SECTION EDITOR: DAVID E. PLEASURE, MD

Emerging Therapies for Relapsing Multiple Sclerosis

Jeffrey A. Cohen, MD

Six agents are currently approved by regulatory agencies to treat relapsing multiple sclerosis. Although these agents are effective and generally safe, some patients have continued disease activity or adverse effects. A sizable number of new agents are under investigation currently. This article reviews emerging agents that have shown promise in phase 2 trials. *Arch Neurol. 2009;66(7):821-828*

Six agents are approved by regulatory agencies to treat relapsing multiple sclerosis (MS). First-line agents include interferon beta-1b, intramuscular or subcutaneous interferon beta-1a, and glatiramer acetate. Pivotal trials and postmarketing experience support the efficacy, tolerability, and safety of these agents. However, all have modest efficacy for patients as a group and are administered by injection. Two agents, mitoxantrone and natalizumab, are more potent and generally well tolerated but typically are second line because of potential safety concerns. In addition, some patients treated with interferon beta or natalizumab develop neutralizing antibodies that abrogate efficacy.

Multiple sclerosis treatment priorities include (1) better understanding of MS pathogenesis and heterogeneity to guide development of better therapies and monitoring methods; (2) additional treatment options for relapsing-remitting MS (RRMS) that are more effective, convenient, and/or tolerable; (3) effective therapies for purely progressive MS; (4) neuroprotective and repair strategies; and (5) more effective treatments for common symptoms such as fatigue, pain, tremor, and cognitive impairment. Potential approaches to improve therapy in relapsing MS are summarized in **Table 1**. This review summarizes emerging therapies for relapsing MS, with a focus on agents with promising phase 2 study results (**Table 2** and **Table 3**).

Author Affiliation: Mellen Center for Multiple Sclerosis Treatment and Research, Cleveland Clinic, Cleveland, Ohio.

MS TREATMENT AGENTS

Alemtuzumab

Alemtuzumab is a humanized monoclonal antibody (mAb) directed against the surface antigen CD52, which is expressed by T cells, B cells, monocytes, natural killer cells, macrophages, eosinophils, and spermatozoa. Intravenous administration produces rapid, profound, and prolonged lymphopenia via complement-mediated lysis and antibody-dependent cellular cytotoxicity. T cells recover for 16 months, while B cells recover for 3 to 6 months. Alemtuzumab is approved to treat B-cell chronic lymphocytic leukemia.

Single-center pilot studies at the neurology unit of the University of Cambridge demonstrated potent suppression of MS relapses and lesion activity on magnetic resonance imaging (MRI) by alemtuzumab but no benefit on disability accrual in progressive MS.[1-3] A multicenter, randomized, evaluator-blind, active-comparator, phase 2 study enrolled 334 treatment-naïve participants with early (onset within 36 months of screening and Expanded Disability Status Scale [EDSS] score ≤3.0), active (≥2 relapses in the previous 2 years) RRMS.[4] Participants were randomized 1:1:1 to receive 44 µg of subcutaneous interferon beta-1a thrice weekly or high-dose (24 mg per day) or low-dose (12 mg per day) in-

Table 1. Potential Strategies to Improve Therapy of Relapsing Multiple Sclerosis

Address comorbidities
Better use of existing agents
Initiate treatment early
Optimize dose
Reduce the incidence or titer of neutralizing antibodies
Distinguish responders/nonresponders
Customize therapy
Combination therapy
Develop new therapies

travenous alemtuzumab for 5 days at month 0, 3 days at month 12, and for some participants, 3 days at month 24. Coprimary outcome measures were EDSS progression sustained for 6 months and relapse rate. There were no significant differences between results of the 2 alemtuzumab doses. Alemtuzumab significantly reduced the rate of sustained disability progression vs interferon beta-1a (9.0% vs 26.2%; hazard ratio, 0.29; $P < .001$). Mean EDSS scores improved 0.39 points vs baseline scores in alemtuzumab-treated participants compared with mean worsening of 0.38 points with interferon beta-1a ($P < .001$). The relapse rate was significantly reduced (0.10 vs 0.36; hazard ratio, 0.26; $P < .001$). A significant benefit was demonstrated for proportion of relapse-free participants, T2-hyperintense MRI lesion volume change, and brain volume.

Infusion reactions and cytokine release syndrome (fever, headache, hypotension, malaise, and urticaria) occur with alemtuzumab treatment initiation but can be attenuated with concomitant administration of antipyretic, antihistamine, and corticosteroid drugs. Serious infusion reactions occurred in 1.4% of subjects treated with alemtuzumab in the phase 2 study.[4] With prolonged lymphopenia, infection is a potential concern. In the phase 2 study, infections were more common with alemtuzumab treatment,[4] but to date increased serious infection-related adverse effects have not been seen in the MS population. The most significant adverse effect is antibody-mediated autoimmunity. Thyroid disorders, particularly Graves disease, occurred in up to 30% of alemtuzumab-treated patients with MS in earlier studies[5] and in 23% in a phase 2 study.[4] In addition, immune thrombocytopenia purpura developed in 6 alemtuzumab-treated participants (2.8%) in a phase 2 study vs 1 participant treated with interferon beta-1a (0.9%) and was fatal in 1 participant who did not seek medical attention. The other 5 participants recovered with medical treatment.

Two randomized, evaluator-blind, 3-arm phase 3 trials of alemtuzumab in early RRMS currently are enrolling subjects.[6] Both trials compare 12 mg of intravenous alemtuzumab per day for 5 consecutive days at month 0 and 3 days at month 12 with 44 μg of subcutaneous interferon beta-1a thrice weekly; time to sustained disability accumulation is the primary outcome measure. One phase 3 study will enroll approximately 525 treatment-naïve participants, and another phase 3 study will enroll approximately 1200 participants with continued disease activity while undergoing interferon beta or glatiramer acetate therapy.

Table 2. Parenteral Multiple Sclerosis Therapies With Positive Phase 2 Results

Agent	Postulated Mechanism of Action	Potential Adverse Effects
Alemtuzumab	Anti-CD52 mAb, T- and B-cell depletion	Antibody-mediated autoimmunity (Graves disease and ITP), infection
Daclizumab	Anti-CD25 mAb, IL-2R antagonist	Cutaneous reactions, infection, autoimmunity
Rituximab	Anti-CD20 mAb, B-cell depletion	Infusion reactions, cytokine release syndrome, infection (including PML)
Dirucotide	Soluble MBP-derived peptide, immune tolerance	Infusion reaction, autoimmunity
BHT-3008	DNA vaccine encoding human MBP, immune tolerance	Autoimmunity

Abbreviations: ITP, immune thrombocytopenia purpura; mAb, monoclonal antibody; MBP, myelin basic protein; PML, progressive multifocal leukoencephalopathy.

Daclizumab

Daclizumab is a humanized mAb directed against IL-2Rα (CD25) that is approved to treat acute renal allograft rejection. It was the third therapeutic mAb to be approved, the first humanized mAb, and the first directed against a cytokine receptor.

Interleukin-2 is postulated to play a central role in MS pathogenesis. The IL-2Rα–encoding gene was recently identified in a whole-genome screen as an MS risk allele.[7] Interleukin-2R blockade is effective in experimental autoimmune encephalomyelitis (EAE). Daclizumab blocks the binding of IL-2 to the high-affinity IL-2R and subsequent IL-2–dependent T-cell and B-cell proliferation, a critical step in the generation and amplification of antigen-specific immune responses. It also leads to down-modulation of IL-2R from the cell surface. Interestingly, studies in MS suggested that daclizumab's clinical benefit was mediated through generation of $CD56^+$ natural killer cells with regulatory effects.[8]

Two pilot studies in MS supported safety, tolerability, and efficacy of intravenous daclizumab.[9,10] A multicenter, randomized, double-blind, placebo-controlled phase 2 study enrolled 230 participants with relapsing MS and continued activity despite treatment with interferon beta.[11] Participants continued to take interferon beta and were randomized to receive 2-mg/kg subcutaneous daclizumab every 2 weeks (n=75), 1-mg/kg daclizumab alternating with placebo, or placebo for 24 weeks, with 48 weeks of subsequent follow-up. There was a 72% reduction ($P=.004$) in the cumulative number of new gadolinium-enhancing (GdE) lesions on MRI scans during 6 months, the primary outcome measure, in the high-dose daclizumab group and a nonsignificant trend in the low-dose group. There was also a nonsignificant reduction of annualized relapse rate, a secondary outcome measure. There was rapid onset of action, rapid loss of efficacy with treatment discontinuation, and no apparent rebound. The magnitude of benefit on MRI was roughly comparable with that seen in previous studies of monthly intravenous daclizumab.

Daclizumab was generally safe and well tolerated in the relatively short studies to date; long-term safety with chronic use needs to be determined. In the phase 2 study, the main adverse effects were cutaneous reactions and possibly increased severity of common infections. The overall incidence of infection was similar in the daclizumab and placebo groups, and there were no opportunistic infections. There were no malignancies or increase in interferon beta toxicity or incidence of anti–interferon beta–neutralizing antibodies. Although IL-2 plays a role in eliminating autoreactive T cells through activation-induced cell death and maintenance of FoxP3+ regulatory T cells, no autoimmune phenomena were observed. The frequency and significance of anti-daclizumab antibodies need further study.

In an ongoing multicenter, randomized, double-blind, placebo-controlled phase 2 study evaluating subcutaneous daclizumab as monotherapy, approximately 297 participants with RRMS will receive 1 of 2 doses of subcutaneous daclizumab or placebo every 4 weeks for 48 weeks.[6]

Rituximab

Rituximab is a chimeric murine/human mAb directed against CD20, a surface antigen expressed on pre–B cells and mature B cells. Intravenous rituximab leads to rapid depletion of circulating B cells by complement-mediated lysis, cell-mediated cytotoxicity, and apoptosis. Rituximab currently is approved to treat non-Hodgkin lymphoma and, combined with methotrexate, to treat rheumatoid arthritis with an inadequate response to anti–tumor necrosis factor agents.

Although MS is traditionally postulated to be a T cell–mediated autoimmune disorder, several lines of evidence support involvement of B cells and humoral immune mechanisms, including intrathecal antibody production, autoreactive antibody in cerebrospinal fluid, complement deposition associated with vesicular myelin disruption in MS lesions, and the presence of B cells in perivascular cuffs and meningeal lymphoid follicles. However, the lack of CD20 expression by plasma cells (the principal source of antibodies) and the rapidity of the clinical and MRI response in MS (circulating antibodies have a relatively long half-life) suggest that rituximab benefit is not mediated by decreasing antibody titers.[12,13] Rather, these observations suggest that the initial benefit results from loss of antigen presentation or production of proinflammatory mediators by B cells.

In a multicenter, randomized, double-blind, placebo-controlled phase 2 trial, 104 participants with RRMS received 1000 mg of intravenous rituximab (n=69) or placebo (n=35) on days 1 and 5 and were followed up for 48 weeks.[14] Benefit favoring rituximab was demonstrated on the primary outcome measure, total GdE lesions at weeks 12, 16, 20, and 24 (mean, 5.5 vs 0.5 lesions, $P<.001$). Benefit was also shown for total new GdE lesions at weeks 12 to 24 (mean 4.5 vs 0.2 lesions, $P<.001$), GdE lesions at 48 weeks ($P<.001$), and proportion of relapsing participants at weeks 24 (14.5% vs 34.3%, $P=.02$) and 48 (20.3% vs 40.0%, $P=.04$). Gadolinium-enhancing lesion inhibition on MRI was apparent as early as week 12.

Initial rituximab infusions produce fever, rigors, tachycardia, dyspnea, headache, pruritus, and rashes, probably due to B-cell lysis and cytokine release. The infusion reactions rarely are severe with acute respiratory distress syndrome, myocardial infarction, or anaphylaxis. Concomitant corticosteroid administration reduces these symptoms. In the phase 2 trial, rituximab was associated with rapid circulating B-cell depletion that remained nearly complete (>95%) until week 24, with gradual partial return thereafter. Therefore, increased risk of infection is a potential concern. Most infections were mild and occurred equally in treatment groups. No opportunistic infections were seen. However, progressive multifocal leukoencephalopathy was reported in patients treated for malignancy, hematologic disorders, systemic lupus erythematosus, and rheumatoid arthritis, typically in the setting of concomitant chemotherapy, immunosuppression, or stem cell transplantation.[15]

In the phase 2 trial, 16 of 65 participants (24.6%) who received rituximab had human antichimeric antibodies at week 48 or early termination. Although there was no relationship to adverse effects or efficacy, these antibodies may be an issue with repeated administration. Therefore, future development in MS will use ocrelizumab, a humanized anti-CD20 mAb. A 6-month phase 2 study comparing 2 doses of intravenous ocrelizumab, pla-

Table 3. Oral Multiple Sclerosis Therapies With Positive Phase 2 Results

Agent	Postulated Mechanism of Action	Potential Adverse Effects
Cladribine	Purine analogue, lymphocytotoxic agent	Myelosuppression and infection
Dimethyl fumaric acid	Nuclear factor E2–related factor-2 transcriptional pathway activator, immunomodulation	Hepatotoxicity
Estriol	Immunomodulation	Vascular events
Fingolimod	Myriocin derivative, S1P receptor agonist/antagonist, altered lymphocyte recirculation	Lymphopenia, infection, bradycardia, increased airway resistance, macular edema, hepatotoxicity
Laquinimod	Roquinimex derivative, immunomodulation	Hepatotoxicity, proinflammation
Minocycline	Matrix metalloproteinase inhibition	None
Statins	3-Hydroxyl-3-methyl-glutaryl coenzyme A reductase inhibitor, immunomodulation	Rhabdomyolysis, hepatotoxicity
Temsirolimus	Rapamycin analogue, cell cycle inhibition	Leukopenia, thrombocytopenia, mucous membrane ulcers
Teriflunomide	Dihydroorotate dehydrogenase inhibitor (pyrimidine synthesis), inhibition of T- and B-cell proliferation	Pancytopenia, hepatotoxicity

Abbreviation: S1P, sphingosine-1-phosphate.

cebo, and intramuscular interferon beta-1a in RRMS is in progress.[6]

Dirucotide

Dirucotide is a synthetic peptide corresponding to amino acids 82 through 98 of human myelin basic protein, an immunodominant T- and B-cell epitope in MS patients with the HLA-DR2 haplotype. Intravenous administration is postulated to induce high-dose tolerance.

Dirucotide is of interest, as it is being tested in secondary progressive MS (SPMS). In a single-center, 24-month, placebo-controlled, double-blind phase 2 trial, 32 participants with progressive MS received 500 mg of intravenous dirucotide or placebo every 6 months.[16] By contingency analysis of EDSS score progression, there was a nonsignificant trend favoring dirucotide on relative rate of progression for the overall study population (relative rate of progression = 0.56; n = 32; $P = .29$). There was significant benefit favoring dirucotide in HLA-DR2–positive and/or DR4-positive participants (relative rate of progression = 0.23, n = 20, $P = .01$). All but 3 dirucotide-treated participants had reduced cerebrospinal fluid anti–myelin basic protein antibody levels, but antibody changes did not predict clinical outcome.

In general, dirucotide was well tolerated with no serious treatment-related adverse effects in this study or previous pilot studies. The most common drug-related adverse effects were injection-site reactions with interstitial injection, facial flushing, and mild blood pressure decrease. There were no clinical or MRI findings suggesting disease activation.

Subject recruitment is complete in 2 ongoing 2-year phase 3 studies comparing dirucotide and placebo in HLA-DR2–positive and/or DR4-positive participants with SPMS.[6] A 15-month, double-blind, placebo-controlled phase 2 study in RRMS will enroll approximately 215 participants.[6]

BHT-3009

BHT-3009 is a DNA vaccine, a plasmid encoding the 18.5-kDa isoform of full-length human myelin basic protein that is intended to generate immune tolerance, both antigen-specific and generalized to other myelin antigens, by bystander suppression. In a multicenter, randomized, double-blind, placebo-controlled, dose-escalation phase 1/2 trial, 30 participants with RRMS or SPMS were treated with BHT-3009, BHT-3009 plus 80 mg of oral atorvastatin daily, or placebo.[17] BHT-3009 was administered in 0.5-, 1.5-, or 3-mg doses intramuscularly at weeks 1, 3, 5, and 9 after randomization. The vaccine was safe and well tolerated, with no indication of disease activation clinically or radiographically. There were favorable trends of GdE lesion number and volume. Immunologic changes included a marked decrease in proliferation of interferon γ–producing myelin-reactive $CD4^+$ T cells in peripheral blood and decreased titers of myelin-specific cerebrospinal fluid antibodies measured by protein microarray. There was no additional benefit from combination with atorvastatin.

In a randomized, double-blind, placebo-controlled phase 2 trial, 289 participants with RRMS were randomized to receive 0.5 mg (n = 104) or 1.5 mg (n = 89) of BHT-3009 intramuscularly or placebo (n = 96) at weeks 0, 2, and 4, then every 4 weeks until week 44.[18] In the analysis population of 267 participants, GdE lesions seen on monthly MRI from weeks 28 to 48, the primary outcome measure, were reduced 50% by the 0.5-mg dose relative to placebo ($P = .07$), which was associated with dramatic reduction in 23 myelin-specific antibodies measured by protein microarray. Interestingly, the higher dose was ineffective on GdE lesions and antibody titers. The DNA vaccine was well tolerated, with no evidence of disease activation by clinical or MRI parameters. Plans for future studies of BHT-3009 in MS have not been announced.

Cladribine

Cladribine is an adenosine deaminase-resistant purine nucleoside analogue. The phosphorylated triphosphate deoxynucleotide accumulates in lymphocytes, leading to apoptosis of resting and dividing lymphocytes and long-lasting lymphocyte depletion preferentially affecting $CD4^+$ T cells.[19] Cladribine is approved to treat hairy cell leukemia. Several trials supported some aspects of efficacy of parenteral cladribine in RRMS[20] and progressive MS.[19,21,22] In general, parenteral cladribine is well tolerated. Infectious complications are the principal concern with prolonged lymphocyte depletion. This issue has been less prominent in MS than in the oncology experience.

Recent development of cladribine has examined an oral formulation. A randomized, double-blind, 3-arm, placebo-controlled phase 3 study assessed oral cladribine for 96 weeks in approximately 1300 participants with RRMS.[6] Participants were randomized to high-dose (1.4 mg/kg in year 1 and 0.7 mg/kg in year 2) or low-dose (0.7 mg/kg in years 1 and 2) oral cladribine or placebo. The primary outcome measure was relapse rate throughout 96 weeks. The results of this completed trial have not yet been reported.

One ongoing 2-year phase 2 study is evaluating the safety, tolerability, and efficacy of oral cladribine combined with interferon beta.[6] There were approximately 200 participants with relapsing MS and at least 1 relapse within 48 weeks of screening during treatment with interferon beta. Participants continue taking interferon beta and are randomized 2:1 to undergo up to 4 cycles of cladribine treatment (0.875 mg/kg per cycle) or placebo at weeks 0, 5, 48, and 52. There is also a randomized, double-blind, placebo-controlled phase 3 trial of oral cladribine in approximately 642 participants with a clinically isolated syndrome at high risk of converting to MS.[6] Participants are randomized to low-dose (1.75 mg/kg per year) or high-dose (3.5 mg/kg/year) cladribine or placebo once a week for 4 weeks each year. The primary outcome measure is time to conversion to MS by revised McDonald criteria.[23]

Dimethyl Fumarate

Oral fumaric acid esters have a long history of use in treating psoriasis. Dimethyl fumarate is a second-generation fumarate derivative with improved tolerability. The primary metabolite, monomethyl fumarate, activates the nuclear factor E2–related factor-2 transcriptional path-

way, which is involved in regulating both immune function and the response to oxidative stress.[24] Immunologic actions include inhibition of T-cell activity by inducing apoptosis in activated T cells and stimulating a Th1 to Th2 shift in cytokine profile. Methyl hydrogen fumarate and dimethyl fumarate inhibit clinical and histopathologic features of EAE through both anti-inflammatory[25] and neuroprotective[24] actions.

In a randomized, double-blind, placebo-controlled, dose-ranging phase 2b study, 257 participants with RRMS were randomized to receive oral placebo (n=65) or 120 mg (n=64), 360 mg (n=64), or 720 mg (n=64) of dimethyl fumarate per day.[26] Seven hundred twenty milligrams of dimethyl fumarate per day reduced the mean cumulative new GdE lesion number on monthly scans from week 12 to 24 compared with placebo (1.4 vs 4.5 lesions, 69% reduction, $P<.001$). The 720-mg dose also produced a 48% reduction in new or enlarging T2 lesions at 24 weeks ($P=.006$), 53% reduction in T1-hypointense lesion number ($P=.01$), and a nonsignificant 32% reduction in relapses. There were nonsignificant trends favoring the 120-mg and 360-mg doses vs placebo on some outcome measures.

Dimethyl fumarate was generally well tolerated. The most common adverse effects were flushing, feeling hot, gastrointestinal symptoms (abdominal pain, nausea, vomiting, and diarrhea), headache, and fatigue. There was rare elevation of liver enzymes that resolved with drug discontinuation. There was no increase in infections and no opportunistic infections. During the double-blind extension, participants who continued active treatment had decreased adverse effects.

There are 2 ongoing 2-year phase 3 trials of dimethyl fumarate.[6] One phase 3 study will randomize approximately 1011 participants with RRMS to receive 720 or 480 mg of dimethyl fumarate per day or placebo. The primary outcome measure is the proportion of relapsing participants. The other phase 3 study will randomize approximately 1232 participants to treatment with 720 or 480 mg of dimethyl fumarate per day, oral placebo, or 20 mg of subcutaneous glatiramer acetate daily. The primary outcome measure is relapse rate.

Estriol

Multiple sclerosis disease activity decreases during pregnancy.[27] A variety of pregnancy-related factors are probably involved, including estrogens, which have immunomodulatory effects in vitro[28] and ameliorate EAE.[29] In an open-label pilot study of 12 women with RRMS or SPMS, the number and volume of GdE lesions on monthly MRI were reduced in months 7 through 12 and 19 through 22 during treatment with 8 mg of oral estriol per day compared with months 1 through 6 and 13 through 18 without treatment. This dose of estriol produced blood levels comparable with those at pregnancy month 6. In general, estriol was well tolerated aside from increased risk of vascular adverse effects. Also, its use is restricted to female patients. A 2-year, multicenter, randomized, double-blind trial of glatiramer acetate combined with 8 mg of oral estriol per day or placebo in RRMS is ongoing.[6] The primary outcome measure is relapse rate.

Fingolimod

Glycerol- and sphingosine-based phospholipids are abundant structural components of cellular membranes plus pleiotropic-soluble mediators of a variety of biologic functions. Sphingosine is generated by deacylation of ceramide by ceramidase then phosphorylated by sphingosine kinase, forming sphingosine-1-phosphate (S1P). Actions of S1P are mediated by 5 G protein–coupled receptors with 7 membrane-spanning domains. The S1P receptor subtype 1 predominates in lymphoid tissue and is highly expressed by resting T cells and B cells. Lymphocyte egress from secondary lymphoid organs is dependent on detection of a chemoattractant S1P gradient mediated by S1P receptor subtype 1.

Fingolimod, an orally active myriocin derivative, is phosphorylated in vivo by sphingosine kinase, forming fingolimod phosphate, a structural analogue of S1P. Fingolimod-phosphate binding leads to aberrant internalization of S1P receptor subtype 1, rendering lymphocytes insensitive to the S1P gradient and blocking egress from secondary lymphoid organs.[30] Fingolimod administration produces a rapid, reversible decrease in circulating lymphocytes (approximately 70%). Interruption of lymphocyte recirculation between central nervous system and secondary lymphoid organs is probably responsible for clinical benefit in MS. Fingolimod does not affect lymphocyte activation or memory T-cell and B-cell responses, so general immunosuppression is not produced. In addition, fingolimod is lipophilic, allowing central nervous system penetration. The S1P receptors are widely expressed by central nervous system cells and mediate a variety of actions that potentially lead to neuroprotective or reparative effects.[31] Interaction of fingolimod with widely expressed S1P receptors in other tissues probably accounts for its adverse effects.

In a randomized, double-blind, placebo-controlled phase 2 trial, 281 participants with RRMS were randomized to 1.25 or 5 mg of oral fingolimod daily or placebo for 6 months.[32] Median total number of GdE lesions on monthly MRI was reduced with 1.25 mg (1 lesion, $P<.001$) and 5 mg (3 lesions, $P=.006$) of fingolimod vs placebo (5 lesions). The annualized relapse rate was also reduced (1.25 mg of fingolimod, 0.35; $P=.009$; and 5 mg of fingolimod, 0.36; $P=.01$; vs placebo, 0.77). In the extension study, benefits were maintained in participants who continued to take fingolimod and were reproduced in participants who switched from placebo.

Adverse effects included nasopharyngitis, dyspnea, headache, diarrhea, nausea, and asymptomatic elevations of liver enzymes. There was 1 case of posterior reversible encephalopathy in the 5-mg group. Fingolimod treatment produced bradycardia with the first dose and a mild decrease in forced expiratory volume in 1 second. As with any immunomodulatory treatment, infections are a potential concern with fingolimod, though no opportunistic infections were seen in the phase 2 study. Possible increased incidence and severity of herpes infections have occurred in ongoing phase 3 studies. Macular edema was seen in the studies of fingolimod in renal transplant.

Ongoing phase 3 studies will evaluate a 1.25-mg and a 0.5-mg dose. A double-blind, double-dummy, random-

ized, 3-arm trial compares 1.25- and 0.5-mg daily doses of oral fingolimod and 30 μg of weekly intramuscular interferon beta-1a in 1292 participants with RRMS.[6] The primary outcome measure is annualized relapse rate during 12 months. The core study is complete, but the results have not yet been reported. A randomized, double-blind, placebo-controlled phase 3 trial of fingolimod in RRMS randomized 1272 participants with RRMS to 1.25 or 0.5 mg of daily oral fingolimod or placebo.[6] The primary outcome measure is annualized relapse rate during 24 months. A parallel placebo-controlled phase 3 study is being conducted in the United States with a 1080-participant target.[6]

Laquinimod

Laquinimod is a once-daily oral immunomodulatory agent derived from linomide. Promising results with linomide were demonstrated in EAE and preliminary clinical trials.[33] However, a phase 3 trial of linomide was terminated shortly after enrollment completion owing to unanticipated toxicity, including pericarditis, pleural effusion, myocardial infarction, possible pulmonary embolism, pancreatitis, and death.[34] Other common adverse effects included arthralgia, myalgia, bursitis, and edema. Laquinimod was identified by extensive screening of linomide derivatives for efficacy in EAE and lack of proinflammatory effects in beagles.

A multicenter, randomized, double-blind, placebo-controlled phase 2 study in RRMS and SPMS demonstrated that daily doses of 0.3 mg of laquinimod reduced cumulative active MRI lesion number during 24 weeks, the primary outcome measure, in the per-protocol cohort (n=187; mean, 5.2 vs 9.4 lesions; 44% reduction; $P=.0498$), with a nonsignificant trend in the intention-to-treat cohort (n=209; mean, 5.5 vs 9.3 lesions; 41% reduction; $P=.17$).[35] The 0.1-mg daily dose was ineffective. Benefit was more prominent in the subgroup of participants with at least 1 GdE lesion at baseline (approximately 70% of the per-protocol group), with a 52% reduction in cumulative active MRI lesions during 24 weeks ($P=.005$).

In a multicenter, randomized, double-blind, placebo-controlled phase 2b trial with 306 participants, a higher laquinimod dose (0.6 mg per day) significantly reduced mean cumulative GdE lesions per scan at weeks 24, 28, 32, and 36 compared with placebo (2.6 vs 4.2 lesions; 40.4% reduction; $P=.005$), while the 0.3-mg/day dose was not effective.[36] Benefit was seen on several other MRI measures with a nonsignificant trend on relapse rate. In an open-label extension study, benefit of 0.6 mg of laquinimod per day was recapitulated in participants who switched from placebo and persisted in participants who continued taking laquinimod. No new safety issues were identified.

In general, laquinimod has been well tolerated. Mild self-limited dose-dependent increases with liver enzymes were seen in both phase 2 studies. A single case of Budd-Chiari syndrome developed after 1 month of exposure in the phase 2b study in a participant with the factor V Leiden mutation. No clinical evidence of a proinflammatory effect was seen.

Two phase 3 trials of 0.6-mg doses of laquinimod per day in RRMS are under way.[6] One study compares laquinimod with placebo in 1000 participants. A second study compares laquinimod with placebo with an internal comparator (weekly intramuscular interferon beta-1a) in 1200 participants.

Minocycline

Minocycline is a semisynthetic tetracycline antibiotic with extensive clinical experience supporting safety and tolerability. Minocycline crosses the blood-brain barrier, and biologic actions of minocycline potentially of benefit in MS include inhibition of microglial activation, apoptosis, inducible nitrous oxide and free radicals, mitogen-activated kinases, proinflammatory cytokine production by T cells, and matrix metalloproteinase activity.[37] In a pilot study of 10 participants with active RRMS, 100 mg of oral minocycline twice daily reduced GdE lesions during 6 months compared with 3 months before therapy.[38] This result was driven by activity in 5 participants. A small, randomized, placebo-controlled phase 2 study assessed minocycline combined with glatiramer acetate in 44 participants with RRMS.[39] At months 8 and 9, GdE lesions were reduced by 63% in the glatiramer acetate plus minocycline group compared with the glatiramer acetate group (mean 1.47 vs 2.95 lesions, $P=.08$) and the number of new T2 lesions were reduced by 65% (mean, 1.84 vs 5.14 lesions, $P=.06$). Future studies will assess the efficacy of minocycline monotherapy following a clinically isolated demyelinating syndrome and minocycline combined with subcutaneous interferon beta-1a in RRMS.

Statins

Several attributes make statins, 3-hydroxy-3-methylglutaryl-coenzyme A inhibitors, attractive MS therapy candidates. They are administered orally, and extensive clinical experience with long-term treatment of hyperlipidemia indicates excellent tolerability and safety. In addition to lipid-lowering actions, statins have many immunomodulating effects that might be beneficial in MS, including decreased production of Th1 cytokines (IL-2, IL-12, interferon γ, and tumor necrosis factor α), increased production of Th2 cytokines (IL-4, IL-5, IL-10, and transforming growth factor β), generation of Th2 cells, decreased major histocompatibility complex class II expression by antigen-presenting cells, decreased costimulatory molecule expression, decreased antigen-specific T-cell activation, and decreased adhesion molecule and chemokine receptor expression by activated lymphocytes.[40-42] Oral statin treatment prevents or reverses EAE.[40]

An open-label, single-arm cross-over study of 30 participants with RRMS showed a 44% reduction in the number of GdE lesions on MRI at months 4, 5, and 6 of treatment with 80 mg of simvastatin daily vs pretreatment (mean, 1.30 vs 2.31 lesions, $P<.001$).[43] A second study with a similar design evaluated 41 participants with RRMS, including 16 taking interferon beta.[42] Treatment with 80 mg of atorvastatin daily produced a 24% reduction in GdE lesions on monthly MRI at

months 6 to 9 compared with months −2 to 0 (mean, 1.52 vs 2.00 lesions, $P=.003$).

The principal adverse effects of statins are gastrointestinal symptoms, hepatotoxicity, and rhabdomyolysis. There has also been concern that aggressive lowering of serum cholesterol may increase risk for hemorrhagic stroke.[44] A recent small, randomized, double-blind, pilot study compared daily oral atorvastatin (40 or 80 mg) with placebo combined with 44 µg of subcutaneous interferon beta-1a 3 times weekly in 26 participants with RRMS.[45] Prior to the study, participants received interferon beta-1a on average for 2 years and were clinically stable for at least 6 months. Surprisingly, during the 9-month study, participants treated with either dose of atorvastatin exhibited significantly increased risk of new T2-hyperintense or GdE MRI lesions, or clinical relapse. Whether this observation reflects potential proinflammatory effects of statins[41,46] or is an artifact resulting from the small sample size remains to be determined.

Additional studies will be necessary to confirm the utility of statins in RRMS both alone and in combination. Enrollment in an ongoing study of atorvastatin in clinically isolated syndrome[6] was closed before reaching the target of 152 subjects because of slow accrual.

Temsirolimus

Temsirolimus is an esterified derivative of sirolimus, a cytostatic rapamycin analogue widely used as an immunosuppressant following organ transplantation. In a multicenter, randomized, double-blind, placebo-controlled phase 2 trial, 296 participants with active RRMS and SPMS received 2, 4, or 8 mg of oral temsirolimus or placebo once daily for 9 months.[47] The 8-mg dose produced a 48% decrease in the mean number of cumulative new GdE lesions ($P=.01$), a 51% reduction in relapses ($P=.02$), and a trend to reduced brain volume loss vs placebo. Adverse effects that were significantly more frequent in the 8-mg group included aphthous stomatitis, mouth ulceration, hyperlipidemia, rash, and menstrual dysfunction. There are no announced plans for additional studies.

Teriflunomide

Teriflunomide is a dihydroorotate dehydrogenase inhibitor that decreases T-cell and B-cell proliferation with resultant immunomodulatory effects. It is the active metabolite of leflunomide, an agent used to treat rheumatoid arthritis. In a multicenter, randomized, double-blind, placebo-controlled phase 2 study, 179 participants with active RRMS or SPMS were treated with 7 mg (n=61) or 14 mg (n=57) of teriflunomide per day or placebo (n=61) for 36 weeks.[48] Median cumulative unique lesions per scan, the primary outcome measure, were reduced in the 7-mg/kg (0.2 lesions, $P<.03$) and 14-mg/kg (0.3 lesions, $P<.01$) groups vs placebo (0.5 lesions). Teriflunomide-treated participants also had reduced GdE lesions, new or enlarging T2 lesions, new T2 lesions, and T2 lesion burden. There was a trend to fewer relapses and significantly fewer participants with disability increase.

Teriflunomide was generally safe and well tolerated. Adverse effects more frequent in teriflunomide-treated participants included nasopharyngitis, alopecia, nausea, limb pain, diarrhea, and arthralgia. Hepatic necrosis and pancytopenia were reported in patients with rheumatoid arthritis who were taking teriflunomide. An additional potential safety issue is teratogenicity in animals. Female subjects are advised not to become pregnant and males are cautioned not to father a child during therapy. Without washout with cholestyramine or activated charcoal, it may take up to 2 years for plasma levels to reach less than 0.02 mg/L, the level expected to present minimal teratogenic risk.

A 2-year, double-blind, placebo-controlled phase 3 study in relapsing MS is in progress.[6] The primary outcome measure is relapse rate. Enrollment was closed with 1088 participants. Other ongoing or planned studies of teriflunomide include a phase 2 study of combination with interferon beta, a phase 2 study of combination with glatiramer acetate, and a placebo-controlled phase 3 trial in clinically isolated syndrome.

COMMENT

A sizable number of promising therapies for relapsing MS are being tested. Anticipated advantages include improved tolerability and convenience, particularly for oral agents, and efficacy that is comparable with or greater than the current standard agents. However, when these agents initially become available, there will be a relative lack of long-term safety and efficacy data. Many of these agents already have potential safety concerns, and, as with all new agents, there is the possibility of unanticipated safety issues. Cost of these agents will include not only the medication but also required monitoring. Which agent has the optimal balance of efficacy, safety, tolerability, and convenience is not clear at present and will depend on results of pivotal trials and clinical experience. It is likely that different agents will be appropriate for different settings.

Accepted for Publication: November 14, 2008.
Correspondence: Jeffrey A. Cohen, MD, Mellen Center for Multiple Sclerosis Treatment and Research, Cleveland Clinic, 9500 Euclid Ave, Cleveland, OH 44195 (cohenj@ccf.org).
Financial Disclosure: Dr Cohen has received personal compensation as a consultant or speaker from Biogen Idec, Eisai, Eli Lilly, Genentech, Genzyme, Glaxo Smith Kline, IMPAX, Incyte, Novartis, Schering Plough, Serono, Teva, and Wyeth. He has received research support paid to his institution from Artielle, Biogen Idec, BioMS, Genzyme, Orchestra Therapeutics, Novartis, and Teva.

REFERENCES

1. Coles AJ, Wing MG, Molyneux P, et al. Monoclonal antibody treatment exposes three mechanisms underlying the clinical course of multiple sclerosis. *Ann Neurol.* 1999;46(3):296-304.
2. Paolillo A, Coles AJ, Molyneux PD, et al. Quantitative MRI in patients with secondary progressive MS treated with monoclonal antibody Campath 1H. *Neurology.* 1999;53(4):751-757.
3. Coles AJ, Cox A, Le Page E, et al. The window of therapeutic opportunity in multiple sclerosis: evidence from monoclonal antibody therapy. *J Neurol.* 2006; 253(1):98-108.
4. CAMMS223 Trial Investigators; Coles AJ, Compston DA, Selmaj KW, et al.

Alemtuzumab vs. interferon beta-1a in early multiple sclerosis. *N Engl J Med*. 2008;359(17):1786-1801.
5. Coles AJ, Wing M, Smith S, et al. Pulsed monoclonal antibody treatment and autoimmune thyroid disease in multiple sclerosis. *Lancet*. 1999;354(9191): 1691-1695.
6. ClinicalTrials.gov. http://clinicaltrials.gov. Accessed November 1, 2008.
7. Hafler DA, Compston A, Sawcer S, et al; International Multiple Sclerosis Genetics Consortium. Risk alleles for multiple sclerosis identified by a genomewide study. *N Engl J Med*. 2007;357(9):851-862.
8. Bielekova B, Catalfamo M, Reichert-Scrivner S, et al. Regulatory CD56(bright) natural killer cells mediate immunomodulatory effects of IL-2Rα-targeted therapy (daclizumab) in multiple sclerosis. *Proc Natl Acad Sci U S A*. 2006;103(15): 5941-5946.
9. Rose JW, Watt HE, White AT, Carlson NG. Treatment of multiple sclerosis with an anti-interleukin-2 receptor monoclonal antibody. *Ann Neurol*. 2004;56(6): 864-867.
10. Bielekova B, Richert N, Howard T, et al. Humanized anti-CD25 (daclizumab) inhibits disease activity in multiple sclerosis patients failing to respond to interferon β. *Proc Natl Acad Sci U S A*. 2004;101:8705-8708.
11. Kaufman MD, Wynn DR, Montalban X. A phase 2 randomized, double-blinded, placebo-controlled, multicenter study of subcutaneous daclizumab, a humanized, anti-CD-25 monoclonal antibody, in patients with active, relapsing forms of multiple sclerosis: week 44 results [abstract PL01.003]. *Neurology*. 2008; 70(suppl 1):A220.
12. McFarland HF. The B cell: old player, new position on the team [editorial]. *N Engl J Med*. 2008;358(7):664-665.
13. Dalakas MC. Inhibition of B cell functions: implications for neurology. *Neurology*. 2008;70(23):2252-2260.
14. Hauser SL, Waubant E, Arnold DL, et al; HERMES Trial Group. B-cell depletion with rituximab in relapsing-remitting multiple sclerosis. *N Engl J Med*. 2008; 358(7):676-688.
15. Calabrese LH, Molloy ES, Huang D, Ransohoff RM. Progressive multifocal leukoencephalopathy in rheumatic diseases: evolving clinical and pathologic patterns of disease. *Arthritis Rheum*. 2007;56(7):2116-2128.
16. Warren KG, Catz I, Ferenczi LZ, Krantz MJ. Intravenous synthetic peptide MBP8298 delayed disease progression in an HLA Class II-defined cohort of patients with progressive multiple sclerosis: results of a 24-month double-blind placebo-controlled clinical trial and 5 years of follow-up treatment. *Eur J Neurol*. 2006; 13(8):887-895.
17. Bar-Or A, Vollmer T, Antel J, et al. Induction of antigen-specific tolerance in multiple sclerosis after immunization with DNA encoding myelin basic protein in a randomized, placebo-controlled phase 1/2 trial. *Arch Neurol*. 2007;64(10): 1407-1415.
18. Garren H, Robinson WH, Krasulova E, et al; BHT-3009 Study Group. Phase 2 trial of a DNA vaccine encoding myelin basic protein for multiple sclerosis. *Ann Neurol*. 2008;63(5):611-620.
19. 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*. 1996;93(4):1716-1720.
20. Brousil JA, Roberts RJ, Schlein AL. Cladribine: an investigational immunomodulatory agent for multiple sclerosis. *Ann Pharmacother*. 2006;40(10):1814-1821.
21. Sipe JC, Romine JS, Koziol JA, McMillan R, Zyroff J, Beutler E. Cladribine in treatment of chronic progressive multiple sclerosis. *Lancet*. 1994;344(8914):9-13.
22. Rice GPA, Filippi M, Comi G; Cladribine MRI Study Group. Cladribine and progressive MS. clinical and MRI outcomes of a multicenter controlled trial. *Neurology*. 2000;54(5):1145-1155.
23. Polman CH, Reingold SC, Edan G, et al. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria." *Ann Neurol*. 2005;58(6):840-846.
24. Lukashev M, Zeng W, Goetz S, et al. Activation of Nrf2 and modulation of disease progression in EAE models by BG00012 (dimethylfumarate) suggests a novel mechanism of action combining anti-inflammatory and neuroprotective modalities [abstract]. *Mult Scler*. 2007;13:A503.
25. Schilling S, Goelz S, Linker R, Luehder F, Gold R. Fumaric acid esters are effective in chronic experimental autoimmune encephalomyelitis and suppress macrophage infiltration. *Clin Exp Immunol*. 2006;145(1):101-107.
26. Kappos L, Gold R, Miller DH, et al; BG-12 Phase IIb Study Investigators. Efficacy and safety of oral fumarate in patients with relapsing-remitting multiple sclerosis: a multicentre, randomized, double-blind, placebo-controlled phase IIb study. *Lancet*. 2008;372(9648):1463-1472.
27. Confavreux C, Hutchinson M, Hours MM, Cortinovis-Tourniaire P, Moreau T; Pregnancy in Multiple Sclerosis Group. Rate of pregnancy-related relapse in multiple sclerosis. *N Engl J Med*. 1998;339(5):285-291.
28. Gilmore W, Weiner LP, Correale J. Effect of estradiol on cytokine secretion by proteolipid protein-specific T cell clones isolated from multiple sclerosis patients and normal control subjects. *J Immunol*. 1997;158(1):446-451.
29. Kim S, Liva S, Dalal MA, Verity MA, Voskuhl RR. Estriol ameliorates autoimmune demyelinating disease: implications for multiple sclerosis. *Neurology*. 1999; 52(6):1230-1238.
30. Massberg S, Von Andrian UH. Fingolimod and sphingosine-1-phosphate - modifiers of lymphocyte migration. *N Engl J Med*. 2006;355(11):1088-1091.
31. Dev KK, Mullershausen F, Mattes H, et al. Brain sphingosine-1-phosphate receptors: Implications for FTY720 in the treatment of multiple sclerosis. *Pharmacol Ther*. 2008;117(1):77-93.
32. Kappos L, Antel J, Comi G, et al; FTY720 D2201 Study Group. Oral fingolimod (FTY720) for relapsing multiple sclerosis. *N Engl J Med*. 2006;355(11):1124-1140.
33. Andersen O, Lycke J, Tollesson PO, et al. Linomide reduces the rate of active lesions in relapsing-remitting multiple sclerosis. *Neurology*. 1996;47(4):895-900.
34. Noseworthy JH, Wolinsky JS, Lublin FD, et al; North American Linomide Investigators. Linomide in relapsing and secondary progressive MS, part I: trial design and clinical results. *Neurology*. 2000;54(9):1726-1733.
35. Polman C, Barkhof F, Sandberg-Wollheim M, Linde A, Nordle O, Nederman T; Laquinimod in Relapsing MS Study Group. Treatment with laquinimod reduces development of active MRI lesions in relapsing MS. *Neurology*. 2005;64(6):987-991.
36. Comi G, Pulizzi A, Rovaris M, et al; LAQ/5062 Study Group. Effect of laquinimod on MRI-monitored disease activity in patients with relapsing-remitting multiple sclerosis: a multicentre, randomised, double-blind, placebo-controlled phase IIb study. *Lancet*. 2008;371(9630):2085-2092.
37. Yong VW, Wells J, Giuliani F, Casha S, Power C, Metz LM. The promise of minocycline in neurology. *Lancet Neurol*. 2004;3(12):744-751.
38. Metz LM, Zhang Y, Yeung M, et al. Minocycline reduces gadolinium-enhancing magnetic resonance imaging lesions in multiple sclerosis [letter]. *Ann Neurol*. 2004;55(5):756.
39. Metz L, Li D, Traboulsee A, et al. Glatiramer acetate combined with minocycline reduces the number of T1 Gd-enhancing and new T2 lesions compared to glatiramer alone [abstract S02.003]. *Neurology*. 2007;68(suppl 1):A84-A85.
40. Youssef S, Stuve O, Patarroyo JC, et al. The HMG-CoA reductase inhibitor, atorvastatin, promotes a Th2 bias and reverses paralysis in central nervous system autoimmune disease. *Nature*. 2002;420(6911):78-84.
41. Neuhaus O, Strasser-Fuchs S, Fazekas F, et al. Statins as immunomodulators: comparison with interferon-β1b in MS. *Neurology*. 2002;59(7):990-997.
42. Paul F, Waiczies S, Wuerfel J, et al. Oral high-dose atorvastatin treatment in relapsing-remitting multiple sclerosis. *PLoS ONE*. 2008;3(4):e1928.
43. Vollmer T, Key L, Durkalski V, et al. Oral simvastatin treatment in relapsing-remitting multiple sclerosis. *Lancet*. 2004;363(9421):1607-1608.
44. Goldstein LB, Amarenco P, Szarek M, et al; SPARCL Investigators. Hemorrhagic stroke in the Stroke Prevention by Aggressive Reduction in Cholesterol Levels study. *Neurology*. 2008;70(24, pt 2):2364-2370.
45. Birnbaum G, Cree B, Altafullah I, Zinser M, Reder AT. Combining beta interferon and atorvastatin may increase disease activity in multiple sclerosis. *Neurology*. 2008;71(18):1390-1395.
46. Kieseier BC, Archelos JJ, Hartung HP. Different effects of simvastatin and interferon beta on the proteolytic activity of matrix metalloproteinases. *Arch Neurol*. 2004;61(6):929-932.
47. Kappos L, Barkhof F, Desmet A, et al. The effect of oral temsirolimus on new magnetic resonance imaging scan lesions, brain atrophy, and the number of relapses in multiple sclerosis: results from a randomized controlled clinical trial [abstract 158]. *J Neurol*. 2005;252(suppl 2):46.
48. O'Connor PW, Li D, Freedman MS, et al; Teriflunomide Multiple Sclerosis Trial Group; University of British Columbia MS/MRI Research Group. A phase II study of the safety and efficacy of teriflunomide in multiple sclerosis with relapses. *Neurology*. 2006;66(6):894-900.