SENTINEL trial in which it was given in combination with intramuscular IFNβ 1a [14]. In the pivotal placebo-controlled phase III trial which led to its approval natalizumab administered in the dose of 300 mg intravenously (i.v.) monthly reduced RR by 68% and sustained progression of

disability at 2 years by 42% [15]and MRI activity by 92% [16]. Natalizumab was reintroduced in 2006 with revised labelling and after the introduction of risk management programs [17]. The PML risk stratification for people with MS on natalizumab takes into account duration of treatment, prior immunosuppressant use and the anti- JC virus (JCV) antibody status reflecting infection with JCV [18,19]. This is allowing further risk stratification during natalizumab treatment [20]. In 4- to 6% of cases natalizumab treatment may induce the formation of persistent neutralising antibodies (NABs), usually within the first 12 months. The NABs are associated with higher rates of infusion-related adverse events and can lower the efficacy of the treatment [21].

Alemtuzumab is a humanized monoclonal antibody targeting CD52 expressed on lymphocytes, natural killer (NK) cells, monocytes, and some granulocytes [22,23]. Alemtuzumab produces rapid and profound lymphopenia lasting for years via antibody-dependent cellular cytotoxicity [13]. Alemtuzumab was compared to IFNβ-1a administered subcutaneously three times a week in two phase III trials of RRMS [24,25]. Alemtuzumab reduced the annualised relapse rate (ARR) by 49%–55%, MRI gadolinium enhancing lesions by 61%–63% and the rate of disability progression by 30%–42% [25,26]. The major safety concern with alemtuzumab are the autoimmune conditions (cumulative risk between 22% and 47%) [27,28] involving mainly the thyroid gland and blood cells (thrombocytopenia, hemolytic anemia, and pancytopenia), and nephropathies (in 0.3% of patients [29]). Prophylaxis of herpetic infections with oral acyclovir is required during and for 28 days after alemtuzumab infusion [28]. In Europe alemtuzumab is licensed as a first-line medication in active RRMS, however some neurologists would use it as a second-line drug because of the risk of secondary autoimmunity [21].

Daclizumab is a humanized neutralizing monoclonal antibody against the interleukin-2 (IL-2) receptor subunit CD25 on T cells [30]. Its effect on reducing CD25+ T cells is minimal but it expands CD56 bright NK cells and this correlates with the clinical effect [30]. Daclizumab is given either intravenously once every four weeks or subcutaneously once every four weeks (daclizumab high-yield process, DAC HYP). Daclizumab showed promising effects on MRI outcomes in randomized double-blind trials (two phase II, one phase III trial) [31-33] either as add-on therapy to IFNB-beta1a or placebo, and there were no indications of rebound effects after treatment interruption. In a phase III trial DAC HYP 150 mg was compared to intramuscular IFNB beta-1a and led to a 45% reduction in AAR but no statistically significant difference in the reduction of sustained disability. An extension phase is ongoing. There had been two deaths in the phase II trials (one from a psoas abscess and one from autoimmune

hepatitis) and one death in the DAC HYP not deemed to be related to the medication. The safety profile includes an increased incidence of cutaneous adverse events, serious cutaneous events, serious infections and elevations in liver function tests [33]. The cutaneous effects are distinctive adverse events of daclizumab. A review of the cutaneous adverse events in the DECIDE trial has recently been published [34]. Cortese et al. followed 31 participants in the phase I study of DAC-HYP (NCT01143441) over 42 months and observed cutaneous adverse events in 77% of patients treated with daclizumab[35]. The majority of skin events consisted of patches of eczema requiring no treatment [35], while moderate to severe rash (some with psoriasiform phenotype) developed in 19% and required treatment discontinuation in 13%. The skin biopsies from the lesions had nonspecific features of eczematous dermatitis, with important CD56+ lymphocytic infiltrates [35]which were not related to the clinical severity and with no histopathologic post-treatment changes [35].

Daclizumab (Zinbryta©) has very recently been approved by the FDA for RRMS [36]. Daclizumab should be given to patients who have had an inadequate response to two or more MS drugs. Because of the risk of side effects, daclizumab has a boxed warning and is available only through a restricted distribution program under a Risk Evaluation and Mitigation Strategy [36]. Monitoring of liver function is required before commencing on daclizumab and monthly before each dose, and then for up to six months after the last dose [36]. The boxed warning highlights the risk of severe liver injury which could be potentially fatal, of non-infectious colitis, skin reactions, and lymphadenopathy [36].

Mitoxantrone is an anthracenedione that inhibits type II topoisomerase and disrupts DNA synthesis. Mitoxantrone was approved by the FDA in 2000 for rapidly worsening RRMS or SPMS after several clinical trials [37,38]. Mitoxantrone can cross the disrupted BBB and may induce microglial death as shown by in vitro studies [39]. Mitoxantrone is given in infusions at doses of 12 mg/m2 monthly however the cumulative dose is limited due to cardiologic and hematologic adverse effects. The use of mitoxantrone has rapidly decreased due to the risk of severe complications such as acute leukemia [40] and the increasing number of alternative highly effective and less toxic treatment options [21].

Oral drugs

Teriflunomide is the metabolite of leflunomide, which has been approved for mild to moderate rheumatoid arthritis. Teriflunomide is given as 14 mg tablets once daily and the oral

bioavailability is almost 100% [41]. It inhibits the rate-limiting mitochondrial enzyme in de novo pyrimidine synthesis dihydroorotate dehydrogenase (DHODH) [41]. This leads to reduced proliferation of cells that need de novo synthesis of pyrimidine to divide. A salvage pathway independent of DHODH is enough for resting lymphocytes, however fast-proliferating cells such as activated lymphocytes are dependent on de novo synthesis thus being a selective target of teriflunomide [42]. Other immunological mechanisms of teriflunomide have been suggested [41].

In two phase III trials in RRMS [43,44] teriflunomide reduced the ARR compared to placebo by 31%–36%, the MRI gadolinium enhancing lesions by about 80% and the rate of disability progression by 26%–27%. Teriflunomide had similar effects on the ARR and on time to a new relapse or termination of treatment compared with subcutaneous IFNβ-1a [45]. Teriflunomide has been tested in a randomized, double-blind, placebo-controlled trial on clinically isolated syndrome (CIS) patients with silent MRI lesions [46] and it delayed the time to a second relapse and a reduction in new MRI lesions. Teriflunomide is generally well tolerated, however common adverse events include alanine aminotransferase (ALT) increase, headache, diarrhoea, hair thinning, and nausea [47]. These are usually mild-to-moderate in intensity and are self-limiting [47]. ALT elevation is the most common reason for treatment discontinuation and a relatively frequent (every second week) ALT screening during the first 6 months of treatment and thereafter every second month is recommended [47].

Delayed-release dimethyl fumarate (DMF) is the most recently approved oral DMT for RRMS. DMF is administered as a 240 mg capsule twice daily. Its mechanisms of action are not completely understood; however, data suggest they are mediated via the activation of the nuclear factor (erythroid-derived 2)-like 2 (Nrf2) pathway [48]. DMF was compared to placebo in two phase III trials in RRMS [49,50] and it reduced the ARR by 44%–53%, the MRI gadolinium-enhancing lesions by about 75%–94% and the rate of disability progression by 22%–32% [51]. DMF reduced the ARR by 24% and the rate of disability progression by 17% compared to GA [50], however the differences were not significant and the study was not powered to detect statistically significant differences in treatment effect. An interim analysis of an ongoing long term extension of the phase III trials showed that treatment with DMF was followed by continuously low clinical and MRI disease activity [52]. Common adverse events include flushing, nausea, diarrhoea and abdominal pain [51], are usually transient and mild to moderate in severity and can limit patient tolerance of DMT [53]. The treatment may induce leucopenia and increase liver transaminases, and regular blood tests are therefore

recommended. Prolonged severe lymphopaenia (<500 cells/mm^3) has been suggested as a risk factor for PML [54]. In 2014, a case of PML was reported in a patient treated with DMF [55]. Four other PML cases have been previously reported in patients with psoriasis who had received fumaderm [56]. In JCV-positive patients with persistent lymphopaenia, DMF should be stopped. Up to now, PML has not been observed during DMF therapy without lymphopaenia and in the presence of appropriate monitoring and drug-discontinuation rules [57].

Fingolimod was the first of the oral agents for relapsing forms of MS to be approved by the FDA in 2010. Fingolimod is administered as a once-daily 0.5-mg capsule. Fingolimod is a nonselective sphingosine-1-phosphate (S1P) receptor modulator [58,59]. It acts as a functional antagonist, by internalising and degrading the S1P1 receptor on lymphocytes (a receptor responsible for T lymphocyte exit from the lymph nodes, circulation, and T cell differentiation)[58]. This leads to sequestration of T lymphocytes in secondary lymphatic tissues and thus reduces inflammation in MS [60]. An additional direct effect on the CNS has been suggested by animal studies [60].

In two phase III trials in RRMS fingolimod compared to placebo educed the ARR by 48%–55%, the MRI gadolinium-enhancing lesions by more than 80% and the rate of disability progression by 25%–30% [61]. Compared to IFNβ-1a 30 lg intramuscularly once weekly fingolimod reduced ARR by 52%, the MRI gadolinium-enhancing lesions by more than 50% and the rate of disability progression by 25% [62]. In a phase 3 trial in PPMS fingolimod failed to delay disability progression compared to placebo [63].

Common adverse events include upper respiratory tract infection, diarrhoea, cough, headache, and back pain [64]. Because it can cause bradycardia and atrioventricular block at first administration it is recommended to have electrocardiogram monitoring continuously for 6 h after the first dose [65]. After one death due to a fulminant primary varicella zoster in one of the phase III trials [66], screening for previous varicella zoster infection is advised in patients due to start fingolimod, and vaccination is recommended if negative. Rare adverse events include elevated liver enzymes and macular oedema and bloods and ophthalmological follow-up are recommended [67].

Agents in trial

Monoclonal antibodies

Three anti-CD20 agents (rituximab, ocrelizumab, and ofatumumab) that deplete pre-B cells and mature B cells without affecting plasma cells or progenitor cells in the bone marrow have been studied in MS [23]. Rituximab is a human–mouse chimeric monoclonal antibody against CD20. Rituximab has been used off label for MS as well as neuromyelitis optica. Rituximab has been approved to treat B cell lymphomas, rheumatoid arthritis, Wegener's polyangiitis and microscopic polyangiitis [23]. In a phase II double-blind, placebo-controlled trial rituximab reduced new MRI gadolinium-enhancing lesions by 91% and the proportion of patients with relapses [68]. 78% of treated patients experienced an infusion-related adverse effect. Infection incidence was similar in both groups [68]. A phase II trial of rituximab in PPMS failed to meet its primary endpoint of delaying confirmed disability progression [69] and showed a 3.5% increase in risk of serious infections in the treated arm. In patients using rituximab for other indications, PML cases have rarely been reported [70]. Although rituximab rapidly and consistently decreases the numbers of peripheral CD20+ and CD19+ cells [71], a small phase II trial of intrathecal rituximab was terminated early because of low efficacy on the CSF biomarkers [72]. Rituximab induced an incomplete and transient depletion of B cells in the CSF, whilst the effects on peripheral B cells were complete and lasting.[72]. No phase III trials of rituximab for MS have yet been performed. Some suggest rituximab can be an option in RRMS patients who have failed to respond to first- and second-line therapies, in those with concomitant autoimmune disorders [73] or in people with stable RRMS who switch from natalizumab to other DMT due to increased PML risk[74].

Ocrelizumab and ofatumumab are humanized anti-CD20 monoclonal antibodies. In a phase II placebo-controlled RRMS trial [75] two doses of ocrelizumab met the primary outcome of reducing the number of MRI enhancing lesions. Infection side effects were equivalent between the two groups, but more infusion related side effects occurred in the ocrelizumab group than in the placebo arm. Two phase 3 clinical trials (OPERA I and II) for relapsing remitting MS were completed in 2015 [76]. The trials compared intravenous ocrelizumab 600 mg every 6 months to subcutaneous IFNBβ-1a. The studies reported that ocrelizumab reduced the annual relapse rate by 46% in OPERA I and by 47% in OPERA II [76]. Also, they showed a reduction of clinical disability by 40 %, as measured by the EDSS. Ocrelizumab reduced the number of T1 gadolinium-enhancing lesions in the brain by 94%, and 95% respectively [76]. Additionally, it resulted in a 77% and 83% reduction of hyperintense T2 lesions compared with IFNBβ-1a. At 96 weeks, 47.9% of patients treated with ocrelizumab in OPERA I and 47.5% in OPERA II achieved the composite measure of 'no evidence of disease activity' (NEDA) defined as no

MS relapses, no confirmed disability progression, and no new or enlarging T2 or gadolinium-enhancing T1 lesions, compared to 29.2% and 25.1% of patients treated with IFNBβ-1a [77].

A recent phase III clinical trial (ORATORIO) tested ocrelizumab for primary progressive MS and compared intravenous ocrelizumab 600 mg every 6 months to placebo [78]. The primary endpoint was time to onset of 12-week confirmed disability progression (CDP), while secondary endpoints included time to onset of 24-week CDP; change in timed 25-foot walk, T2 lesion volume, change in whole brain volume and safety [78]. Ocrelizumab was the first trialed drug to meet primary and key secondary efficacy outcomes in a phase III PPMS study. Ocrelizumab significantly reduced the relative risk of 12-week CDP by 24% and 24-week CDP by 25% [78], decreased the volume of T2 hyperintense lesions and reduced the whole brain volume loss compared with placebo [78]. The frequency of adverse events and serious adverse events was similar in both groups [78]. The results of the OPERA I, OPERA II and ORATORIO trials will be submitted for the approval of ocrelizumab to the FDA.

Ofatumumab is a fully human monoclonal antibody currently used for lymphocytic leukemia, which interacts with the early activation of the B lymphocyte and has lower potential for antigenicity. It was tested in a small phase II clinical trial with promising results, showing a 99% reduction of MRI activity, with no serious adverse events [79]. A larger phase 2 trial, the MIRROR trial, tested the safety and effectiveness of ofatumumab compared to placebo in 232 patients with RRMS [80]. This study also showed a 90% reduction of MRI lesions after 12 weeks of treatment [80]. Five serious adverse events were reported in the 60mg dose regimen, but no cases of PML or opportunistic infections were reported [80]. The authors concluded the results support the further study of ofatumumab in clinical RRMS trials [80].

Cladribine

Cladribine is a cytotoxic drug, an adenosine deaminase-resistant purine nucleoside, used as a first-line chemotherapeutic agent in the treatment of hairy cell leukemia and other neoplasms, in its parenteral formulation [81]. Cladribine enters cells via purine nucleoside transporters [82]. Cladribine works preferentially on lymphocytes and monocytes by disrupting cellular metabolism resulting in cell death [83], being incorporated into the DNA of the dividing cells. It selectively depletes the number of circulating T cells and B lymohocytes, having only a minor effect on NK cells [84]. The study of cladribine in MS started more than 20 years ago. Intravenous cladribine was initially evaluated for progressive MS (PPMS, SPMS) in two randomized double-blind clinical trials [85,86] with some promising results. Later, cladribine

was tested as an oral agent for RRMS, tablets being taken in two cycles of few days per year [84]. Cladribine for RRMS was tested in the placebo-controlled CLARITY trial and reduced both the frequency and severity of relapses, and suppressing the MRI enhancing lesions at 6 months [87]. The recently reported results of a 120-week extension demonstrated that in a majority of patients, the clinical benefits on relapses and disability as well as on MRI outcome measures of 3.5mg/kg cladribine given in the first two years of the trial can be maintained for at least 4years [88,89]. ONWARD was a 2-year, randomized, double-blind phase IIb study of cladribine tablets (3.5mg/kg) as an add-on to IFN-β therapy in relapsing MS patients (including SPMS with ongoing relapses). The mean number of relapses was lower (23%) in patients treated with cladribine 3.5mg/kg and IFNβ than in patients on placebo plus IFNβ (56%) [90]. The mean numbers of new enhancing T1 lesions and active T2 were reduced in the cladribine+IFNβ treatment arm vs. placebo plus IFNβ [90]. In the phase III trial ORACLE (Oral Cladribine in Early Multiple Sclerosis) 616 CIS patients received 1:1:1 cladribine 5.25 mg/kg, cladribine 3.5 mg/kg, or placebo [91]. Both doses of cladribine considerably delayed MS diagnosis compared with placebo [91]. A more recent analysis applied the 2010 McDonald criteria to the patient cohort at baseline and showed that the risk of further relapse and disability worsening was significantly reduced with cladribine 3.5 mg/kg compared to placebo [92]. Cladribine failed to get regulatory approval by the EMA because of concerns over the risks of cancers in the CLARITY active arm [93]. Cladribine was used for RRMS treatment in 2010 in Russia and Australia, but was withdrawn afterwards. However, a recent meta-analysis of phase III trials of licensed DMTs for RRMS and the CLARITY trial did not support an increased cancer risk from cladribine in the doses used in CLARITY and ORACLE MS, which previously contributed to refusal of market authorization of cladribine by EMA [94]. The authors concluded that longer-term follow-up is required to assess the safety profile of both cladribine or of the currently approved DMDs, to definitively assess cancer risk [94]. PREMIERE (NCT01013350), an observational prospective study of patients who have participated in clinical trials with cladribine or other DMTs is ongoing [94].

Laquinimod

Laquinimod is an orally available carboxamide derivative, derived from linomide, a drug that was proved to reduce activity in RRMS, but with the cost of severe adverse events [95]. Laquinimod was or currently is tested for neurodegenerative disease such as Huntington's disease and also for relapsing remitting and progressive MS [96]. Studies on the animal model of MS (experimental autoimmune encephalomyelitis, EAE) showed that laquinimod decreased

inflammation, demyelination and axonal injury [97]. It appears to reduce infiltration of CD4+ T cells and macrophages into the central nervous system (CNS), it also seems to increase the serum level of brain-derived neurotrophic factor which may protect against neuronal injury [98]. Laquinimod efficacy in RRMS patients has been assessed in two Phase III trials, ALLEGRO (Assessment of Oral Laquinimod in Preventing Progression in Multiple Sclerosis) [99] and BRAVO (Benefit–Risk Assessment of Avonex and Laquinimod) [100]. In ALLEGRO laquinimod was compared to placebo and showed a modest, but significant reduction of the annual relapse rate, and also a reduced rate of progression [99]. Initially the primary outcome measure of reducing annual relapse rate in BRAVO was not reached [100], but following an adjustment for an imbalance between the groups in the number of patients with enhancing lesions and of mean T2 lesion volume, both predictors of relapses, a reduction in relapse rate in the laquinimod arm (21% reduction vs placebo, p=0.026) was obtained. Overall laquinimod had more pronounced effects on disability progression and brain atrophy than on relapses and new MRI lesion formation [101]. Two ongoing studies are evaluating the safety and efficacy of laquinimod: CONCERTO (phase III, RRMS; NCT01707992), and ARPEGGIO (phase II, PPMS; NCT02284568). Both aimed to compare two doses of laquinimod, 0.6 and 1.5 mg/day, to placebo, however, in January 2016 TEVA announced the discontinuation of higher doses of laquinimod after the occurrence of cardiovascular events (none fatal), in eight subjects [102]. The study of lower-dose laquinimod will continue in both trials.

Siponimod and ozanimod

Siponimod (BAF312) is an oral modulator of sphingosine more selective than figolimod - acts selectively on S1P-1 and S1P-5 [103]. In a phase II trial in RRMS, siponimod was shown to reduce brain MRI lesions and relapses by up to 80 % compared to placebo [104]. The results of a phase III trial in SPMS (NCT01665144) were reported at the 32nd ECTRIMS Congress in London in September 2016. Patients with SPMS on siponimod had the risk of 3-month confirmed disability progression reduced by 21% compared with patients on placebo (p=0.013) Another oral selective S1P receptor modulator, ozanimod, was recently successfully tested in a phase 2 trial with positive MRI outcomes [24].

Agents in trial for progressive MS

Finding effective treatments for progressive MS is a major priority and a challenge. The current treatment candidates and approaches for progressive MS have been recently reviewed in detail by Shirani et al [105]. An important point in testing drugs for progressive forms of MS is to

adapt trial methodology and clinically meaningful outcomes to this particular phenotype of MS, based on lessons learned from prior clinical trials [106].

It has been hypothesized that high-dose biotin can act on demyelinating axons by increasing energy production promoting synthesis of myelin [107]. Treatment with high-dose biotin was tested in an open-label pilot study [108] and a randomized, double-blind, placebo-controlled trial in progressive MS (23 and 154 patients, respectively) [109]. Both studies reported positive results on disability progression and a good safety profile [107]. Biotin can interfere with some laboratory tests (eg. false positive or negative thyroid tests [110,111]) and teratogenicity was reported in rabbits. Further studies are needed to clarify the duration of response to biotin over time and the responder profile in people with progressive MS.

Autologous Bone Marrow Transplantation

Hematopoietic stem cell transplantation (HSCT) has been reviewed in detail elsewhere [112-116]. Studies on animal models showed that strong immunosuppression followed by syngeneic bone marrow transplantation can induce long term antigen-specific tolerance [117]. In the last 15 years, due to the high rate of complications related to the procedure, autologous bone marrow transplantation was reserved only to MS patients that failed all other therapies and had a poor prognosis [116]. There is evidence that high-dose immune ablation and autologous HSCT could renew the immune system repertoire and reinforce immune tolerance mechanisms [118] thus having a clinical impact. Phase I clinical trials have shown that autologous hematopoietic stem cell transplantation may improve the quality of life of MS patients [112,116]. A recent phase II trial of HSCT vs mitoxantrone in RRMS and SPMS showed that HSCT reduced the number of new T2 and enhancing lesions and the AAR as compared to mitoxantrone [119]. In a very recent phase II trial, 70% of the patients who received an aggressive immune-ablative treatment followed by a HSCT graft depleted of autoreactive lymphocytes did not have any signs of disease activity (relapses, new MRI lesions, or EDSS progression) after a median follow-up of 6-7 years [120]. Although these results are promising, and progress has been made over the last decade in mitigating risks, there are many unknowns regarding the use of HSCT as a possible second-line therapy for refractory MS [121,122]. There is lack of consensus on the optimal conditioning regimen, patient selection (including the stage of the disease or what other prior treatments should they have failed to etc.) and the HSCT graft manipulation. Different conditioning regimens following the harvesting of the stem cells were used it the studies of autologous HSCT in people with MS [123]. The choice

of the conditioning regimens (myeloablative or non-myeloablative) can impact the outcomes both in terms of efficacy and toxicity [113]. The myeloablative conditioning regimens can completely eliminate the activated immune cells before the HSCT treatment, but would expose the patients to aplasia-related complications and death[113]. The use of lymphoablative but non-myeloablative conditioning regimens would be in keeping with "the rationale of auto-HSCT […] to revive an antigen-naive immune system from the patient's HSCs" [113] whilst mitigating adverse effects such as neurotoxicity[124]. Freedman recently noted that, although systematic comparison of regimens is lacking, the available data would suggest that the more intense conditioning regimens are followed by more durable responses but with more toxicity as well [121]. In their recently published study, Atkins et al removed the mature lymphocytes from the graft prior to transplantation by an ex vivo cell selection technology, avoiding graft-mediated immune effects and thus apparently impacting disease activity[120]. However, the benefits of this procedure would only be seen with intense conditioning regimens which achieve a near-complete immune ablation[121]. Intense immune ablation with a regimen including a cytotoxic agent crossing the BBB would translate in an accelerated whole-brain atrophy rate, although the rate of atrophy would further slow to that expected from normal aging [125]. In a recent editorial, Sorensen suggests that, taking into account the HSCT benefit-risk profile and the availability of highly-effective treatments with monoclonal antibodies which can achieve disease control in patients with active disease, intense immunosuppression with HSCT should remain a third-line therapy [122]. However, as Ellen Mowry notes in an editorial in the same journal [123], the comparison in terms of efficacy between the phase 3 studies with monoclonal antibodies and the observational or single-arm HSCT studies does not provide evidence for or against the use of HSCT in MS, and further studies designed to comparing these treatments are warranted [123]. It is likely that the place of HSCT in MS will be re-evaluated over the next years, in light of the continuously-growing spectrum of available therapies and of new pragmatic, prospective, controlled multicentre trials.

Remyelination strategies

Remyelination occurs initially in MS lesions but is inadequate, and the mechanism of repair in the CNS fails with time, especially in chronic disease stages [126]. The differentiation of oligodendrocyte precursor cells (OPC) into mature cells is essential [126]. Remyelination develops in two steps: the colonization of the lesions by the OPCs, and the OPC differentiation

into mature oligodendrocytes able to generate functional myelin sheath [127]. The factors that could interfere with the OPC abilities to remyelinating in MS have been reviewed [128-130]. The immunological-OPC crosstalk has specific features in MS [131,132]. Age and disease duration matter. In older animals remyelination is significantly slowed, possibly because of the decreased response of monocytoid immune cells which are necessary for the clearance of the myelin debris that inhibit remyelination [133]. Neurodegeneration itself occurs early in the course of the disease and repeated episodes of demyelination could conceivably lead to local wearing out of myelin forming OPCs [134]. Most information on these processes comes from studies on animal models (EAE and the cuprizone model of MS) [135]. Remyelination can be promoted either by intrinsic (altering intrinsic signaling pathways) or extrinsic (acting on lesion environment) repair mechanisms [136,137].

Intrinsic targets for remyelination**.** A way of promoting remyelination is modulation of specific signaling pathways such as Notch, the Wnt/β-catenin pathway and the retinoid X-receptor (RXR) signaling pathway [136] within oligodendrocytes to outweigh the inhibition of remyelination. Tocopherol derivative TFA-12, a synthetic long-chain fatty alcohol and a member of the vitamin E family, with anti-inflammatory properties [138] stimulates OPC differentiation and myelin repair in experimental models of MS through the inhibition of the Notch/Jagged1 intrinsic signaling pathway [138]. Lithium chloride stimulates myelin gene expression in oligodendrocytes via Wnt/β-catenin and Akt/CREB pathways [139]. Indomethacin, a non-steroidal anti-inflammatory drug that penetrates the blood brain barrier promotes the differentiation of OPC into mature cells, hence stimulating remyelination in animal models via modulation of Wnt/β-catenin pathway [140]. In vitro studies showed that the action of indomethacin relies on the GSK3β activity [140]. The nuclear retinoid X-receptor (RXR)-γ regulates positively the endogenous remyelination, by stimulating OPC differentiation [141]. RXR-γ binds to receptors inside the OPC, including the vitamin D receptor, and the complex RXR-vitamin D receptor enhances OPC differentiation [142]. Hence, vitamin D might have a role in remyelination. Finally, miconazole and clobetasol have been recently shown to enhance the generation of human oligodendrocytes from human OPC in vitro, possibly through mitogen-activated protein kinase and glucocorticoid receptor signalling, respectively [143].

Extrinsic targets for remyelination. Leucine-rich repeat and immunogloobulin domain-containing 1 (LINGO-1) is a nervous-system specific transmembrane protein that may be a therapeutic target for remyelination. LINGO-1 is expressed by oligodendrocytes and was

shown to inhibit their ability to differentiate and myelinate; it is also expressed on axons where it limits axonal regeneration [144,145]. Antibodies blocking LINGO-1 promote OPC differentiation in demyelinated lesions, reduce axonal damage and restore function in EAE, cuprizone and lysolecithin animal models [146,147]. BIIB033 (Li 81; opicinumab) is a fully human monoclonal antibody that binds LINGO-1. BIIB033 enhances remyelination and restores function in animal models, although brain concentrations were of <0.5% of those in blood [148]. Anti-LINGO-1 antibodies have been the first remyelinating therapy evaluated in humans. Two randomized placebo-controlled phase I trials tested the safety, tolerability and pharmacokinetics of BIIB033 administered via IV infusion or subcutaneous injection in healthy volunteers and people with MS [149]. In these studies, one or two doses up to 100 mg/kg were tolerated, with no serious adverse events and low immunogenicity [149]. RENEW was a randomized, double-blind, placebo-controlled phase II trial evaluating the ability of anti-LINGO-1 to promote the repair of an acute optic nerve lesion after a first episode of acute optic neuritis and examining the effects on remyelination over 24 weeks through the measurement of the latency of nerve conduction between the retina and the visual cortex [150]. The treatment with anti-LINGO-1 in acute optic neuritis prevented the amplitude loss of the multifocal visual evoked potential observed in the fellow eye visual pathway of placebo-treated subjects over 32 weeks and had some possible positive effects on amplitude preservation on the affected eye [150].

Another phase II trial (SYNERGY) testing the safety and effectiveness of opicinumab in association with IFNβ-1a injections once weekly in people with RRMS or SPMS (NCT01864148) has recently completed and the results presented at the 32nd ECTRIMS Congress in London in September 2016. The trial involved 416 participants receiving IFNβ-1a weekly and one of five different doses of anti-LINGO-1 per kg body weight or a placebo, once every 4 weeks for 72 weeks. The trial missed its primary endpoint (the percentage of subjects with confirmed improvement of neuro-physical and/or cognitive function and/or disability).

Wang et al. differentiated OPCs from human induced pluripotent stem cells (iPS) and engrafted them in a myelin-deficient mouse model [151]. The transplanted OPCs differentiated into astrocytes and oligodendrocytes, myelinated the brains of the animals, and increased their survival [151]. However, since MS is a multifocal disease it would probably require repeated transplantation of the OPC in all the demyelinated regions [134]. These techniques are still under study, and their safety and efficacy are yet unknown. OPC recruitment is reduced in MS lesions and chemotactic molecules such as Sema3A receptor neuropilin-1 could be a new group

of drug targets to improve remyelination [152]. It is not yet clear whether remyelination completely prevents neurodegeneration but it does appear to restore neuronal function and at least limit neuronal degeneration [134], therefore remyelination strategies are likely to be part of the MS treatments in the future (Table 2).

Mesenchymal stem cells

Mesenchymal stem cells (MSC) can be harvested from adult bone marrow and can be transplanted securely without the need for immunosuppression and with a low risk of aberrant proliferation. The IV route is preferred as it is less invasive has fewer adverse effects compared to intrathecal administration. It is likely that the potential therapeutic efficacy of MSC could be based on systemic effects as recently shown [153]. Mechanisms of action would include immunoregulation and anti-inflammatory changes of the cellular environment. The phase II trials of MSC in MS did not report major side effects related to treatment [154]. A phase 1/2 open-safety clinical trial in patients with MS and with amyotrophic lateral sclerosis showed that intrathecal and intravenous administration of autologous MSCs is a clinically feasible and a relatively safe procedure which produced immediate immunomodulatory effects [155].

T-cell directed strategies

It was suggested that the immune response in MS is directed at least in part against myelin proteins including basic protein (MBP), myelin oligodendrocyte protein (MOG) and proteolipid protein (PLP) [156-159]. Although there are differences in the activation state or precursor frequencies of T cells from patients with MS and healthy subjects, people without MS also have immune responses against such antigens. However, it was suggested based on indirect evidence that molecular mimicry, epitope spreading and bystander activation are possible mechanisms to initiate and maintain disease activity. Consequently, an alternative treatment approach in MS could aim to selectively restore self-tolerance to auto-antigens via immunization [160] and epitope-specific induction of T cell tolerization [161] or specifically by targeting regulatory T cells (Treg) signaling [162]. Antigen-specific therapies in MS have been reviewed [163,164]. A first phase I trial in humans published in 2013 showed that the antigen-coupled cell tolerization in MS is feasible and safe [165].

A number of animal studies and human clinical trials of T-cell vaccination in MS have been conducted and reviewed [166,167]. TCV include an attenuation step to preventing the encephalitogenicity of myelin-reactive T cells and is safe. Two phase II, placebo-controlled clinical trials of TCV in MS were reported [167,168]. Fox et al. showed that treatment with

myelin-reactive T-cells against up to six peptides of MBP, MOG and PLP was safe in 100 RRMS and CIS patients, of which 44% were previously treated with DMTs [168]. Although no statistically significant clinical or radiological benefits were obtained when compared with the placebo arm, some post-hoc evidence of clinical efficacy in the active arm in the subgroup of patients DTM-naïve was seen[168]. Karussis et al. performed the first placebo-controlled, double blind TCV trial in progressive MS[169]. 26 patients with relapsing-progressive MS were included in the trial, of which 19 were treated with four injections with a mixture of attenuated autologous T-cell lines reactive to three to nine different peptides from the MBP, PLP and MOG sequences[169]. The TCV was safe, without serious adverse events. There was a strong clinical effect on relapses and disability measured by EDSS and the timed 10-meter walking test. The authors suggested that the use of multiple vaccinations, peptides and several anti-myelin cell lines accounted for the positive clinical effects obtained in this trial [169]. The ongoing or completed clinical trials using T-cell directed approaches are listed in Table 1.

Drug repurposing in MS

The failure to deliver successful neuroprotective therapies in MS has led to alternative strategies such as drug repurposing [170]. Vesterinen et al. list some of the factors contributing to this failure: "the incomplete understanding of disease biology, pathogenic complexity and heterogeneity, limited predictive value of animal models, […] a lack of established trial methodologies compounded by a wider context of chronically declining productivity in drug development based on target-based approaches, declining resources for drug development in the neurosciences, and the growing costs of clinical trials" [170]. In a phase 2 trial, high-dose simvastatin compared with placebo reduced the annualized rate of whole-brain atrophy [171]. Encouraging recent data suggest that phenytoin in acute optic neuritis [172] and amiloride in PPMS [173] could have a neuroprotective effect. We and others showed that re-purposing molecules such as angiotensin converting enzyme inhibitors or retinoids can be effective in the animal model of MS or promote remyelination [141,174-176]. It is probable that repurposing drug development will play a bigger role in the future despite the inherent pitfalls which impact its feasibility [177].

Choice of treatment strategy

The best treatment strategy in MS is still debated. Escalation strategy implies starting with less effective but safer treatments and escalating to more efficacious but riskier DMTs if the inflammation is not controlled [178,179]. This approach would select only patients with a more active MS to be commenced on riskier drugs. The downside of this approach is that often in practice some patients have already accumulated some degree of disability at the time of escalation, which may be irreversible [180]. This disadvantage is taken into account by those advocating starting treatment with a more potent drug from the outset (induction strategy). This approach applied indiscriminately would in turn expose people with less active MS to unwanted drug side-effects. The treatment selection should take into account how aggressive and active the inflammation is; medications such as natalizumab and alemtuzumab would be preferred in people with active MS. However, alemtuzumab was EMA registered for patients "with active disease defined by clinical or imaging features" [29] and this allows treatment in people with early disease and moderate relapse rate if active disease is demonstrated (defined as at least 2 relapses within the prior 2 years).

A new strategy aims to attain 'no evidence of disease activity' (NEDA). NEDA emerged as therapeutic expectations and targets evolved to enclose possible remission from evolving disease. NEDA is defined as absence of relapses, progression of disability and MRI activity such as new or newly enlarging T2 lesions and new enhancing lesions [181]. Achieving the NEDA status at 2 years has a good prognostic value over a 7-year period [182]. NEDA is criticized of not reflecting patients' needs in clinical practice [183]. The absence of brain atrophy measured by MRI as a marker of neuroaxonal loss was proposed as an additional criterion for NEDA (NEDA-4)[184]. The effects of MS treatments on disability progression relate with the effects on atrophy over 2 years, independently from the effect on active MRI lesions[185]. This would support the use of brain atrophy as a surrogate marker of disability progression [186]. However, routinely assessing atrophy is difficult due to logistical and technical restraints (for example variability between scans and scanners). As Zimmsen et al. note, "the increasing focus on NEDA as an aim of MS therapy implies that regular, systematic, monitoring should be a central aspect of the management of the condition" [179]. This raises the issue of disease monitoring: how often and what measures to use. How each of the detected MRI changes would entail a specific and timely change in the treatment strategy is still a matter of debate. Moreover, it is not clear yet if an escalation approach aiming of achieving NEDA would certainly improve disease outcome over the long term [180]. Possibly additional outcome measures (ie. cognitive impairment) and MS patient reported measures may prove

useful to identify non-responders to DMT and therefore could be candidate measures to be incorporated in NEDA. Nevertheless, data from clinical trials back the idea of a benefit of early initiation of treatment in MS [187-190] [191]. Early treatment would have an impact on long-term prognosis in terms of mortality [192]. Although it is intuitive that MRI measures can reflect breakthrough disease, there is a lack of clinical consensus on the quantitative aspects of these measures. Two new enhancing lesions were predictive of disability worsening in a 15-year follow-up study of patients enrolled in a pivotal IFNβ trial [193]. A recent systematic review of studies examining differential response to IFNβ showed that patients with 2 or more new hyperintense T2 lesions or new gadolinium-enhancing lesions had significantly increased risk of both future relapses and progression [194]. The Rio score was developed by the Barcelona group and included the number of clinical relapses, disability progression and new T2 or enhancing MRI lesions [195]. Patients with RRMS who met at least two of these criteria (but not relapses or MRI criteria alone) had a higher risk of having relapses or disability progression in the next 2 years. A new version of the Rio score was validated in the original data set and classified patients in low-, medium- and high-risk of progression based on relapses or MRI activity only [196,197]. A study by Prosperini et al. suggested that MRI alone could be a 4-year good predictor of outcome after the first year of treatment with IFNβ-beta [198]. While changes in clinical and MRI measures of disease activity can lead to a treatment change, the disease-related outcomes reported by the patient could too. It is probable that over the next years more information from real-life experience regarding specific drug efficacy, long term safety concerns, cost-benefit considerations and patient-perceived therapy burden, will be available.

Five year view

In a recent review, Coles [180] describes the current era of treatments of MS as ‘the era of complexity’. This followed the ‘era of nihilism’ when no DMT were available and the ‘era of modest efficacy’ which started after 1993 with the IFNβ-beta and glatiramer acetate trials (which were proven modestly effective on relapse rate but safer than immunosuppressants such as mitoxantrone and cyclophosphamide) [180]. The current ‘era of treatment’ started with the introduction of natalizumab and the first reports of PML as the main drug side-effect which mitigated the enthusiasm for this medication [180]. Over the next decade, the oral drugs fingolimod, dimethyl fumarate and teriflunomide, and the injectable alemtuzumab have

become available in many countries. As mentioned above, it is likely that daclizumab and ocrelizumab will soon follow. The development of the newer therapies for MS raises new challenges to the treating neurologist. The questions the doctors are facing regard the correct sequence of drugs to be offered to a given patient; and the appropriate and safe sequence of switching between drugs. Since all the available DMTs are directed against the inflammatory phase of MS, the major unmet need at this time is for treatments for progressive disease. It is likely that in the next and fourth era of DMTs in MS the efforts of tackling progressive disease will become successful [180]. It is also likely that more evidence would become available to support the different strategies for the sequencing and timing of treatments. Clarifying the monitoring strategies for adverse effects and breakthrough MS would be crucial. The development of radiological and biological biomarkers could allow in the future personalizing treatment and precision medicine in MS [199]. Features such as detection of central veins in the MS lesions could improve diagnostic quality [200]. Biomarkers such as neurofilament light chains [201] and chitinase 3-like proteins in the CSF [202] could be used as potential prognostic markers after CIS while markers such as CSF lipid-specific IgM bands[203] and L-selectin[204] could be incorporated in the PML risk stratification in patients under natalizumab [203].

Over the past 50 years, the largest impact on increasing life expectancy in the general population came from primary prevention. MS is the result of the interaction between the genetic background and environmental factors. Among the latter, vitamin D deficiency, infection with the Epstein-Barr virus (EBV), cigarette smoking, obesity in youth and lack of exposure to intestinal parasites are environmental predictors of MS risk [205]. Considering that research into preventing MS currently has a high priority, developments in prevention strategies are anticipated. Studies of anti-EBV vaccination in people at risk of developing MS, clinical trials of smoking cessation in MS and more clinical trials with vitamin D are expected. The prevalence of MS is inversely correlated with helminth infections and studies of helminth treatments in MS could help in understanding the mechanisms of parasite-induced immunomodulation [206].

Treating the right patient with the right drug early in the disease course, before disability has been acquired, could yield long term benefits. It is realistic to expect over the next decade more data from approaches aiming to prevent MS and on methodologies to protect neurons or promote remyelination. Finally, the future of MS treatments would rely on a better understanding of the immunopathogenesis of MS.

Key issues

- MS is an immune condition of the CNS characterized by focal damage (inflammation) which manifests clinically mainly as relapses and diffuse damage (neurodegeneration) and brain volume loss) which are both responsible for disability.
- There is no cure for MS. A number of disease modifying treatments (DMTs) have been approved which are primarily directed against inflammation. There are a number of additional DMTs in trial or being submitted for approval.
- There are different treatment strategies in MS. In patients with active disease despite treatment it has been recommended to switch early to a therapy of higher efficacy.
- Despite important advances in the treatment of MS, the burden of progressive disability and premature mortality remains considerable. There is no currently approved treatment for progressive MS, but recently a monoclonal antibody (ocrelizumab) was reported to be effective in a primary progressive MS trial.
- New treatment strategies involving remyelination or neuroprotection are under study. It is likely that the next decade brings substantial changes in the understanding and ways of approaching the treatment in MS.

References

1. Federation MSI. Atlas of MS 2013: Mapping Multiple Sclerosis Around the World. (Ed.^(Eds) (London, 2013)
2. Compston A, Coles A. Multiple sclerosis. *Lancet*, 372(9648), 1502-1517 (2008).
3. Lublin FD, Reingold SC, Cohen JA *et al.* Defining the clinical course of multiple sclerosis: the 2013 revisions. *Neurology*, 83(3), 278-286 (2014).
4. Tanasescu R, Ionete C, Chou IJ, Constantinescu CS. Advances in the treatment of relapsing-remitting multiple sclerosis. *Biomed J*, 37(2), 41-49 (2014).
5. Thomas RH, Wakefield RA. Oral disease-modifying therapies for relapsing-remitting multiple sclerosis. *Am J Health Syst Pharm*, 72(1), 25-38 (2015).
6. Ali R, Nicholas RS, Muraro PA. Drugs in development for relapsing multiple sclerosis. *Drugs*, 73(7), 625-650 (2013).
7. Boster AL, Ford CC, Neudorfer O, Gilgun-Sherki Y. Glatiramer acetate: long-term safety and efficacy in relapsing-remitting multiple sclerosis. *Expert Rev Neurother*, 15(6), 575-586 (2015).
8. Johnson KP, Brooks BR, Cohen JA *et al.* Copolymer 1 reduces relapse rate and improves disability in relapsing-remitting multiple sclerosis: results of a phase III multicenter, double-blind, placebo-controlled trial. 1995. *Neurology*, 57(12 Suppl 5), S16-24 (2001).
9. Lugaresi A, di Ioia M, Travaglini D, Pietrolongo E, Pucci E, Onofrj M. Risk-benefit considerations in the treatment of relapsing-remitting multiple sclerosis. *Neuropsychiatr Dis Treat*, 9, 893-914 (2013).
10. Palace J, Duddy M, Bregenzer T *et al.* Effectiveness and cost-effectiveness of interferon beta and glatiramer acetate in the UK Multiple Sclerosis Risk Sharing Scheme at 6 years: a clinical cohort study with natural history comparator. *Lancet Neurol*, 14(5), 497-505 (2015).
11. Ford C, Goodman AD, Johnson K *et al.* Continuous long-term immunomodulatory therapy in relapsing multiple sclerosis: results from the 15-year analysis of the US prospective open-label study of glatiramer acetate. *Mult Scler*, 16(3), 342-350 (2010).
12. Giovannoni G, Southam E, Waubant E. Systematic review of disease-modifying therapies to assess unmet needs in multiple sclerosis: tolerability and adherence. *Mult Scler*, 18(7), 932-946 (2012).
13. Craddock J, Markovic-Plese S. Immunomodulatory therapies for relapsing-remitting multiple sclerosis: monoclonal antibodies, currently approved and in testing. *Expert Rev Clin Pharmacol*, 8(3), 283-296 (2015).
14. Rudick RA, Stuart WH, Calabresi PA *et al.* Natalizumab plus interferon beta-1a for relapsing multiple sclerosis. *N Engl J Med*, 354(9), 911-923 (2006).
15. Polman CH, O'Connor PW, Havrdova E *et al.* A randomized, placebo-controlled trial of natalizumab for relapsing multiple sclerosis. *N Engl J Med*, 354(9), 899-910 (2006).
16. Miller DH, Soon D, Fernando KT *et al.* MRI outcomes in a placebo-controlled trial of natalizumab in relapsing MS. *Neurology*, 68(17), 1390-1401 (2007).
17. Rommer PS, Zettl UK, Kieseier B *et al.* Requirement for safety monitoring for approved multiple sclerosis therapies: an overview. *Clin Exp Immunol*, 175(3), 397-407 (2014).
18. McGuigan C, Craner M, Guadagno J *et al.* Stratification and monitoring of natalizumab-associated progressive multifocal leukoencephalopathy risk: recommendations from an expert group. *J Neurol Neurosurg Psychiatry*, 87(2), 117-125 (2016).

** An algorithm of stratification and monitoring of patients at risk for PML

19. Bloomgren G, Richman S, Hotermans C *et al.* Risk of natalizumab-associated progressive multifocal leukoencephalopathy. *N Engl J Med*, 366(20), 1870-1880 (2012).

20. Plavina T, Subramanyam M, Bloomgren G *et al.* Anti-JC virus antibody levels in serum or plasma further define risk of natalizumab-associated progressive multifocal leukoencephalopathy. *Ann Neurol*, 76(6), 802-812 (2014).
21. Torkildsen O, Myhr KM, Bo L. Disease-modifying treatments for multiple sclerosis - a review of approved medications. *Eur J Neurol*, 23 Suppl 1, 18-27 (2016).
22. Jones JL, Coles AJ. Mode of action and clinical studies with alemtuzumab. *Exp Neurol*, 262 Pt A, 37-43 (2014).
23. Singer BA. Parenteral Treatment of Multiple Sclerosis: The Advent of Monoclonal Antibodies. *Semin Neurol*, 36(2), 140-147 (2016).
24. Cohen JA, Arnold DL, Comi G *et al.* Safety and efficacy of the selective sphingosine 1-phosphate receptor modulator ozanimod in relapsing multiple sclerosis (RADIANCE): a randomised, placebo-controlled, phase 2 trial. *Lancet Neurol*, 15(4), 373-381 (2016).
25. Coles AJ, Twyman CL, Arnold DL *et al.* Alemtuzumab for patients with relapsing multiple sclerosis after disease-modifying therapy: a randomised controlled phase 3 trial. *Lancet*, 380(9856), 1829-1839 (2012).
26. Cohen JA, Coles AJ, Arnold DL *et al.* Alemtuzumab versus interferon beta 1a as first-line treatment for patients with relapsing-remitting multiple sclerosis: a randomised controlled phase 3 trial. *Lancet*, 380(9856), 1819-1828 (2012).
27. Cossburn M, Pace AA, Jones J *et al.* Autoimmune disease after alemtuzumab treatment for multiple sclerosis in a multicenter cohort. *Neurology*, 77(6), 573-579 (2011).
28. Tuohy O, Costelloe L, Hill-Cawthorne G *et al.* Alemtuzumab treatment of multiple sclerosis: long-term safety and efficacy. *J Neurol Neurosurg Psychiatry*, 86(2), 208-215 (2015).
29. http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/003718/human_med_001678.jsp&mid=WC0b01ac058001d124.).
30. Bielekova B, Catalfamo M, Reichert-Scrivner S *et al.* Regulatory CD56(bright) natural killer cells mediate immunomodulatory effects of IL-2Ralpha-targeted therapy (daclizumab) in multiple sclerosis. *Proc Natl Acad Sci U S A*, 103(15), 5941-5946 (2006).
31. Wynn D, Kaufman M, Montalban X *et al.* Daclizumab in active relapsing multiple sclerosis (CHOICE study): a phase 2, randomised, double-blind, placebo-controlled, add-on trial with interferon beta. *Lancet Neurol*, 9(4), 381-390 (2010).
32. Gold R, Giovannoni G, Selmaj K *et al.* Daclizumab high-yield process in relapsing-remitting multiple sclerosis (SELECT): a randomised, double-blind, placebo-controlled trial. *Lancet*, 381(9884), 2167-2175 (2013).
33. Giovannoni G, Gold R, Selmaj K *et al.* Daclizumab high-yield process in relapsing-remitting multiple sclerosis (SELECTION): a multicentre, randomised, double-blind extension trial. *Lancet Neurol*, 13(5), 472-481 (2014).
34. Krueger JG, Kircik L, Hougeir F *et al.* Cutaneous Adverse Events in the Randomized, Double-Blind, Active-Comparator DECIDE Study of Daclizumab High-Yield Process Versus Intramuscular Interferon Beta-1a in Relapsing-Remitting Multiple Sclerosis. *Adv Ther*, 33(7), 1231-1245 (2016).
35. Cortese I, Ohayon J, Fenton K *et al.* Cutaneous adverse events in multiple sclerosis patients treated with daclizumab. *Neurology*, 86(9), 847-855 (2016).
36. http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm504000.htm.).
37. Millefiorini E, Gasperini C, Pozzilli C *et al.* Randomized placebo-controlled trial of mitoxantrone in relapsing-remitting multiple sclerosis: 24-month clinical and MRI outcome. *J Neurol*, 244(3), 153-159 (1997).
38. Hartung HP, Gonsette R, Konig N *et al.* Mitoxantrone in progressive multiple sclerosis: a placebo-controlled, double-blind, randomised, multicentre trial. *Lancet*, 360(9350), 2018-2025 (2002).

39. Li JM, Yang Y, Zhu P, Zheng F, Gong FL, Mei YW. Mitoxantrone exerts both cytotoxic and immunoregulatory effects on activated microglial cells. *Immunopharmacol Immunotoxicol*, 34(1), 36-41 (2012).
40. Tanasescu R, Debouverie M, Pittion S, Anxionnat R, Vespignani H. Acute myeloid leukaemia induced by mitoxantrone in a multiple sclerosis patient. *J Neurol*, 251(6), 762-763 (2004).
41. Tanasescu R, Evangelou N, Constantinescu CS. Role of oral teriflunomide in the management of multiple sclerosis. *Neuropsychiatr Dis Treat*, 9, 539-553 (2013).
42. Li L, Liu J, Delohery T, Zhang D, Arendt C, Jones C. The effects of teriflunomide on lymphocyte subpopulations in human peripheral blood mononuclear cells in vitro. *J Neuroimmunol*, 265(1-2), 82-90 (2013).
43. O'Connor P, Wolinsky JS, Confavreux C *et al.* Randomized trial of oral teriflunomide for relapsing multiple sclerosis. *N Engl J Med*, 365(14), 1293-1303 (2011).
44. Confavreux C, O'Connor P, Comi G *et al.* Oral teriflunomide for patients with relapsing multiple sclerosis (TOWER): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Neurol*, 13(3), 247-256 (2014).
45. Vermersch P, Czlonkowska A, Grimaldi LM *et al.* Teriflunomide versus subcutaneous interferon beta-1a in patients with relapsing multiple sclerosis: a randomised, controlled phase 3 trial. *Mult Scler*, 20(6), 705-716 (2014).
46. Miller AE, Wolinsky JS, Kappos L *et al.* Oral teriflunomide for patients with a first clinical episode suggestive of multiple sclerosis (TOPIC): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Neurol*, 13(10), 977-986 (2014).
47. Comi G, Freedman MS, Kappos L *et al.* Pooled safety and tolerability data from four placebo-controlled teriflunomide studies and extensions. *Mult Scler Relat Disord*, 5, 97-104 (2016).
48. Linker RA, Gold R. Dimethyl fumarate for treatment of multiple sclerosis: mechanism of action, effectiveness, and side effects. *Curr Neurol Neurosci Rep*, 13(11), 394 (2013).
49. Gold R, Kappos L, Arnold DL *et al.* Placebo-controlled phase 3 study of oral BG-12 for relapsing multiple sclerosis. *N Engl J Med*, 367(12), 1098-1107 (2012).
50. Fox RJ, Miller DH, Phillips JT *et al.* Placebo-controlled phase 3 study of oral BG-12 or glatiramer in multiple sclerosis. *N Engl J Med*, 367(12), 1087-1097 (2012).
51. Havrdova E, Hutchinson M, Kurukulasuriya NC *et al.* Oral BG-12 (dimethyl fumarate) for relapsing-remitting multiple sclerosis: a review of DEFINE and CONFIRM. Evaluation of: Gold R, Kappos L, Arnold D, et al. Placebo-controlled phase 3 study of oral BG-12 for relapsing multiple sclerosis. N Engl J Med 2012;367:1098-107; and Fox RJ, Miller DH, Phillips JT, et al. Placebo-controlled phase 3 study of oral BG-12 or glatiramer in multiple sclerosis. N Engl J Med 2012;367:1087-97. *Expert Opin Pharmacother*, 14(15), 2145-2156 (2013).
52. Gold R, Arnold DL, Bar-Or A *et al.* Long-term effects of delayed-release dimethyl fumarate in multiple sclerosis: Interim analysis of ENDORSE, a randomized extension study. *Mult Scler*, (2016).
53. Prosperini L, Pontecorvo S. Dimethyl fumarate in the management of multiple sclerosis: appropriate patient selection and special considerations. *Ther Clin Risk Manag*, 12, 339-350 (2016).
54. Longbrake EE, Naismith RT, Parks BJ, Wu GF, Cross AH. Dimethyl fumarate-associated lymphopenia: Risk factors and clinical significance. *Mult Scler J Exp Transl Clin*, 1 (2015).
55. Rosenkranz T, Novas M, Terborg C. PML in a patient with lymphocytopenia treated with dimethyl fumarate. *N Engl J Med*, 372(15), 1476-1478 (2015).
56. Sweetser MT, Dawson KT, Bozic C. Manufacturer's response to case reports of PML. *N Engl J Med*, 368(17), 1659-1661 (2013).
57. Reich K, Hartung HP, Lebwohl M. More on PML in Patients Treated with Dimethyl Fumarate. *N Engl J Med*, 374(3), 294-295 (2016).
58. Brinkmann V, Davis MD, Heise CE *et al.* The immune modulator FTY720 targets sphingosine 1-phosphate receptors. *J Biol Chem*, 277(24), 21453-21457 (2002).

59. Tanasescu R, Constantinescu CS. Pharmacokinetic evaluation of fingolimod for the treatment of multiple sclerosis. *Expert Opinion on Drug Metabolism & Toxicology*, 10(4), 621-630 (2014).
60. Groves A, Kihara Y, Chun J. Fingolimod: direct CNS effects of sphingosine 1-phosphate (S1P) receptor modulation and implications in multiple sclerosis therapy. *J Neurol Sci*, 328(1-2), 9-18 (2013).
61. Kappos L, Radue EW, O'Connor P *et al.* A placebo-controlled trial of oral fingolimod in relapsing multiple sclerosis. *N Engl J Med*, 362(5), 387-401 (2010).

- The first approved oral medication in MS.

62. Cohen JA, Barkhof F, Comi G *et al.* Oral fingolimod or intramuscular interferon for relapsing multiple sclerosis. *N Engl J Med*, 362(5), 402-415 (2010).
63. Lublin F, Miller DH, Freedman MS *et al.* Oral fingolimod in primary progressive multiple sclerosis (INFORMS): a phase 3, randomised, double-blind, placebo-controlled trial. *Lancet*, 387(10023), 1075-1084 (2016).
64. Ayzenberg I, Hoepner R, Kleiter I. Fingolimod for multiple sclerosis and emerging indications: appropriate patient selection, safety precautions, and special considerations. *Ther Clin Risk Manag*, 12, 261-272 (2016).
65. http://www.fda.gov/Drugs/DrugSafety/ucm303192.htm FFdscrrfcmauomsdGfF.).
66. Cohen JA, Barkhof F, Comi G *et al.* Oral Fingolimod or Intramuscular Interferon for Relapsing Multiple Sclerosis. *New England Journal of Medicine*, 362(5), 402-415 (2010).
67. Khatri BO. Fingolimod in the treatment of relapsing-remitting multiple sclerosis: long-term experience and an update on the clinical evidence. *Ther Adv Neurol Disord*, 9(2), 130-147 (2016).
68. Hauser SL, Waubant E, Arnold DL *et al.* B-cell depletion with rituximab in relapsing-remitting multiple sclerosis. *N Engl J Med*, 358(7), 676-688 (2008).
69. Hawker K, O'Connor P, Freedman MS *et al.* Rituximab in patients with primary progressive multiple sclerosis: results of a randomized double-blind placebo-controlled multicenter trial. *Ann Neurol*, 66(4), 460-471 (2009).
70. Clifford DB, Ances B, Costello C *et al.* Rituximab-associated progressive multifocal leukoencephalopathy in rheumatoid arthritis. *Arch Neurol*, 68(9), 1156-1164 (2011).
71. Topping J, Dobson R, Lapin S *et al.* The effects of intrathecal rituximab on biomarkers in multiple sclerosis. *Mult Scler Relat Disord*, 6, 49-53 (2016).
72. Komori M, Lin YC, Cortese I *et al.* Insufficient disease inhibition by intrathecal rituximab in progressive multiple sclerosis. *Ann Clin Transl Neurol*, 3(3), 166-179 (2016).
73. Berenguer-Ruiz L, Sempere AP, Gimenez-Martinez J *et al.* Rescue Therapy Using Rituximab for Multiple Sclerosis. *Clin Neuropharmacol*, (2016).
74. Alping P, Frisell T, Novakova L *et al.* Rituximab versus fingolimod after natalizumab in multiple sclerosis patients. *Ann Neurol*, 79(6), 950-958 (2016).
75. Kappos L, Li D, Calabresi PA *et al.* Ocrelizumab in relapsing-remitting multiple sclerosis: a phase 2, randomised, placebo-controlled, multicentre trial. *Lancet*, 378(9805), 1779-1787 (2011).
76. Hauser S CG, H.-P. Hartung , K. Selmaj , A. Traboulsee , A. Bar-Or , D.L. Arnold , G. Klingelschmitt , A. Kakarieka , F. Lublin , H. Garren , L. Kappos , on behalf of the OPERA I and II clinical investigators. Efficacy and safety of ocrelizumab in relapsing multiple sclerosis - results of the interferon-beta-1a-controlled, double-blind, Phase III OPERA I and II studies. In: *ECTRIMS.* (Ed.^(Eds) (Barcelona, Spain, 2015)
77. Traboulsee AL AD, Bar-Or A, et al. . Ocrelizumab no evidence of disease activity (NEDA) status at 96 weeks in patients with relapsing multiple sclerosis: analysis of the phase III double-blind, double-dummy, interferon beta-1a-controlled OPERA I and OPERA II studies. . In: *The 68th Annual Meeting of the American Academy of Neurology.* (Ed.^(Eds) (Vancouver, British Columbia., 2016)

78. Montalban X HB, Rammohan K, Giovannoni G et al. Efficacy and Safety of Ocrelizumab in Primary Progressive Multiple Sclerosis: Results of the Phase III Double-Blind, Placebo-Controlled ORATORIO Study In: *The 68th Annual Meeting of the American Academy of Neurology.* (Ed.^(Eds) (Vancouver, British Columbia, Canada, 2016)

- The first successful placebo-controlled trial of a monoclonal antibody in primary progressive MS.

79. Sorensen PS, Lisby S, Grove R *et al.* Safety and efficacy of ofatumumab in relapsing-remitting multiple sclerosis: a phase 2 study. *Neurology*, 82(7), 573-581 (2014).
80. Bar-Or A GR, Austin D et al. The MIRROR Study: A Randomized, Double-Blind, Placebo-Controlled, Parallel-Group, Dose-Ranging Study to Investigate the Safety and MRI Efficacy of Subcutaneous Ofatumumab in Subjects with Relapsing-Remitting Multiple Sclerosis (RRMS). In: *The 66th Annual Meeting of the American Academy of Neurology*(Ed.^(Eds) (2014)
81. Huynh E, Sigal D, Saven A. Cladribine in the treatment of hairy cell leukemia: initial and subsequent results. *Leuk Lymphoma*, 50 Suppl 1, 12-17 (2009).
82. Liliemark J. The clinical pharmacokinetics of cladribine. *Clin Pharmacokinet*, 32(2), 120-131 (1997).
83. Beutler E. Cladribine (2-chlorodeoxyadenosine). *Lancet*, 340(8825), 952-956 (1992).
84. Thone J, Ellrichmann G. Oral available agents in the treatment of relapsing remitting multiple sclerosis: an overview of merits and culprits. *Drug Healthc Patient Saf*, 5, 37-47 (2013).
85. Beutler E, Sipe JC, Romine JS, Koziol JA, McMillan R, Zyroff J. The treatment of chronic progressive multiple sclerosis with cladribine. *Proc Natl Acad Sci U S A*, 93(4), 1716-1720 (1996).
86. Sipe JC, Romine JS, Koziol JA, McMillan R, Zyroff J, Beutler E. Cladribine in treatment of chronic progressive multiple sclerosis. *Lancet*, 344(8914), 9-13 (1994).
87. Cook S, Vermersch P, Comi G *et al.* Safety and tolerability of cladribine tablets in multiple sclerosis: the CLARITY (CLAdRIbine Tablets treating multiple sclerosis orallY) study. *Mult Scler*, 17(5), 578-593 (2011).
88. Giovannoni G CG, Cook S, Rieckmann P et al. Clinical Efficacy of Cladribine Tablets in Patients with Relapsing-Remitting Multiple Sclerosis (RRMS): Final Results from the 120-Week Phase IIIb Extension Trial to the CLARITY Study. In: *The 68th Annual Meeting of the American Academy of Neurology.* (Ed.^(Eds) (Vancouver, British Columbia., 2016)
89. Comi G GG, Cook S et al. Magnetic Resonance Imaging (MRI) Outcomes in Patients with Relapsing-Remitting Multiple Sclerosis (RRMS) Treated with Cladribine Tablets: Results from the 120-Week Phase IIIb Extension of the CLARITY. In: *The 68th Annual Meeting of the American Academy of Neurology.* (Ed.^(Eds) (Vancouver, British Columbia, 2016)
90. Montalban X CB, Leist T, Moses H et al. Efficacy of Cladribine Tablets as Add-On to IFN-beta Therapy in Patients with Active Relapsing MS: Final Results from the Phase II ONWARD Study In: *The 68th Annual Meeting of the American Academy of Neurology*(Ed.^(Eds) (Vancouver, British Columbia, 2016)
91. Leist TP, Comi G, Cree BA *et al.* Effect of oral cladribine on time to conversion to clinically definite multiple sclerosis in patients with a first demyelinating event (ORACLE MS): a phase 3 randomised trial. *Lancet Neurol*, 13(3), 257-267 (2014).
92. Freedman M LT, Comi G, Cree B et al. Efficacy of Cladribine Tablets in ORACLE Study Patients Who Retrospectively Met 2010 McDonald Multiple Sclerosis (MS) Criteria at Baseline. In: *The 68th Annual Meeting of the American Academy of Neurology.* (Ed.^(Eds) (Vancouver, British Columbia., 2016)
93. Agency EM. Refusal of the marketing authorization for Movectro (cladribine): outcome of re-examination. . (Ed.^(Eds) (January 20, 2011)

94. Pakpoor J, Disanto G, Altmann DR *et al.* No evidence for higher risk of cancer in patients with multiple sclerosis taking cladribine. *Neurol Neuroimmunol Neuroinflamm*, 2(6), e158 (2015).
- The evidence on cancer-related cladribine reappraised.

95. Brueck W, Wegner C. Insight into the mechanism of laquinimod action. *Journal of the Neurological Sciences*, 306(1-2), 173-179 (2011).
96. Kim W, Zandona ME, Kim SH, Kim HJ. Oral disease-modifying therapies for multiple sclerosis. *J Clin Neurol*, 11(1), 9-19 (2015).
97. Yang HS, Xu LY, Xiao BG, Hedlund G, Link H. Laquinimod (ABR-215062) suppresses the development of experimental autoimmune encephalomyelitis, modulates the Th1/Th2 balance and induces the Th3 cytokine TGF-beta in Lewis rats. *Journal of Neuroimmunology*, 156(1-2), 3-9 (2004).
98. Thone J, Gold R. Laquinimod: a promising oral medication for the treatment of relapsing-remitting multiple sclerosis. *Expert Opin Drug Metab Toxicol*, 7(3), 365-370 (2011).
99. Filippi M, Rocca MA, Pagani E *et al.* Placebo-controlled trial of oral laquinimod in multiple sclerosis: MRI evidence of an effect on brain tissue damage. *J Neurol Neurosurg Psychiatry*, (2013).
100. Vollmer TL, Sorensen PS, Selmaj K *et al.* A randomized placebo-controlled phase III trial of oral laquinimod for multiple sclerosis. *J Neurol*, 261(4), 773-783 (2014).
101. Varrin-Doyer M, Zamvil SS, Schulze-Topphoff U. Laquinimod, an up-and-coming immunomodulatory agent for treatment of multiple sclerosis. *Exp Neurol*, 262 Pt A, 66-71 (2014).
102. Teva and Active Biotech Announce Discontinuation of Higher Doses of Laquinimod in Two Multiple Sclerosis Trials. CONCERTO and ARPEGGIO Trials Continue Study of Lower-dose Laquinimod. (Ed.^(Eds) (2016)
103. Gonzalez-Cabrera PJ, Brown S, Studer SM, Rosen H. S1P signaling: new therapies and opportunities. *F1000Prime Rep*, 6, 109 (2014).
104. Selmaj K, Li DK, Hartung HP *et al.* Siponimod for patients with relapsing-remitting multiple sclerosis (BOLD): an adaptive, dose-ranging, randomised, phase 2 study. *Lancet Neurol*, 12(8), 756-767 (2013).
105. Shirani A, Okuda DT, Stuve O. Therapeutic Advances and Future Prospects in Progressive Forms of Multiple Sclerosis. *Neurotherapeutics*, 13(1), 58-69 (2016).
106. Ontaneda D, Fox RJ, Chataway J. Clinical trials in progressive multiple sclerosis: lessons learned and future perspectives. *Lancet Neurol*, 14(2), 208-223 (2015).
107. Peyro Saint Paul L, Debruyne D, Bernard D, Mock DM, Defer GL. Pharmacokinetics and pharmacodynamics of MD1003 (high-dose biotin) in the treatment of progressive multiple sclerosis. *Expert Opin Drug Metab Toxicol*, 12(3), 327-344 (2016).
108. Sedel F, Papeix C, Bellanger A *et al.* High doses of biotin in chronic progressive multiple sclerosis: a pilot study. *Mult Scler Relat Disord*, 4(2), 159-169 (2015).
109. Tourbah AL-F, C. Edan, G et al. MD1003 (high doses of biotin) in progressive multiple sclerosis: subgroup analyses of the MS-SPI trial. In: *ECTRIMS* (Ed.^(Eds) (Barcelona, Spain, 2015)
110. Kummer S, Hermsen D, Distelmaier F. Biotin Treatment Mimicking Graves' Disease. *N Engl J Med*, 375(7), 704-706 (2016).
111. Elston MS, Sehgal S, Du Toit S, Yarndley T, Conaglen JV. Factitious Graves' disease due to biotin immunoassay interference - A case and review of the literature. *J Clin Endocrinol Metab*, jc20161971 (2016).
112. Karussis D, Petrou P, Vourka-Karussis U, Kassis I. Hematopoietic stem cell transplantation in multiple sclerosis. *Expert Rev Neurother*, 13(5), 567-578 (2013).
113. Bakhuraysah MM, Siatskas C, Petratos S. Hematopoietic stem cell transplantation for multiple sclerosis: is it a clinical reality? *Stem Cell Res Ther*, 7, 12 (2016).

114. Reston JT, Uhl S, Treadwell JR, Nash RA, Schoelles K. Autologous hematopoietic cell transplantation for multiple sclerosis: a systematic review. *Mult Scler*, 17(2), 204-213 (2011).
115. Pfender N, Saccardi R, Martin R. Autologous hematopoietic stem cell transplantation as a treatment option for aggressive multiple sclerosis. *Curr Treat Options Neurol*, 15(3), 270-280 (2013).
116. Radaelli M, Merlini A, Greco R *et al.* Autologous bone marrow transplantation for the treatment of multiple sclerosis. *Curr Neurol Neurosci Rep*, 14(9), 478 (2014).
117. Karussis D, Vourka-Karussis U, Mizrachi-Koll R, Abramsky O. Acute/relapsing experimental autoimmune encephalomyelitis: induction of long lasting, antigen-specific tolerance by syngeneic bone marrow transplantation. *Mult Scler*, 5(1), 17-21 (1999).
118. Muraro PA, Douek DC. Renewing the T cell repertoire to arrest autoimmune aggression. *Trends Immunol*, 27(2), 61-67 (2006).
119. Mancardi GL, Sormani MP, Gualandi F *et al.* Autologous hematopoietic stem cell transplantation in multiple sclerosis: a phase II trial. *Neurology*, 84(10), 981-988 (2015).
120. Atkins HL, Bowman M, Allan D *et al.* Immunoablation and autologous haemopoietic stem-cell transplantation for aggressive multiple sclerosis: a multicentre single-group phase 2 trial. *Lancet*, 388(10044), 576-585 (2016).
- A phase II trial of HSCT in MS with positive clinical and radiological outcomes.

121. Freedman M, Atkins HL. Haematopoietic stem cell transplants should be a second-line therapy for highly active MS - YES. *Mult Scler*, (2016).
122. Soelberg Sorensen P. Haematopoietic stem cell transplants should be a second-line therapy for highly active MS - NO. *Mult Scler*, (2016).
123. Mowry EM. Haematopoietic stem cell transplants should be a second-line therapy for highly active MS - Commentary. *Mult Scler*, (2016).
124. Atkins HL, Freedman MS. Hematopoietic stem cell therapy for multiple sclerosis: top 10 lessons learned. *Neurotherapeutics*, 10(1), 68-76 (2013).
125. Lee H, Narayanan S, Brown RA *et al.* Brain atrophy after bone marrow transplantation for treatment of multiple sclerosis. *Mult Scler*, (2016).
126. Franklin RJ, Kotter MR. The biology of CNS remyelination: the key to therapeutic advances. *J Neurol*, 255 Suppl 1, 19-25 (2008).
127. Chari DM. Remyelination in multiple sclerosis. *Int Rev Neurobiol*, 79, 589-620 (2007).
128. Harlow DE, Macklin WB. Inhibitors of myelination: ECM changes, CSPGs and PTPs. *Exp Neurol*, 251, 39-46 (2014).
129. Lau LW, Cua R, Keough MB, Haylock-Jacobs S, Yong VW. Pathophysiology of the brain extracellular matrix: a new target for remyelination. *Nat Rev Neurosci*, 14(10), 722-729 (2013).
130. Harlow DE, Honce JM, Miravalle AA. Remyelination Therapy in Multiple Sclerosis. *Front Neurol*, 6, 257 (2015).
131. Peferoen L, Kipp M, van der Valk P, van Noort JM, Amor S. Oligodendrocyte-microglia cross-talk in the central nervous system. *Immunology*, 141(3), 302-313 (2014).
132. Foote AK, Blakemore WF. Inflammation stimulates remyelination in areas of chronic demyelination. *Brain*, 128(Pt 3), 528-539 (2005).
133. Ruckh JM, Zhao JW, Shadrach JL *et al.* Rejuvenation of regeneration in the aging central nervous system. *Cell Stem Cell*, 10(1), 96-103 (2012).
134. Munzel EJ, Williams A. Promoting remyelination in multiple sclerosis-recent advances. *Drugs*, 73(18), 2017-2029 (2013).
135. Zendedel A, Beyer C, Kipp M. Cuprizone-induced demyelination as a tool to study remyelination and axonal protection. *J Mol Neurosci*, 51(2), 567-572 (2013).
136. Rodgers JM, Robinson AP, Miller SD. Strategies for protecting oligodendrocytes and enhancing remyelination in multiple sclerosis. *Discov Med*, 16(86), 53-63 (2013).

137. Keough MB, Yong VW. Remyelination therapy for multiple sclerosis. *Neurotherapeutics*, 10(1), 44-54 (2013).
138. Blanchard B, Heurtaux T, Garcia C *et al.* Tocopherol derivative TFA-12 promotes myelin repair in experimental models of multiple sclerosis. *J Neurosci*, 33(28), 11633-11642 (2013).
139. Meffre D, Massaad C, Grenier J. Lithium chloride stimulates PLP and MBP expression in oligodendrocytes via Wnt/beta-catenin and Akt/CREB pathways. *Neuroscience*, 284, 962-971 (2015).
140. Preisner A, Albrecht S, Cui QL *et al.* Non-steroidal anti-inflammatory drug indometacin enhances endogenous remyelination. *Acta Neuropathol*, 130(2), 247-261 (2015).
141. Huang JK, Jarjour AA, Nait Oumesmar B *et al.* Retinoid X receptor gamma signaling accelerates CNS remyelination. *Nat Neurosci*, 14(1), 45-53 (2011).
142. de la Fuente AG, Errea O, van Wijngaarden P *et al.* Vitamin D receptor-retinoid X receptor heterodimer signaling regulates oligodendrocyte progenitor cell differentiation. *J Cell Biol*, 211(5), 975-985 (2015).
143. Najm FJ, Madhavan M, Zaremba A *et al.* Drug-based modulation of endogenous stem cells promotes functional remyelination in vivo. *Nature*, 522(7555), 216-220 (2015).
144. Trifunovski A, Josephson A, Ringman A, Brene S, Spenger C, Olson L. Neuronal activity-induced regulation of Lingo-1. *Neuroreport*, 15(15), 2397-2400 (2004).
145. Mi S, Lee X, Shao Z *et al.* LINGO-1 is a component of the Nogo-66 receptor/p75 signaling complex. *Nat Neurosci*, 7(3), 221-228 (2004).
146. Rudick RA, Mi S, Sandrock AW, Jr. LINGO-1 antagonists as therapy for multiple sclerosis: in vitro and in vivo evidence. *Expert Opin Biol Ther*, 8(10), 1561-1570 (2008).
147. Wang CJ, Qu CQ, Zhang J, Fu PC, Guo SG, Tang RH. Lingo-1 inhibited by RNA interference promotes functional recovery of experimental autoimmune encephalomyelitis. *Anat Rec (Hoboken)*, 297(12), 2356-2363 (2014).
148. Pepinsky RB, Shao Z, Ji B *et al.* Exposure levels of anti-LINGO-1 Li81 antibody in the central nervous system and dose-efficacy relationships in rat spinal cord remyelination models after systemic administration. *J Pharmacol Exp Ther*, 339(2), 519-529 (2011).
149. Tran JQ, Rana J, Barkhof F *et al.* Randomized phase I trials of the safety/tolerability of anti-LINGO-1 monoclonal antibody BIIB033. *Neurol Neuroimmunol Neuroinflamm*, 1(2), e18 (2014).
150. Cadavid D KA, Chai Y et al. on behalf of the RENEW Investigators. Evidence that the anti-LINGO-1 monoclonal antibody BIIB033 protects against multifocal visual evoked potential amplitude loss in the fellow eye of subjects with unilateral acute optic neuritis. In: *ECTRIMS.* (Ed.^(Eds) (Barcelona, Spain, 2015)
151. Wang S, Bates J, Li X *et al.* Human iPSC-derived oligodendrocyte progenitor cells can myelinate and rescue a mouse model of congenital hypomyelination. *Cell Stem Cell*, 12(2), 252-264 (2013).
152. Boyd A, Zhang H, Williams A. Insufficient OPC migration into demyelinated lesions is a cause of poor remyelination in MS and mouse models. *Acta Neuropathol*, 125(6), 841-859 (2013).
153. Abramowski P, Krasemann S, Ernst T *et al.* Mesenchymal Stromal/Stem Cells Do Not Ameliorate Experimental Autoimmune Encephalomyelitis and Are Not Detectable in the Central Nervous System of Transplanted Mice. *Stem Cells Dev*, (2016).
154. Meamar R, Nematollahi S, Dehghani L *et al.* The role of stem cell therapy in multiple sclerosis: An overview of the current status of the clinical studies. *Adv Biomed Res*, 5, 46 (2016).
155. Karussis D, Karageorgiou C, Vaknin-Dembinsky A *et al.* Safety and immunological effects of mesenchymal stem cell transplantation in patients with multiple sclerosis and amyotrophic lateral sclerosis. *Arch Neurol*, 67(10), 1187-1194 (2010).
156. Amor S, Groome N, Linington C *et al.* Identification of epitopes of myelin oligodendrocyte glycoprotein for the induction of experimental allergic encephalomyelitis in SJL and Biozzi AB/H mice. *J Immunol*, 153(10), 4349-4356 (1994).

157. Fritz RB, Chou CH, McFarlin DE. Relapsing murine experimental allergic encephalomyelitis induced by myelin basic protein. *J Immunol*, 130(3), 1024-1026 (1983).
158. Tuohy VK, Sobel RA, Lees MB. Myelin proteolipid protein-induced experimental allergic encephalomyelitis. Variations of disease expression in different strains of mice. *J Immunol*, 140(6), 1868-1873 (1988).
159. Johns TG, Kerlero de Rosbo N, Menon KK, Abo S, Gonzales MF, Bernard CC. Myelin oligodendrocyte glycoprotein induces a demyelinating encephalomyelitis resembling multiple sclerosis. *J Immunol*, 154(10), 5536-5541 (1995).
160. Fissolo N, Montalban X, Comabella M. DNA-based vaccines for multiple sclerosis: current status and future directions. *Clin Immunol*, 142(1), 76-83 (2012).
161. Billetta R, Ghahramani N, Morrow O *et al.* Epitope-specific immune tolerization ameliorates experimental autoimmune encephalomyelitis. *Clin Immunol*, 145(2), 94-101 (2012).
162. Spence A, Klementowicz JE, Bluestone JA, Tang Q. Targeting Treg signaling for the treatment of autoimmune diseases. *Curr Opin Immunol*, 37, 11-20 (2015).
163. Lutterotti A, Martin R. Antigen-specific tolerization approaches in multiple sclerosis. *Expert Opin Investig Drugs*, 23(1), 9-20 (2014).
164. Bittner S, Wiendl H. Neuroimmunotherapies Targeting T Cells: From Pathophysiology to Therapeutic Applications. *Neurotherapeutics*, 13(1), 4-19 (2016).
165. Lutterotti A, Yousef S, Sputtek A *et al.* Antigen-specific tolerance by autologous myelin peptide-coupled cells: a phase 1 trial in multiple sclerosis. *Sci Transl Med*, 5(188), 188ra175 (2013).
166. Hellings N, Raus J, Stinissen P. T-cell vaccination in multiple sclerosis: update on clinical application and mode of action. *Autoimmun Rev*, 3(4), 267-275 (2004).
167. Vandenbark AA, Abulafia-Lapid R. Autologous T-cell vaccination for multiple sclerosis: a perspective on progress. *BioDrugs*, 22(4), 265-273 (2008).
168. Fox E, Wynn D, Cohan S, Rill D, McGuire D, Markowitz C. A randomized clinical trial of autologous T-cell therapy in multiple sclerosis: subset analysis and implications for trial design. *Mult Scler*, 18(6), 843-852 (2012).
169. Karussis D, Shor H, Yachnin J *et al.* T cell vaccination benefits relapsing progressive multiple sclerosis patients: a randomized, double-blind clinical trial. *PLoS One*, 7(12), e50478 (2012).
170. Vesterinen HM, Connick P, Irvine CM *et al.* Drug repurposing: a systematic approach to evaluate candidate oral neuroprotective interventions for secondary progressive multiple sclerosis. *PLoS One*, 10(4), e0117705 (2015).
171. Chataway J, Schuerer N, Alsanousi A *et al.* Effect of high-dose simvastatin on brain atrophy and disability in secondary progressive multiple sclerosis (MS-STAT): a randomised, placebo-controlled, phase 2 trial. *Lancet*, 383(9936), 2213-2221 (2014).
172. Raftopoulos R, Hickman SJ, Toosy A *et al.* Phenytoin for neuroprotection in patients with acute optic neuritis: a randomised, placebo-controlled, phase 2 trial. *Lancet Neurol*, 15(3), 259-269 (2016).
173. Arun T, Tomassini V, Sbardella E *et al.* Targeting ASIC1 in primary progressive multiple sclerosis: evidence of neuroprotection with amiloride. *Brain*, 136(Pt 1), 106-115 (2013).
174. Constantinescu CS, Goodman DB, Ventura ES. Captopril and lisinopril suppress production of interleukin-12 by human peripheral blood mononuclear cells. *Immunol Lett*, 62(1), 25-31 (1998).
175. Constantinescu CS, Ventura E, Hilliard B, Rostami A. Effects of the angiotensin converting enzyme inhibitor captopril on experimental autoimmune encephalomyelitis. *Immunopharmacol Immunotoxicol*, 17(3), 471-491 (1995).
176. Abtahi Froushani SM, Delirezh N, Hobbenaghi R, Mosayebi G. Synergistic effects of atorvastatin and all-trans retinoic acid in ameliorating animal model of multiple sclerosis. *Immunol Invest*, 43(1), 54-68 (2014).

177. Giovannoni G, Baker D, Schmierer K. The problem with repurposing: Is there really an alternative to Big Pharma for developing new drugs for multiple sclerosis? *Mult Scler Relat Disord*, 4(1), 3-5 (2015).
178. Multiple Sclerosis Therapy Consensus G, Wiendl H, Toyka KV *et al.* Basic and escalating immunomodulatory treatments in multiple sclerosis: current therapeutic recommendations. *J Neurol*, 255(10), 1449-1463 (2008).
179. Ziemssen T, Derfuss T, de Stefano N *et al.* Optimizing treatment success in multiple sclerosis. *J Neurol*, (2015).
180. Coles A. Newer therapies for multiple sclerosis. *Ann Indian Acad Neurol*, 18(Suppl 1), S30-34 (2015).
181. Giovannoni G, Turner B, Gnanapavan S, Offiah C, Schmierer K, Marta M. Is it time to target no evident disease activity (NEDA) in multiple sclerosis? *Mult Scler Relat Disord*, 4(4), 329-333 (2015).
182. Rotstein DL, Healy BC, Malik MT, Chitnis T, Weiner HL. Evaluation of no evidence of disease activity in a 7-year longitudinal multiple sclerosis cohort. *JAMA Neurol*, 72(2), 152-158 (2015).
183. Stangel M, Penner IK, Kallmann BA, Lukas C, Kieseier BC. Towards the implementation of 'no evidence of disease activity' in multiple sclerosis treatment: the multiple sclerosis decision model. *Ther Adv Neurol Disord*, 8(1), 3-13 (2015).
184. Kappos L, De Stefano N, Freedman MS *et al.* Inclusion of brain volume loss in a revised measure of 'no evidence of disease activity' (NEDA-4) in relapsing-remitting multiple sclerosis. *Mult Scler*, (2015).
185. Sormani MP, Arnold DL, De Stefano N. Treatment effect on brain atrophy correlates with treatment effect on disability in multiple sclerosis. *Ann Neurol*, 75(1), 43-49 (2014).
186. Ziemssen T, De Stefano N, Pia Sormani M, Van Wijmeersch B, Wiendl H, Kieseier BC. Optimizing therapy early in multiple sclerosis: An evidence-based view. *Mult Scler Relat Disord*, 4(5), 460-469 (2015).
187. Kappos L, Freedman MS, Polman CH *et al.* Effect of early versus delayed interferon beta-1b treatment on disability after a first clinical event suggestive of multiple sclerosis: a 3-year follow-up analysis of the BENEFIT study. *Lancet*, 370(9585), 389-397 (2007).
188. Kappos L, Freedman MS, Polman CH *et al.* Long-term effect of early treatment with interferon beta-1b after a first clinical event suggestive of multiple sclerosis: 5-year active treatment extension of the phase 3 BENEFIT trial. *Lancet Neurol*, 8(11), 987-997 (2009).
189. Coles AJ, Cox A, Le Page E *et al.* The window of therapeutic opportunity in multiple sclerosis: evidence from monoclonal antibody therapy. *J Neurol*, 253(1), 98-108 (2006).
190. Kappos L, Edan G, Freedman MS *et al.* The 11-year long-term follow-up study from the randomized BENEFIT CIS trial. *Neurology*, (2016).
191. Kappos L, O'Connor P, Radue EW *et al.* Long-term effects of fingolimod in multiple sclerosis: the randomized FREEDOMS extension trial. *Neurology*, 84(15), 1582-1591 (2015).
192. Goodin DS, Reder AT, Ebers GC *et al.* Survival in MS: a randomized cohort study 21 years after the start of the pivotal IFNbeta-1b trial. *Neurology*, 78(17), 1315-1322 (2012).
- The long-term impact of treatment with IFNbeta-1b on survival in MS.

193. Bermel RA, You X, Foulds P *et al.* Predictors of long-term outcome in multiple sclerosis patients treated with interferon beta. *Ann Neurol*, 73(1), 95-103 (2013).
194. Dobson R, Rudick RA, Turner B, Schmierer K, Giovannoni G. Assessing treatment response to interferon-beta: is there a role for MRI? *Neurology*, 82(3), 248-254 (2014).
195. Rio J, Rovira A, Tintore M *et al.* Relationship between MRI lesion activity and response to IFN-beta in relapsing-remitting multiple sclerosis patients. *Mult Scler*, 14(4), 479-484 (2008).
196. Sormani MP, De Stefano N. Defining and scoring response to IFN-beta in multiple sclerosis. *Nat Rev Neurol*, 9(9), 504-512 (2013).

197. Sormani MP, Rio J, Tintore M *et al.* Scoring treatment response in patients with relapsing multiple sclerosis. *Mult Scler*, 19(5), 605-612 (2013).
198. Prosperini L, Mancinelli CR, De Giglio L, De Angelis F, Barletta V, Pozzilli C. Interferon beta failure predicted by EMA criteria or isolated MRI activity in multiple sclerosis. *Mult Scler*, 20(5), 566-576 (2014).
199. Comabella M, Sastre-Garriga J, Montalban X. Precision medicine in multiple sclerosis: biomarkers for diagnosis, prognosis, and treatment response. *Curr Opin Neurol*, 29(3), 254-262 (2016).
200. Mistry N, Abdel-Fahim R, Samaraweera A *et al.* Imaging central veins in brain lesions with 3-T T2*-weighted magnetic resonance imaging differentiates multiple sclerosis from microangiopathic brain lesions. *Mult Scler*, (2015).
201. Fialova L, Bartos A, Svarcova J, Zimova D, Kotoucova J, Malbohan I. Serum and cerebrospinal fluid light neurofilaments and antibodies against them in clinically isolated syndrome and multiple sclerosis. *J Neuroimmunol*, 262(1-2), 113-120 (2013).
202. Mollgaard M, Degn M, Sellebjerg F, Frederiksen JL, Modvig S. Cerebrospinal fluid chitinase-3-like 2 and chitotriosidase are potential prognostic biomarkers in early multiple sclerosis. *Eur J Neurol*, 23(5), 898-905 (2016).
203. Villar LM, Costa-Frossard L, Masterman T *et al.* Lipid-specific immunoglobulin M bands in cerebrospinal fluid are associated with a reduced risk of developing progressive multifocal leukoencephalopathy during treatment with natalizumab. *Ann Neurol*, 77(3), 447-457 (2015).
204. Schwab N, Schneider-Hohendorf T, Pignolet B *et al.* PML risk stratification using anti-JCV antibody index and L-selectin. *Mult Scler*, 22(8), 1048-1060 (2016).
205. Ascherio A, Munger KL. Epidemiology of Multiple Sclerosis: From Risk Factors to Prevention-An Update. *Semin Neurol*, 36(2), 103-114 (2016).
206. Tanasescu R, Constantinescu CS. Helminth Therapy for MS. *Curr Top Behav Neurosci*, 26, 195-220 (2015).