Oral Therapies for Multiple Sclerosis

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Multiple sclerosis treatment faces tremendous changes owing to the approval of new medications, some of which are available as oral formulations. Until now, the four orally available medications, fingolimod, dimethylfumarate (BG-12), teriflunomide, and cladribine have received market authorization, whereas laquinimod is still under development. Fingolimod is a sphingosine-1-phosphate inhibitor, which is typically used as escalation therapy and leads to up to 60% reduction of the annualized relapse rate, but might also have neuroprotective properties. In addition, there are three more specific S1P agonists in late stages of development: siponimod, ponesimod, and ozanimod. Dimethylfumarate has immunomodulatory and cytoprotective functions and is used as baseline therapy. Teriflunomide, the active metabolite of the rheumatoid arthritis medication leflunomide, targets the dihydroorotate dehydrogenase, thus inhibiting the proliferation of lymphocytes by depletion of pyrimidines. Here we will review the mechanisms of action, clinical trial data, as well as data about safety and tolerability of the compounds.

During the last decade, the treatment of multiple sclerosis (MS) made tremendous advances with the development of new therapeutics, which show greater efficacy than the “old” first-line therapies, interferons (IFNs), and glatiramer acetate. By now, clinicians have an armamentarium of more than ten compounds at their disposal. In 2009 and/or 2010, fingolimod received market authorization by the U.S. Food and Drug Administration (FDA) as the first orally available medication for relapsing remitting MS (RRMS). This was welcomed, especially by patients who preferred the oral way of administration. Since approval of fingolimod, a number of new medications have been developed and approved such as dimethylfumarate (DMF) and teriflunomide or are under development such as laquinimod and more specific S1P-agonists. Here we will review the mechanisms of action

of orally available MS therapeutics, the pivotal clinical trial data, as well as safety and tolerability of the compounds.

CLADRIBINE

Cladribine is a purine nucleoside that is phosphorylated in cells with a high amount of deoxycytidine kinase to the active form, 2-chlorodeoxyadenosine-ATP, which accumulates in the nucleus (Beutler 1992). This leads to a disturbance of the cellular metabolism and DNA damage, hence, inducing cell death. This process occurs mostly in lymphocytes as they contain high amounts of deoxycytidine kinase, thereby mediating beneficial effects in RRMS. In 2011, cladribine was refused market authorization in Europe and the United States because of an unfavorable efficacy/side effect profile mainly

Editors: Howard L. Weiner and Vijay K. Kuchroo

Additional Perspectives on Multiple Sclerosis available at www.perspectivesinmedicine.org

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Cite this article as *Cold Spring Harb Perspect Med* 2019;9:a032011

based on a high rate of secondary malignancies. In 2016, Merck applied again for market authorization in the European Union, because follow-up data and the ORACLE study in clinically isolated syndrome (CIS) patients did not confirm the malignancy risk. Cladribine was approved in summer 2017 for highly active relapsing MS.

Mechanisms

Cladribine crosses the blood–brain barrier and also exerts effects on central nervous system (CNS) cells. Microglia treated with cladribine show reduced proliferation and apoptosis (Singh et al. 2012). In experimental autoimmune encephalomyelitis (EAE), an animal model of MS, cladribine did not show an effect on microglial or astroglial activation, but reduced glutamatergic synaptopathy which is associated with central inflammation (Musella et al. 2013). Cladribine also affects the T-cell priming capacity of dendritic cells, hence, resulting in reduced induction of IFN- γ and tumor necrosis factor α (TNF- α) (Kraus et al. 2014). The molecule also leads to a decrease of circulating B cells and CD4⁺ T helper (Th) cells (Mitosek-Szewczyk et al. 2013).

Clinical Studies

Cladribine showed effectiveness in the phase III placebo-controlled CLARITY trial (Giovannoni et al. 2010). Patients were assigned to cladribine 3.5 mg or 5.25 mg or placebo. The annualized relapse rate (ARR) was significantly lower with 0.14/0.15 in the treatment groups and 0.33 in the placebo group. The risk of disability progression was significantly reduced (hazard ratio [HR] 0.67/0.69, respectively) as well as the brain lesion count on magnetic resonance imaging (MRI). Cladribine treatment was associated with a higher risk of grades 1 and 2 lymphocytopenia (21.6% 3.5 mg group, 31.5% 5.25 mg group). Moreover, there was a considerable number of patients who developed herpes zoster (20 in the treatment groups vs. none in the placebo group). The follow-up study, which assessed the long-term effects of cladribine therapy, showed that over a period of 96 weeks, 44%

45% of the patients (3.5 mg/kg, 5.25 mg/kg group, respectively) were free from disease activity, whereas in the placebo group only 16% of the patients did not have disease activity (Giovannoni et al. 2011). The ORACLE MS study in patients with a first demyelinating event investigated the potential of cladribine to prevent the conversion to clinically definite MS. Cladribine therapy reduced the risk with a hazard ratio of 0.38 (5.25 mg/kg group) and 0.33 (3.5 mg/kg group) (Leist et al. 2014). Cladribine might also have indirect neuroprotective effects. Further analysis of the CLARITY study revealed that cladribine-treated patients had a reduced rate of percentage brain volume change compared with the placebo group (cladribine, 0.56%/0.57%, 3.5 mg/kg/5.25 mg/kg vs. 0.7% in the placebo group) (De Stefano et al. 2017). More importantly, there was a significant correlation of disability progression with reduced percentage of brain volume change. Long-term effects will have to be evaluated in further studies, as will the question whether the rate of patients progressing might be beneficially altered using cladribine and whether cladribine has a role as induction therapy, with patients stabilized for many years after the initial cycles of cladribine.

Side Effect Profile and Tolerability

Concerns about the safety profile of cladribine were the reason for stopping the process of authorization by the European Medicines Agency (EMA). Some of the most important serious adverse events of cladribine therapy include myelosuppression and secondary malignancies. In the CLARITY study, the most common adverse events included lymphocytopenia, headache, nasopharyngitis, and upper respiratory tract infection. Serious adverse events included infections with herpes zoster in three of the patients and the occurrence of neoplasms. Those included leiomyomas ($n = 5$), a melanoma and carcinoma of the pancreas and ovary. One death, which occurred in the cladribine treatment group, was because of cardiopulmonary arrest secondary to exacerbation of latent tuberculosis. Although there was a higher number of neoplasms in the treatment group, the authors

could not establish an enhanced risk for their occurrence as neoplasms were found in several organ systems (Giovannoni et al. 2010). In a meta-analysis of the CLARITY and ORACLE MS trial, no increased risk for neoplasms could be shown (Pakpoor et al. 2015). Data from patients treated for mastocytosis showed especially lymphopenia (82%), neutropenia (47%), and opportunistic infections (13%) (Barete et al. 2015). In the studies conducted in MS, no case of progressive multifocal leukoencephalopathy (PML) occurred, but cladribine therapy has been associated with the development of PML in hematological disorders. Until now, three patients have been reported who have been treated for hairy cell leukemia (Aletti et al. 2011), follicular lymphoma (Berghoff et al. 2013), and systemic mastocytosis (Alstadhaug et al. 2016).

FINGOLIMOD

Fingolimod (FTY720, brand name Gilenya) is an oral medication that is typically used as escalation therapy. Fingolimod modulates the sphingosine 1-phosphate receptor (S1PR), which leads to internalization and degradation of the receptor. Hence, the ability of autoreactive T lymphocytes to leave secondary lymphoid organs, especially lymph nodes, is impaired, which leads to reduced influx of lymphocytes into the CNS. In addition to S1PR, other types of S3PR and S5PR are also activated by fingolimod, and it is not yet solved whether this contributes to its therapeutic efficacy.

Mechanisms

Fingolimod has effects on various tissues that express the S1PR, such as cells of the lymphoid lineage but also microglia, astrocytes, and endothelial cells (Hunter et al. 2016). Astrocytes are important contributors of CNS scar formation and express the S1P3 and S1P1 and also S1P2 to a lesser degree. Fingolimod also has beneficial effects on microglia. Microglia express S1P1 receptors and treatment with FTY leads to reduced release of proinflammatory cytokines, such as TNF- α , interleukin (IL)-1 β , and IL-6 (Noda et al. 2013). Moreover, the production of

brain-derived neurotrophic factor (BDNF) and glial cell-derived neurotrophic factor is increased (Noda et al. 2013), suggesting that fingolimod might normalize microglial cytokine release and influence the microglial phenotype. Fingolimod has neuroprotective effects as it reduces excitotoxicity by targeting the p38 MAP kinase (MAPK) stress signaling pathway in microglia (Cipriani et al. 2015). It also ameliorates neurodegenerative processes in the cuprizone model, which is a model of demyelination (Slowik et al. 2015). In the same model, cerebellar remyelination is not influenced (Alme et al. 2015). This could be explained by the finding that fingolimod activates extracellular signal-regulated kinase 1/2 and Akt with reduced apoptosis in oligodendrocyte progenitors together with oligodendrocyte differentiation (Coelho et al. 2007). Studies in other neurodegenerative models, such as models of cerebral ischemia, supported neuroprotective effects of fingolimod. In rodent models of cerebral artery occlusion, the infarct size was reduced on treatment with fingolimod accompanied by reduced microglial/macrophage activation (Wei et al. 2011). Fingolimod did not show direct effects against glutamate excitotoxicity to neurons, suggesting that neuroprotection is mediated rather via anti-inflammatory mechanisms. Neuroprotection was shown also in other models, for example in a rat model of autism (Wu et al. 2017), hemorrhage (Rolland et al. 2017), and Parkinson's disease (Zhao et al. 2017). Hence, it remains unclear whether the neuroprotective effects of fingolimod are linked to its anti-inflammatory properties or whether neuroprotection can be achieved by the medication alone.

Clinical Studies

Fingolimod had been tested in several phase II and III clinical trials before its approval by the FDA and EMA in 2010. Interestingly, the empirical initial dosing of 5 mg per day could be reduced 10-fold and still full activity was maintained. The first pivotal study that showed efficacy of fingolimod was the FREEDOMS trial. Here, fingolimod reduced the ARR significantly, either in a dosage of 0.5 mg or in a dosage of

1.25 mg compared with placebo (ARR placebo 0.4; 0.5 mg fingolimod 0.18; 1.25 mg 0.16) (Kappos et al. 2010). The risk of disability progression was also reduced (HR 0.70 and 0.68 [both dosages] vs. placebo). MRI parameters were significantly better regarding the number of new T2 weighted lesions, gadolinium-enhancing lesions, and brain volume loss. The FREEDOMS II study investigated the effect of fingolimod 0.5 mg or 1.25 mg versus placebo. After a review of other phase III clinical trials, patients in the placebo group were switched to the 0.5 mg group. Treatment with fingolimod reduced the ARR by 48% but had no effect on percent brain volume change (Table 1) (Calabresi et al. 2014). Owing to the high range of effective doses of fingolimod, a trial with 0.25 mg is still ongoing in North America.

Now, fingolimod has been used for more than 5 years as a therapeutic agent in 160,000 MS patients, hence long-term data get more into the focus of clinicians. Fingolimod has been evaluated in the extension from the randomized TRANSFORMS study, which investigated the effect of switching from IFN- β 1a to fingolimod. Switching led to a 50% reduction of ARR (0.4 vs. 0.2) concomitant with reduced MRI activity and reduced rate of brain volume loss (Cohen et al. 2016b). Fingolimod might even have neuroprotective effects, as 12-month therapy leads to a significant reduction of neurofilament light-chain levels in the cerebrospinal fluid (CSF) (reduction of 326 pg/mL, $p = 0.002$) (Kuhle et al. 2015). However, fingolimod was also tested in a phase III clinical trial in primary progressive MS (PPMS) (oral fingolimod in PPMS [INFORMS]) in which the rate of disease progression was not altered compared with placebo (Lublin et al. 2016). Reasons for the failure of fingolimod are still speculative. Nonetheless, fingolimod might also show neuroprotective properties, as suggested by MRI analyses. De Stefano and colleagues analyzed MRI data from FREEDOMS I and II, and revealed that the percent brain volume change was reduced by 48.2% after 24 months compared with the placebo control (De Stefano et al. 2016). Using a regression model, they suggested that 54% of the effect was independent of its effect on visible

focal damage, suggesting that both inflammatory and neurodegenerative processes are targeted (Table 2) (De Stefano et al. 2016).

Side Effect Profile and Tolerability

Fingolimod is relatively well tolerated. One of the most important side effects of the medication includes a reduction of the heart rate, which was 12.4 beats per minute (SD 8.41) in mean (1.25 mg group; maximum) and 8.5 beats per minute (SD 7.84) 5 h post application of the medication in the FREEDOMS II trial (Calabresi et al. 2014). The percentage of patients with atrioventricular (AV) block I did not differ between treatment and placebo groups and there was no patient with AV block II on treatment. However, there was a higher incidence of Mobitz type I AV block and 2:1 AV block in the fingolimod group. In the FREEDOMS II trial, fingolimod treatment was associated with lymphopenia (8%, 0.5 mg group; 0% placebo), increase of the alanine aminotransferase (8%, 0.5 mg group; 2% placebo), herpes zoster infection (3%, 0.5 mg group; 1% placebo), hypertension (9%, 0.5 mg group; 3% placebo), and bradycardia after the administration of the first dose (1%, 0.5 mg group; <0.5% placebo) (Calabresi et al. 2014). The number of serious adverse events was similar in the treatment group (15%) and the placebo group (13%) and included basal cell carcinoma (3% vs. 1%), macular edema (both 1%), infections (3% vs. 1%), and neoplasms (4% vs. 2%) (Calabresi et al. 2014). In the phase IIIb study FIRST, it could be shown that even for patients at risk for cardiac side effects safety precautions with a 6 h continuous electrocardiogram (ECG) recording are sufficient to avoid severe cardiac side effects (Gold et al. 2014).

Immunosuppression usually raises questions about the ability of the compromised immune system to mount immune responses against novel and recall antigens such as vaccinations. Kappos and colleagues investigated, in a blinded, randomized, multicenter, placebo-controlled trial, the response after influenza vaccination and tetanus toxoid booster dose. Six weeks postvaccination, the response rate was reduced for both influenza vaccination (43% vs.

Table 1. Clinical trial data

Compound	Additional information	Annualized relapse rate (ARR)	Reduction of ARR	Risk of disability progression	T2- and Gd-enhancing lesions	Atrophy	Study name	References
Cladribine	-	0.14 (3.5 mg/kg) 0.15 (5.25 mg/kg) 0.33 (placebo)	57%	HR 0.67 (3.5 mg/kg) HR 0.69 (5.25 mg/kg)	-85.7/87.9% reduction Gd-enhancing lesions; -73.4/76.9% reduction T2 lesions	0.56% (3.5 mg/kg) 0.57% (5.25 mg/kg) 0.7% (placebo)	CLARITY	Giovannoni et al. 2010
Fingolimod	-	0.18 (0.5 mg) 0.16 (1.25 mg) 0.4 (placebo)	60%	HR 0.7/0.68	T2, Gd, brain volume loss better	Reduced brain volume reduction	FREEDOMS	Kappos et al. 2010
-	-	0.21 (0.5 mg) 0.4 (placebo)	48%	HR 0.83 (ns)	Reduced T2 volume	-0.41 PBVC	FREEDOMS II	Calabresi et al. 2014
Switch from IFN- β -1a	0.2 (fingolimod) 0.4 (IFN- β -1a)	50% reduction after switching	No difference	Reduced MRI activity	Reduced rate of brain volume loss		TRANSFORMS	Cohen et al. 2016b
-	-	-	-	-	-	48.2% reduced brain volume change	FREEDOMS and FREEDOMS II	De Stefano et al. 2016
DMF	-	0.27 (twice daily) 0.26 (thrice daily) 0.46 (placebo)	43%	38% (twice daily) 34% (thrice daily) reduction	Reduced number of patients with Gd-enhancing lesions		DEFINE	Gold et al. 2012
-	-	0.22 (twice daily) 0.20 (thrice daily) 0.29 (GA) 0.4 (placebo)	44% (twice) 51% (thrice) 29% (GA)	21% (ns) 24% (ns) 7% (ns)	Less new or enlarging T2 lesions, less new T1 hypointense lesions		CONFIRM	Fox et al. 2012

Continued

Table 1. *Continued*

Compound	Additional information	Annualized relapse rate (ARR)	Reduction of ARR	Risk of disability progression	T2- and Gd-enhancing lesions	Atrophy	Study name	References
Lacquinimod (LQ)	-	0.3 (LQ)	23%	11.1% (LQ)	Less Gd-enhancing lesions and new T2 lesions	Less brain atrophy within 12 months, but not persistent after 24 months	ALLEGRO	Comi et al. 2012; Filippi et al. 2014
	-	0.39 (placebo)		15.7% (LQ) (HR 0.64)		28% reduced brain atrophy ($p < 0.001$)	BRAVO	Vollmer et al. 2014
Teriflunomide	-	0.37 (7 mg)	31.2% (7 mg)	23.7% (7 mg, ns)	Gd-enhancing lesions reduced by 80%, reduction of total lesion volume 67%	No change	TEMPO	O'Connor et al. 2011
	-	0.37 (14 mg)	31.5% (14 mg)	29.8% (14 mg, $p = 0.03$)				
	-	0.54 (placebo)						
	-	0.39 (7 mg)	22% (7 mg)	HR 0.95 (7 mg, ns)	Not included	Not included	TOWER	Confavreux et al. 2014
	-	0.32 (14 mg)	36% (14 mg)	HR 0.68 (14 mg, $p = 0.04$)				
	-	0.5 (placebo)						

DMF, Dimethylfumarate; HR, hazard ratio; Gd, gadolinium; PBVC, percent brain volume change; IFN, interferon; MRI, magnetic resonance imaging; ns, not significant; GA, glatiramer acetate.

Table 2. Risk of conversion from clinically isolated syndrome (CIS) to definite multiple sclerosis (MS)

Compound	Risk reduction to develop definite MS	Study	References
Cladribine	HR 0.38 (5.25 mg/kg group) HR 0.33 (3.5 mg/kg group)	ORACLE MS	Leist et al. 2014
Teriflunomide	HR 0.65	TOPIC	Miller et al. 2014

HR, Hazard ratio.

75% [0.25; 0.11–0.57]) and tetanus toxoid boosting (38% vs. 49% [0.62; 0.29–1.33]) (Kappos et al. 2015). Thus, although roughly 40% of the patients were able to mount an immune response against vaccination, the response was not as strong as in the placebo group.

Currently, three more selective S1P agonists are in their final stage of study evaluation: ponesimod, ozanimod, and siponimod. Siponimod is currently investigated in the phase II BOLD study (NCT00879658) and the phase III EXPAND trial (NCT01665144). Ozanimod is investigated in the phase II/III RADIANCE (NCT01628393) and phase III SUNBEAM trial (NCT02294058). Ponesimod is investigated in the phase III OPTIMUM (NCT02425644) and POINT trials (NCT02907177). Those molecules have shorter half-lives than fingolimod and are reported to lead to a faster recovery of lymphocyte counts after discontinuation of treatment (Selmaj et al. 2013; Olsson et al. 2014; Cohen et al. 2016a). Whether they offer a superior profile compared with fingolimod or just imitate the prototype fingolimod is currently unclear.

BG-12, DIMETHYLFUMARATE

Fumarates were first introduced as therapeutics for the treatment of psoriasis as Fumaderm containing 56% DMF (120 mg) and 44% ethylhydrogenfumarate (95 mg of three different salts). The first anecdotal reports about coincidental amelioration of MS in psoriasis patients treated with Fumaderm led to a prospective clinical observation of ten RRMS patients treated with fumarates over 72 weeks (Schimrigk et al. 2006).

Immunomodulatory Mechanisms of DMF

DMF has pleiotropic effects on immune cells, glia, and neurons as it induces immunomodula-

tion on different immune cell subsets and shows cytoprotective properties. Fumaric acid esters are able to modify the expression of genes involved in antioxidative mechanisms. Monomethylfumarate (MMF), which is the active metabolite of DMF, modifies the Kelch-like erythroid cell-derived associated protein-1 (KEAP-1) via binding. In turn, this leads to modification of the KEAP-1/nuclear factor (erythroid-derived 2)-like 2 (Nrf2) complex. The translocation of Nrf2 to the nucleus modifies the transcription of antioxidative genes, ultimately promoting cytoprotective effects in chronic EAE (Linker et al. 2011). In a later study conducted in acute EAE Nrf2^{-/-} C57BL/6 mice were still protected on treatment with DMF (Schulze-Topphoff et al. 2016), suggesting more pathways to be active. DMF induced a reduction of circulation IFN- γ and IL-17 producing CD4⁺ cells and induced anti-inflammatory M2 monocytes (Schulze-Topphoff et al. 2016). The investigators discussed that neuroprotective effects, which are important to prevent chronification, are rather mediated by Nrf2, whereas immunomodulation in the acute phase seems to be mediated by a shift of the immune cell composition. Immunomodulation was also shown ex vivo in DMF-treated patients, as analyses of T-cell subsets showed a shift to a Th2 cell type on DMF treatment (Gross et al. 2016). DMF also influences B-cell subsets. In patients with RRMS, DMF treatment decreased the number of circulating mature/differentiated B cells, mostly on memory rather than naïve B cells (Li et al. 2017). This was accompanied by a shift of the cytokine profile to an anti-inflammatory phenotype. DMF reduces the release of proinflammatory cytokines in activated microglia and rescues mitochondrial deficits in neurons (Peng et al. 2016). DMF modulates microglia through the activation of the HCAR2 pathway, which leads to the modulation of neu-

roinflammation and restores synaptic alterations in EAE (Parodi et al. 2015). The combination with IFN- β even shows enhanced neuroprotection in EAE (Reick et al. 2014).

There is also evidence that DMF might have beneficial effects in other models of autoimmunity or neurodegeneration. Pitarokoili et al. (2015) showed clinical improvement in conjunction with less demyelination and axonal degeneration in experimental autoimmune neuritis (EAN), a model of Guillain-Barré syndrome (GBS). Another EAN study showed a shift of macrophage phenotypes with less proinflammatory M1 macrophages and more anti-inflammatory M2 macrophages (Han et al. 2016). This was accompanied by reduced messenger RNA (mRNA) of IFN- γ , TNF- α , IL-6, and IL-17 and up-regulation of anti-inflammatory IL-4 and IL-10 (Han et al. 2016). The anti-inflammatory properties of DMF might also support neuroprotection as DMF treatment ameliorates locomotor function in a model of spinal cord injury in conjunction with increases of neurotrophic factors BDNF, glial-derived neurotrophic factor (GDNF), and neurotrophin 3 (NT-3) (Cordaro et al. 2016). DMF inhibits the activation of neutrophils, the ability to produce reactive oxygen species, and phagocytic activity, which might be of interest in neutrophil-mediated diseases such as epidermolysis bullosa (Muller et al. 2016). In summary, DMF modulates immune cells to a protective phenotype and shows antioxidative and neuroprotective properties.

Clinical Studies

Efficacy of fumarates in MS was shown the first time in a small open-label observation with ten patients (Schimrigk et al. 2006). This was followed by large phase III clinical trials. The DEFINE trial compared BG-12 240 mg twice or thrice daily to placebo (Gold et al. 2012). The study showed a significant reduction of relapses (41%/43% reduction of ARR in the twice, thrice daily group, respectively). The proportion of patients with confirmed disability progression was reduced by 38%/34%, respectively, compared with placebo. This was corroborated by a reduced number of patients with gadolinium-en-

hancing lesions (90/73%) and new/enlarging T2 lesions (85/74%). Data about the clinical and paraclinical efficacy of DMF were reproduced in the CONFIRM study (Fox et al. 2012). Differing from the DEFINE study, the CONFIRM study also investigated the comparison to glatiramer acetate as active treatment arm only powered against placebo. In CONFIRM, significant differences could be shown for the ARR (BG-12 thrice daily), new T2 lesions, and new T1 lesions. Those two studies were the basis for the approval of the FDA and EMA. The ongoing extension of the DEFINE/CONFIRM studies, ENDORSE, which was conducted over 5 years, showed continuous low ARR (0.202, 0.163, 0.139, 0.143, and 0.138 (years 1–5, respectively) and low rates of new gadolinium-enhancing lesions (Gold et al. 2016a).

Because of the aforementioned antioxidative effects with cytoprotective and neuroprotective properties, the medication was also tested in PPMS. In a small observational study in 26 patients treated with Fumaderm ($n = 18$) or DMF ($n = 8$), 75% of the patients either remained stable or slightly ameliorated (Strassburger-Krogias et al. 2014). In November 2016, the “Dimethyl Fumarate Treatment of Primary Progressive Multiple Sclerosis” (FUMAPMS) phase II clinical trial investigating DMF in patients with PPMS started at Copenhagen MS Center (ClinicalTrials.gov; identifier: NCT02959658; the estimated study completion is December 2019). The primary outcome of the study will be neurofilament heavy chain as a marker of neurodegeneration.

Side Effect Profile and Tolerability

The phase III clinical trials and real-world data from the past years have shown a good safety and side effect profile for DMF. DMF treatment is related to significantly more gastrointestinal side effects such as diarrhea, nausea, and upper abdominal pain. In addition, patients report about flushes, which occur usually within the first hour after drug intake. Those can be resolved by intake of aspirin. PML, one of the most feared side effects of immunomodulatory substances, has been reported until now in five

patients out of 230,000, which have been treated with DMF. The first patient had long-term lymphocytopenia with lymphocyte counts of 290–580 cells mm⁻³ over a period of 3.5 years, with unfortunately a fatal outcome (Rosenkranz et al. 2015). In the studies about DMF in RRMS lymphocytes dropped on average about 30% and only a small proportion of 2% had counts <500 cells mm⁻³ (Fox et al. 2016). In addition, several patients that have been treated with Fumaderm for psoriasis have been diagnosed with PML (Ermis et al. 2013; van Oosten et al. 2013; Stoppe et al. 2014; Hoepner et al. 2015; Nieuwkamp et al. 2015). In one patient who had negative John Cunningham virus (JCV)-DNA in the CSF, a brain biopsy revealed typical histopathological alterations for PML and JCV-positive neurons (Bartsch et al. 2015). In this patient, total lymphocyte counts were not altered but analysis of lymphocyte subsets documented reduced CD8⁺ T-cell subsets, which might be involved in PML pathology (Bartsch et al. 2015). An overall age above 50 years and an early drop of lymphocytes in the first 6 months are associated with an increased risk for PML.

Summarizing, DMF is a safe and relatively well-tolerated medication with a strong effect on ARR and neuroprotective properties. Larger trials have to prove whether the medication might also be effective in progressive MS. Follow-up developments such as XP28829 and ALKS8700 offer different fumarate compounds with putatively reduced gastrointestinal side effects. ALKS8700 is an aminoethyl ester of MMF, which is rapidly converted to MMF (Naismith et al. 2016) and is currently investigated in the phase III EVOLVE-MS-1 study (NCT02634307). Especially, gastrointestinal side effects were lower in a phase I study compared with DMF treatment (8.3% vs. 41.7%) (Naismith et al. 2016).

LAQUINIMOD

Mechanism of Action

Laquinimod is an orally available quinoline-3-carboxamide, which is under development as therapeutic for RRMS, PPMS, and Huntington's disease. Laquinimod gets readily into the CNS,

achieving 7% of the blood concentration (measured in healthy rats) (Brück and Wegner 2011). Laquinimod showed first efficacy in EAE with reduction of clinical signs, inflammation, and demyelination (Wegner et al. 2010). This was accompanied by down-regulation of IL-17. Within lesions, axonal damage was also reduced, hence providing early evidence that the molecule might show neuroprotective properties. Preclinical and clinical data proved that laquinimod has effects on various immune cells. Laquinimod reduces CD11c⁺CD4⁺ dendritic cells, inhibits the expansion of T cells, and suppresses the formation of germinal center B cells, hence reducing disability progression in spontaneous EAE in the 2D2 model (Varrin-Doyer et al. 2016). Laquinimod also modifies the phenotype of B cells with an up-regulation of regulatory molecules CD25, IL-10, and CD86 in accordance with decreased IL-4 and increased transforming growth factor β (TGF- β) (Toubi et al. 2012). Early studies investigated the question whether the effect might be mediated by IFN- β . Taking advantage of IFN- β knockout mice, it could be excluded that the effect in EAE is mediated by IFN- β (Runstrom et al. 2006). The presence of high-affinity BDNF receptors is clearly linked to the activity of the drug (Thone et al. 2012). The immunomodulatory effect of laquinimod is mediated by the aryl hydrocarbon receptor, whereas neuroprotective effects of the medication are maybe mediated via other mechanisms (Berg et al. 2016). Laquinimod reduces the number of dendritic cells in the blood as well and alters their maturation (Jolivel et al. 2013). This effect is mediated by inhibition of nuclear factor (NF)- κ B signaling (Jolivel et al. 2013). EAE data indicate that laquinimod also inhibits proinflammatory monocytes to enter the CNS, hence reducing spinal cord inflammation with reduced CD62L and matrix metalloproteinase (MMP)-9 (Mishra et al. 2012).

Possible neuroprotective effects of the medication are supported by reports about efficacy in a model of Huntington's disease, the YAC128 model. Here, striatal and cortical atrophy could be rescued in accordance with improvement of motor function and depressive-like behavior (Garcia-Miralles et al. 2016). Neuroprotective

properties are mediated by the inhibition of the Bax pathway, which is involved in apoptosis, hence leading to reduced caspase-6 activity in neurons (Ehrnhoefer et al. 2016). Laquinimod also has effects in the cuprizone model, which is a model of demyelination. Laquinimod treatment reduces axonal spheroid formation, activation of microglia/macrophages, and apoptosis of oligodendrocytes together with reduced demyelination (Kramann et al. 2016). Brück et al. (2012) showed that this is mediated by reduced NF- κ B signaling in astrocytes. In EAN, laquinimod exerted positive clinical effects within a small-dose range in accordance with less demyelination and inflammation (Pitarokoili et al. 2014).

Clinical Studies

The clinical studies conducted so far show inconsistent results. In the ALLEGRO phase III clinical trial, laquinimod significantly reduced the ARR by 23% from 0.39 in the placebo group to 0.3 in the laquinimod arm (Comi et al. 2012). The risk of disability progression was also reduced (11.1% vs. 15.7%). Laquinimod especially showed promising results regarding brain atrophy in this trial. After 12 months, the rate of atrophy in several brain regions was reduced on laquinimod treatment (Filippi et al. 2014). This effect persisted, however, not up to 24 months. In a follow-up study, the BRAVO phase III clinical trial, laquinimod 0.6 mg once daily did not differ from placebo regarding the ARR and also failed regarding disability progression (Vollmer et al. 2014). However, laquinimod had a significant effect on the reduction of brain atrophy. Blood samples of laquinimod-treated MS patients had increased levels of BDNF, which might explain in part the neuroprotective properties of the medication. Thus, laquinimod showed inconsistent results regarding measures of inflammation but good effects on MRI parameters of brain atrophy. Therefore, the medication might be interesting as a treatment option for progressive MS.

Currently, laquinimod is investigated in the CONCERTO trial in RRMS (NCT01707992) and ARPEGGIO in PPMS. In January 2016, the higher dosage arms of 1.2 or 1.5 laquinimod

were stopped in both trials because of cardiac events, reminiscent of the mother drug linoimide, which induced coronary vasculitis as an unwanted side effect. Results from CONCERTO are expected in April 2017 and for ARPEGGIO in October 2017.

Side Effect Profile and Tolerability

Apart from the aforementioned cardiac side effects in the two ongoing trials, laquinimod was well tolerated in the studies that have been completed so far. The ALLEGRO trial showed an increased risk for alanine transaminase elevation of more than three times more than the upper limit by 2.6-fold compared with placebo. The elevations were of transient nature and not associated with clinical, imaging, or laboratory signs of liver failure (Comi et al. 2012). In the BRAVO trial, the most important side effects included headache (3%), increased alanine transaminase (2%), and nausea (0.4%) (Vollmer et al. 2014). No clear signs for coagulation disorders were observed.

TERIFLUNOMIDE

Teriflunomide was approved as oral therapy for RRMS in September 2012 (FDA) in a dosage of 14 mg per day. The compound is the active metabolite of leflunomide, which has been used for the treatment of rheumatoid arthritis after approval in the European Union in 1998 (ARAVA, Sanofi-Aventis). Teriflunomide leads to the inhibition of the dihydroorotate dehydrogenase (DHODH), hence inhibiting the proliferation of activated lymphocytes (Cherwinski et al. 1995; Ringshausen et al. 2008). As there exists a DHODH-independent salvage pathway, established immune responses are not impaired and neuronal cells do not suffer from pyrimidine deficiency.

Mechanism of Action

Teriflunomide inhibits the dihydroorotate dehydrogenase, which leads to reduced proliferation of B cells and T cells without inducing cytotoxicity (Li et al. 2013). The molecule also reduces

the release of proinflammatory cytokines IL-6, IL-8, and MCP-1 from peripheral blood mononuclear cells (PBMCs) (Li et al. 2013). This effect was not reversible by the addition of exogenous uridine. Teriflunomide showed effectiveness in several in vivo models. In EAE in Dark Agouti rats, the medication reduced clinical signs accompanied by less infiltration of T cells, macrophages, natural killer cells, and neutrophils (Ringheim et al. 2013). Teriflunomide also shows beneficial effects on glial cells in vitro. It reduces proliferation of microglia by about 30% in a concentration of 5 μ M in vitro, enhances the expression of IL-10 (Wostradowski et al. 2016), and modulates microglial-monocyte interaction in HIV infection with improved neurotoxicity (Ambrosius et al. 2017). Interestingly, leflunomide can inhibit viral replication (Teschner et al. 2009; Bernhoff et al. 2010), which speaks for safety in terms of opportunistic infections.

Clinical Studies

The effectiveness of teriflunomide has been proven in three phase III placebo-controlled clinical trials (O'Connor et al. 2011; Confavreux et al. 2014; Miller et al. 2014). In TEMSO, 1088 patients were included and randomized into three arms (teriflunomide 7 or 14 mg, placebo). The ARR was reduced by 31.2% and 31.5%, respectively (7 and 14 mg group, respectively). Disability progression was reduced by 30% (O'Connor et al. 2011). MRI parameters also showed improvement compared with placebo. The number of T1 gadolinium-enhancing lesions was reduced by 80%; the total lesion volume was reduced by 67%.

In TOWER, 1169 patients were included and randomized in the same manner as in the TEMSO trial. Data about effectiveness were comparable with a 36% reduction of the ARR and a reduced progression of disability (HR 0.68; 14 mg group) (Confavreux et al. 2014). The TOPIC study investigated 618 patients with a clinically isolated syndrome and the risk to convert to a clinical definite MS. Treatment with teriflunomide led to a significant risk reduction for the occurrence of a second demyelinating event (HR 0.65) (Miller et al. 2014).

Teriflunomide was also investigated in CIS in the TOPIC trial. Here, teriflunomide reduced the risk of a relapse or a new MRI lesion at both dosages 14 mg (HR 0.651 [95% CI 0.515–0.822]; $p = 0.0003$) and 7 mg (0.686 [0.540–0.871]; $p = 0.0020$). More recently, the phase IV Teri-PRO study focusing on patient reported outcomes reinforced the clinical value of teriflunomide (Gold et al. 2016b).

Side Effect Profile and Tolerability

There exists long-term experience with teriflunomide regarding safety and side effects of the compound. The extension of the pivotal phase III TEMSO trial followed 742 patients (O'Connor et al. 2016). At the time point of publication, the compound was well tolerated with continued exposure. Patients treated with teriflunomide encounter elevations of liver function enzymes with an increase of the alanine transaminase of >1 times higher than normal (54.0% and 57.3% [7 mg and 14 mg group, respectively] compared to 35.9% in the placebo group). The percentage of patients with increases of ALT >3 times the upper limit did not differ. Teriflunomide-treated patients had significantly more gastrointestinal symptoms such as nausea and diarrhea. Another considerable side effect is alopecia/hair thinning, which has been reported in 13.9% of teriflunomide-treated patients compared to 5.1% in the placebo group. Neoplasms were not reported on teriflunomide treatment. In 1%–2% of patients, a sensory neuropathy developed. Mounting an immune response after a vaccination is an important consideration for the treatment with immunosuppressive therapies. A small study conducted in patients treated with teriflunomide in both dosages and IFN- β showed that 61% in the 14 mg group were able to mount an immune response against seasonal influenza (Bar-Or et al. 2013). The response of the 7 mg group (78%) was comparable to the group treated with IFN- β 1 (82%).

CONCLUDING REMARKS

During the last decade, the armamentarium of MS therapies has considerably grown. Nowadays

it is possible to choose from a group of several injectables and compounds that are available as oral formulations. Monoclonal antibodies, used for therapy escalation together with fingolimod and reviewed elsewhere, complement the treatment possibilities. Oral therapeutics satisfy the need of patients to use medications with a pleasant way of administration although they do not enhance the adherence to therapy compared with injectables (Munsell et al. 2017). However, one of the biggest advantages with the growing armamentarium of therapeutic possibilities is that it is now possible to stratify for the optimal treatment regime before starting an immunomodulatory therapy based on knowledge about other underlying autoimmune conditions. For instance, it might be worthwhile to treat a patient with coincidental psoriasis with DMF or another patient with rheumatoid arthritis with teriflunomide. Another considerable advantage of modern MS therapy is the possibility to switch treatments without having to escalate in case the first oral therapeutic does not stabilize the disease or patients want to withdraw because of side effects. One of the biggest unmet needs of MS therapy is progression, which is up to now without authorized treatment options. Preclinical data about some of the molecules reviewed here are promising in the sense that neuroprotection might be feasible in progressive MS. In case laquinimod gets authorization for RRMS, the therapeutic armamentarium will grow even further and provide clinicians with more options for oral MS therapy.

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