



Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: [www.elsevier.com/locate/yexnr](http://www.elsevier.com/locate/yexnr)

## Review

## Laquinimod, an up-and-coming immunomodulatory agent for treatment of multiple sclerosis

Michel Varrin-Doyer, Scott S. Zamvil<sup>\*</sup>, Ulf Schulze-Topphoff

Department of Neurology, University of California, San Francisco, CA 94158, USA

## ARTICLE INFO

## Article history:

Received 19 December 2013

Revised 3 April 2014

Accepted 4 April 2014

Available online xxx

## Keywords:

Multiple sclerosis

Experimental autoimmune encephalomyelitis

Laquinimod

Disease modifying therapy

Immunomodulation

## ABSTRACT

Laquinimod is a novel oral drug that is currently being evaluated for the treatment of relapsing–remitting multiple sclerosis (RRMS). Although the mode of action of laquinimod remains to be fully elucidated, current knowledge indicates that laquinimod exerts beneficial activities both on the peripheral immune system and within the central nervous system (CNS). The immunomodulatory properties have been deciphered primarily from studies of laquinimod in the animal model of multiple sclerosis, experimental autoimmune encephalomyelitis (EAE). Data indicate that laquinimod has a primary effect on innate immunity. Laquinimod modulates the function of various myeloid antigen presenting cell populations, which then downregulate proinflammatory T cell responses. Further, data also indicate that laquinimod acts directly on resident cells within the CNS to reduce demyelination and axonal damage. Results from clinical trials that tested laquinimod in RRMS demonstrated that it reduced relapse rate and the mean cumulative number of active lesions, and had a more marked reduction in disability progression than relapse rate. Laquinimod treatment was associated with an excellent safety and tolerability profile. These data indicate that laquinimod will offer a valuable new treatment option for RRMS patients.

© 2014 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/3.0/>).

## Contents

Introduction . . . . .	0
Mode of action . . . . .	0
Clinical studies . . . . .	0
Conclusions. . . . .	0
Acknowledgments and disclosures . . . . .	0
References . . . . .	0

## Introduction

The number of drugs licensed for multiple sclerosis (MS) has expanded rapidly. Since 2010, three oral therapies have been approved: fingolimod (Gilenya®), teriflunomide (Aubagio®) and BG-12 (Tecfidera®). Although efficacious, each of these medications has been associated with potential toxicities (Bruck, et al., 2013). Laquinimod is a novel oral agent with immunomodulatory

properties that is currently under evaluation for the treatment of relapsing–remitting multiple sclerosis (RRMS) and other autoimmune diseases (Bomback and Appel, 2010; Comi et al., 2008; Polman et al., 2005). Laquinimod is structurally related to roquinimex (linomide), which demonstrated efficacy in MS (Wolinsky et al., 2000), although its development was halted after unanticipated serious adverse events occurred in a Phase III trial (Nosworthy et al., 2000). Laquinimod, which was identified by screening a large number of chemically modified quinoline-3-carboxamides in the MS model, experimental autoimmune encephalomyelitis (EAE), exhibited greater efficacy than linomide without apparent toxicities (Jonsson et al., 2004). Laquinimod has since shown efficacy in Phase II and

<sup>\*</sup> Corresponding author at: University of California, San Francisco, 675 Nelson Rising Lane, NS-215A, San Francisco, CA 94158, USA. Fax: +1 415 502 8512.  
E-mail address: [zamvil@ucsf.neuroimmunol.org](mailto:zamvil@ucsf.neuroimmunol.org) (S.S. Zamvil).

Phase III MS clinical trials, without evident immunosuppression or significant toxicities (Comi et al., 2008, 2010; Polman et al., 2005). Laquinimod significantly reduced relapse rate, disability progression, development of new active MRI lesions and brain atrophy. Because laquinimod treatment showed a greater effect on disability progression than the relapse rate, and slowed brain atrophy, a measure that correlates with disability, it is thought that laquinimod may be beneficial in the progressive phases of MS.

### Mode of action

Laquinimod is a small molecule that enters different tissue compartments. In plasma, 98% is bound to proteins. From animal studies, laquinimod concentration in the CNS reaches approximately 8% of peripheral blood exposure in naive animals, while it peaks at 13% during CNS inflammation (Bruck and Wegner, 2011). Although precise molecular targets are not well defined, current knowledge points towards dual modes of action, in the peripheral immune system as well as in the central nervous system (CNS) itself.

The influence of laquinimod on the immune system became evident when it was studied in EAE, an autoimmune disease mediated by proinflammatory myelin-reactive lymphocytes that results in CNS inflammation and may be associated with demyelination and axonal loss (Steinman and Zamvil, 2005). Laquinimod was shown to suppress clinical signs in both acute and chronic EAE models (Brunmark et al., 2002; Schulze-Topphoff et al., 2012; Wegner et al., 2010; Yang et al., 2004). Specifically, these studies revealed that laquinimod was able not only to prevent the development of EAE when administered from the time of immunization, but also to inhibit the occurrence of relapses when treatment was initiated after disease was established. Laquinimod has also been effective in the treatment of experimental autoimmune neuritis (EAN), an inflammatory autoimmune demyelinating disease of the peripheral nervous system that has been used as an animal model of Guillain-Barré syndrome (Zou et al., 2002). A common characteristic of CNS autoimmune diseases, such as MS or its animal model, is that autoantigen-reactive T cells must undergo several discrete steps in order to cause disease. In EAE, T cells become activated in the peripheral immune compartment, then differentiate into a pathogenic effector phenotype and express appropriate adhesion molecules that permit entry into the target organ, the CNS. Within the CNS, T cells must recognize autoantigen in order to become reactivated and cause tissue injury (Kuchroo et al., 2002; Zamvil and Steinman, 1990). Thus, laquinimod could act in a number of different steps within this pathogenic cascade.

Initial signals that direct T cell activation and differentiation are provided by antigen presenting cells (APC). The myeloid subpopulations, monocytes/macrophages and dendritic cells (DC), which represent cells within the innate immune compartment, are well-recognized APC. We recently reported that in vivo laquinimod treatment of mice was associated with alterations in the frequency of these myeloid subpopulations that included a reduction in CD4<sup>+</sup> DC (Schulze-Topphoff et al., 2012), potent APC for CD4<sup>+</sup> T-cell responses (Dudziak et al., 2007). Laquinimod treatment also promoted the development of anti-inflammatory type II monocytes and DC, which are associated with reduced production of proinflammatory IL-6, IL-12/IL-23 (p40) and TNF, and increased production of anti-inflammatory IL-10. Interestingly, transfer of monocytes from laquinimod-treated donor wild-type mice, but not monocytes from vehicle-treated wild-type mice, ameliorated clinical and histological signs in recipient mice that already had developed EAE. The examination of the cellular pathway involved revealed that laquinimod treatment suppressed inducible STAT1, a transcription factor that participates in the expression of several proinflammatory cytokines, but did not alter activation of p38 MAPK that can be regulated independently or coordinately with STAT1 (Schulze-Topphoff et al., 2012). Laquinimod-induced alterations of APC functions resulted in anti-inflammatory T cell polarization manifested by a reduction in the frequencies of proinflammatory Th1 and Th17 cells in vivo, and by an

increase in regulatory T cells (Treg) (Schulze-Topphoff et al., 2012). Although laquinimod-mediated alterations of T cell responses were previously reported (Wegner et al., 2010; Yang et al., 2004; Zou et al., 2002), Schulze-Topphoff et al. demonstrated that such immune modulation was exerted through laquinimod's effects on APC, and not on T cells directly. Such action of laquinimod on APC was also described by other studies (Jolivel et al., 2013; Thone et al., 2012). Further, Jolivel and colleagues reported a modulation of human dendritic cells by laquinimod. Specifically, in laquinimod-treated MS patients, frequency and proinflammatory cytokine secretion were reduced in circulating conventional CD1c<sup>+</sup> DC, considered an equivalent of murine CD8<sup>−</sup>CD11b<sup>+</sup> splenic DC. In addition, laquinimod-treated human monocyte-derived DC inhibited proliferation of CD4<sup>+</sup> T cells and pro-inflammatory cytokine secretion. These immunomodulatory effects appeared to correspond to an impaired NF-κB pathway in human APC (Gurevich et al., 2010; Jolivel et al., 2013). Further, a high-throughput analysis of in vitro laquinimod-treated peripheral mononuclear cells (PBMC) from MS patients and healthy controls also demonstrated that laquinimod down-regulated MHC class II genes, pivotal for antigen presentation, and inhibited expression of inflammatory pathways (Gurevich et al., 2010).

Recent pathological and clinical studies indicate that B cells have a critical role in the development of MS (Hauser et al., 2008; Magliozzi et al., 2007). Ectopic meningeal B cell follicles are commonly detected in secondary progressive MS. B cell-depleting agents have demonstrated marked efficacy in MS therapy. Recently, it was observed that B cells provide a critical cellular function in the pathogenesis of CNS autoimmunity that is separate from their humoral involvement (Molnarfi et al., 2013). Specifically, mice selectively deficient in B cell MHC II expression were resistant to EAE induced by recombinant human MOG, a T cell- and B cell-dependent autoantigen, and exhibited diminished Th1 and Th17 responses. Further, spontaneous opticospinal EAE and meningeal follicle-like structures were observed when transgenic mice that express MOG-specific B cell receptor (BCR), but cannot secrete antibodies, were crossed with MOG-specific T cell receptor (TCR) transgenic mice. These results demonstrated that B cell APC function is necessary and sufficient for induction of CNS autoimmunity. Interestingly, B cells from MS patients that were exposed to laquinimod in vitro, exhibited alterations in the expression of genes involved in the NF-κB pathway and T cell activation (Gurevich et al., 2010). Another study of in vitro laquinimod treatment observed a modest increase in the frequency of IL-10 positive B cells (Toubi et al., 2012). Laquinimod treatment of human B cells also resulted in an augmented expression of CD86, a costimulatory molecule that has been associated with anti-inflammatory Th2 responses (Kuchroo et al., 1995; Weber et al., 2007). These findings suggest that laquinimod might modulate B cell APC function. Laquinimod treatment has been associated with increased numbers of splenic B cells in mice (Brunmark et al., 2002). Although such modulation was not observed in human PBMC (Lund et al., 2013), one might envisage that laquinimod's effect on B cells may be tissue-dependent. B cells have also been implicated in MS pathogenic mechanisms through the secretion of CNS autoantigen-specific antibodies. Thus, it will be important to investigate whether laquinimod influences the production of CNS antigen-specific autoantibodies.

Through functional alterations induced in APC, laquinimod treatment modulated the differentiation and associated cytokine production of CD4<sup>+</sup> T cells. The drug inhibited T cell secretion of pro-inflammatory cytokines such as interferon-gamma (IFNγ), interleukin (IL)-17, granulocyte-macrophage colony-stimulating factor (GM-CSF) and tumor necrosis factor-alpha (TNFα) (Jolivel et al., 2013; Schulze-Topphoff et al., 2012). There has been report of laquinimod-induced increased CD4<sup>+</sup> T production of the anti-inflammatory cytokine IL-4, suggesting that laquinimod might drive a shift towards Th2 T cell responses (Gurevich et al., 2010), although other data have questioned such a shift (Jolivel et al., 2013; Toubi et al., 2012). Nevertheless, an increased frequency of Treg was observed in the spleen of laquinimod-treated mice, further supporting its potential to induce anti-inflammatory

T cell polarization (Aharoni et al., 2012; Schulze-Topphoff et al., 2012). Such an increase was not detected in PBMC from treated MS patients (Lund et al., 2013). However, it must be acknowledged that, in murine studies, laquinimod-induced immune modulation was evaluated following antigen-specific T cell activation, obtained upon direct immunization with myelin peptides or proteins, a situation obviously not encountered in MS. Interestingly, it was observed that laquinimod treatment of human monocyte-derived dendritic cells altered more profoundly the production of certain lymphocytic cytokines, when human T cells were stimulated in an antigen-specific manner (Jolivel et al., 2013). Therefore, evaluating whether laquinimod treatment of MS modulates cell function in the absence of active antigenic stimulation may be more challenging.

Following polarization, T cell migration represents a key step in CNS inflammation. Laminar flow analyses revealed that laquinimod treatment reduced the responsiveness of VLA-4, a key adhesion molecule in T cell migration (Wegner et al., 2010). Specifically, the ability of the CCR7-binding chemokine, CCL21, to stimulate VLA-4 adhesiveness to its natural ligand, VCAM-1, was inhibited in splenic T cells isolated from immunized mice that received treatment. Interestingly, laquinimod treatment was also associated with a significant reduction of CNS inflammation, as demonstrated by decreased numbers of T cells (Schulze-Topphoff et al., 2012; Wegner et al., 2010). In line with these observations, the *in vitro* secretion of several chemokines participating in the recruitment of leucocytes to inflammatory tissue sites, including MIP-1 $\alpha$ , MIP-1 $\beta$  and MIG, was reduced after laquinimod treatment. Such modulations were observed in mature murine bone marrow-derived dendritic cells, human mature monocyte-derived dendritic cells, as well as conventional CD1c<sup>+</sup> dendritic cells upon lipopolysaccharide stimulation (Jolivel et al., 2013). Whether laquinimod acts similarly *in vivo* is unknown.

In addition to its modulatory action on the immune system, laquinimod therapy demonstrated potential neuroprotective properties by modulating the production of brain-derived neurotrophic factor (BDNF) that is essential for the development and maintenance of the CNS (Kalb, 2005), and also mediates axon protection in EAE (Bruck and Zamvil, 2012; Linker et al., 2010). In EAE, which was associated with a significant decrease of BDNF mRNA expression, laquinimod treatment restored BDNF production to levels that were seen in naive mice (Aharoni et al., 2012; Thone et al., 2012). In patients with RRMS, laquinimod treatment induced direct and sustained upregulation of bioactive brain-derived neurotrophic factor (BDNF), as demonstrated by the evaluation of serum levels (Thone et al., 2012). Further, the therapeutic administration of laquinimod treatment to mice after they developed EAE appeared beneficial to the CNS (Aharoni et al., 2012; Wegner et al., 2010). Although demyelination and acute axonal injury were observed in both laquinimod- and vehicle-treated groups, laquinimod induced a significant reduction of lesions. Similar levels of axonal loss within lesions were however identified in both groups. In the cuprizone-induced demyelination model, laquinimod treatment prevented demyelination, microglial activation, axonal transections, reactive gliosis and oligodendroglial apoptosis, regardless of whether CNS infiltrating immune cells were present (Bruck et al., 2012). Of CNS resident cells, astrocytes appeared to be the principal cell type affected by laquinimod treatment in cuprizone-induced CNS demyelination. These cells exhibited decreased production of proinflammatory factors and reduced NF- $\kappa$ B activation upon *in vitro* laquinimod treatment.

### Clinical studies

Several clinical trials have evaluated the pharmacological effects of laquinimod, including two Phase I studies, two Phase II studies associated with extensions of up to 42 months, and two Phase III clinical trials (Table 1). The clinical Phase I studies were funded by Active Biotech AB (Lund, Sweden), where laquinimod was initially developed.

Although neither of these Phase I studies were published, the results were briefly discussed and summarized by Polman et al. (2005). Those Phase I investigations provided safety and pharmacokinetic data that guided the selection of 0.1 and 0.3 mg oral daily doses for the first Phase II study, a 24-week, multicenter, double-blind, randomized, placebo-controlled trial in patients ( $n = 209$ ) with RRMS or secondary progressive MS (SPMS) (Polman et al., 2005). The study design included three groups: patients administered placebo, and patients treated with laquinimod at 0.1 mg daily or 0.3 mg daily. Patients were required to have an EDSS (Expanded Disability Status Scale) score no greater than 5.5 and active disease as defined by specific MRI criteria. The primary objective of the study was to determine the difference in the cumulative number of active lesions (new gadolinium-enhanced [GdE] and new T2 lesions) between the placebo group and the 0.3-mg laquinimod group over the 24-week treatment period. MRI scans were obtained at baseline, weeks 4, 8, 24, and 8 weeks post discontinuation of therapy with triple dose gadolinium infusions used for the detection of enhancing lesions. The results showed that the mean cumulative number of active lesions was reduced significantly by 44% in the 0.3-mg laquinimod group compared with placebo. There was no significant difference in the cumulative number of active lesions between the 0.1-mg group and the placebo group. With regard to the second objective, laquinimod showed a favorable safety profile based on clinical and laboratory variables. Overall, both doses of laquinimod were well tolerated and the proportion of patients with adverse events was similar in the three groups.

The Phase IIb study was a 36-week, multicenter, double-blind, placebo-controlled trial of patients with RRMS ( $n = 306$ ), initiated to evaluate efficacy, tolerability, and safety of 0.3 and 0.6 mg laquinimod doses (Comi et al., 2008). The primary efficacy outcome measure was the cumulative number of GdE lesions over the last four scans at weeks 24, 28, 32 and 36. In the 0.6-mg group, a significant 40.4% reduction in the cumulative number of GdE lesions compared with placebo was observed. However, there was no statistically significant reduction in the 0.3-mg group compared with placebo. Further, patients who received a 0.6-mg dose also had a statistically significant 51% reduction in the cumulative number of new hypointense T1 lesions compared with placebo. Again, a significant reduction was not observed in the 0.3-mg group. Consistent with the initial Phase II study (Polman et al., 2005), both doses of laquinimod showed an excellent safety profile.

The beneficial effects of oral laquinimod in these Phase II studies prompted the development of two global, multicenter, multinational, 2-year, placebo-controlled Phase III trials of 0.6 mg laquinimod in patients with RRMS. The ALLEGRO (Assessment of Oral Laquinimod in Preventing Progression in Multiple Sclerosis) study was a randomized (1:1) double-blind, placebo-controlled Phase III trial designed to evaluate the efficacy, safety and tolerability of oral 0.6 mg laquinimod versus placebo in the treatment of patients ( $n = 1106$ ) with RRMS (Comi et al., 2012). Recruitment criteria included an EDSS score no greater than 5.5 and either one or more relapse in the previous year, or two or more in the previous two years in association with at least one gadolinium-enhancing lesion. The ALLEGRO study showed that treatment with laquinimod as compared with placebo was associated with a modest, but significant, reduction in the mean ( $\pm$ SE) annualized relapse rate (ARR), used as the primary end point, and a reduction in the risk of confirmed disability progression (11.1 vs 15.7%). The mean cumulative number of gadolinium-enhancing lesions and new or enlarging lesions on T2-weighted images appeared significantly reduced in patients receiving laquinimod compared to those receiving placebo ( $1.33 \pm 0.14$  vs  $2.12 \pm 0.22$  and  $5.03 \pm 0.08$  vs  $7.14 \pm 0.07$ , respectively). Further, laquinimod-treated patients showed reduced brain atrophy, observed in both white and gray matter at 12 and 24 months, as compared with placebo. Laquinimod also slowed thalamic atrophy at month 12 and month 24 and reduced the number of permanent black holes at 12 and 24 months evolving from active lesions. These findings

**Table 1**  
Clinical and imaging results in laquinimod trials.

Clinical study	Groups (daily dose)	Patients started/ completed (n)	Primary endpoint	ARR	Disease progression (% patients)	Mean cumulative number of GdE lesions ± SE	Mean cumulative number of T2 lesions ± SE	Brain volume change (%)	Ref.
Phase II	Placebo	67/64	Cumulative number of GdE lesions	n.d.	n.d.	9.4 ± 17.3 <sup>¶</sup>	n.d.	n.d.	Polman et al. (2005)
	LAQ 0.1 mg <sup>#</sup>	68/65				6.4 ± 9.7 [−32%] n.s.			
Phase IIb	Placebo	102/91	Cumulative number of GdE lesions	0.77 ± 1.25	n.d.	5.2 ± 9.9	2.4 ± 3.3 <sup>≈</sup>	n.d.	Comi et al. (2008)
	LAQ 0.3 mg <sup>#</sup>	74/69				4.2 ± 9.2 <sup>≈</sup> 3.9 ± 5.5 p = 0.0498			
Phase IIb	Placebo	102/91	Cumulative number of GdE lesions	0.76 ± 1.02	n.d.	2.6 ± 5.3	2.5 ± 3.2	n.d.	Comi et al. (2008)
	LAQ 0.3 mg <sup>≠</sup>	98/92				2.6 ± 5.3 [−40.4%] <sup>◇</sup> p = 0.0048			
Phase IIb	Placebo	106/100	Cumulative number of GdE lesions	0.52 ± 0.92	n.d.	1.6 ± 3.7	1.6 ± 3.7	n.d.	Comi et al. (2008)
	LAQ 0.6 mg <sup>≠</sup>	106/100				1.6 ± 3.7 [−44%] p > 0.1			
Phase III ALLEGRO	Placebo	556/427	Annualized relapse rate	0.39 ± 0.03	15.7% <sup>∞</sup>	2.12 ± 0.22 <sup>§</sup>	7.14 ± 0.07 <sup>§</sup>	−1.30	Comi et al. (2012)
	LAQ 0.6 mg	550/437				0.30 ± 0.02 [−23%] p = 0.002			
Phase III BRAVO	Placebo	450/359	Annualized relapse rate	0.34 ± 0.03	10% <sup>∞</sup>	2.34 ± 0.25 <sup>§</sup>	13.03 ± 1.1 <sup>§</sup>	−1.03	Vollmer et al. (2014)
	LAQ 0.6 mg	434/353				0.28 ± 0.03 [−18%] p = 0.075			
Phase III BRAVO	Placebo	450/359	Annualized relapse rate	0.26 ± 0.02	8%	0.90 ± 0.10	6.37 ± 0.51	−1.14	Vollmer et al. (2014)
	LAQ 0.6 mg	434/353				0.26 ± 0.02 [−26%] p = 0.007			
Phase III BRAVO	Placebo	450/359	Annualized relapse rate	0.26 ± 0.02	8%	0.90 ± 0.10	6.37 ± 0.51	−1.14	Vollmer et al. (2014)
	IFNβ-1a IM	447/378				0.26 ± 0.02 [−26%] p = 0.007			

LAQ laquinimod, ARR annualized relapse rate, GdE gadolinium-enhancing, SE standard error, n.s. not significant, n.d. not determined. [..] Treatment effect compared with placebo in percent. p values were calculated in comparison with placebo group.

<sup>#</sup> Per-protocol cohort.

<sup>≠</sup> Intention-to-treat cohort.

<sup>◇</sup> Based on adjusted mean.

<sup>≈</sup> Number of lesions per scan in the last four scans.

<sup>∞</sup> Percentage of patients with confirmed expanded disability status scale (EDSS) progression at 3 months.

<sup>∞</sup> Percentage of patients with confirmed EDSS progression at 6 months.

<sup>§</sup> Cumulative number of lesions at 12 and 24 months.

<sup>¶</sup> Cumulative number of lesions at 24 weeks.

suggested that laquinimod might also modulate some of the more destructive pathological processes in RRMS patients (Filippi et al., 2013).

The BRAVO trial assessed the efficacy, safety and tolerability of laquinimod compared with placebo in patients with RRMS and descriptively compared the benefit/risk profile of laquinimod to IFN- $\beta$ 1a (Avonex®). RRMS patients (n = 1331) were randomized 1:1:1 to receive once-daily oral laquinimod (0.6 mg), placebo (once daily), or IFN- $\beta$ 1a (30  $\mu$ g once weekly) (Vollmer et al., 2014). The results from the BRAVO trial indicated that laquinimod did not reach its end point, as illustrated by the lack of statistical significance in the reduction of relapses. However, the examination of the number of patients with GdE lesions and of mean T2 lesion volume, both predictors of relapses, revealed a significant imbalance between the laquinimod and the placebo arms at baseline. When preplanned analyses were carried out to adjust for these imbalances, there was a significant reduction in the relapse rate for the laquinimod arm (21% reduction vs placebo, p = 0.026), as well as a significant decreased risk of disability progression at 3 months as measured by the EDSS (33.5% reduction vs placebo, p = 0.044) and a reduced cumulative number of new enlarged T2 lesions (19% reduction vs placebo, p = 0.037) (Vollmer et al., 2014). Overall, laquinimod's effects on disability progression and brain atrophy were more pronounced than its effect on relapses and new lesion formation (Comi et al., 2012; Vollmer et al., 2011, 2014).

For both ALLEGRO and BRAVO studies, the most common adverse events in the laquinimod group were abdominal pain (5.8% vs. 2.9% in the placebo group), back pain (16.4% vs. 9.0%), and cough (7.5% vs. 4.5%). These adverse events were rarely associated with discontinuation of the study (3% and 1% of the laquinimod and placebo groups, respectively) (Comi et al., 2012; Vollmer et al., 2014). In addition, transient elevations in alanine aminotransferase levels to greater than three times the upper limit of the normal range were observed in 24 patients receiving laquinimod (5%) and 8 receiving placebo (2%). An ongoing open-label extension of ALLEGRO will provide further useful safety information. Further, continued analysis of the ALLEGRO and BRAVO Phase III studies revealed that one third of the patients who progressed were relapse-free, suggesting that disability progression and relapses are not solely mediated through a common pathway (Comi et al., 2013). Laquinimod reduced disability progression relative to placebo with a treatment effect of 26.7% in relapsing patients and 38.9% effect in relapse-free patients. These findings, which support an effect of laquinimod on both inflammation and neuroprotection, combined with laquinimod's modest action on reducing ARR, led Teva pharmaceuticals to develop a new Phase III study, CONCERTO (Vollmer et al., 2013). This latter study will evaluate effects of two doses of laquinimod (0.6 mg and 1.2 mg) or placebo in approximately 1800 people with RRMS for up to 24 months. Disease progression will be evaluated as a primary end point.

## Conclusions

In contrast with the approved oral therapies, which have potential serious toxicities (Bruck et al., 2013), laquinimod has an excellent safety profile. Laquinimod has shown greater beneficial effect on disability and brain atrophy than relapse rate, suggesting it could have efficacy on disability progression both in RRMS and SPMS. It will be important to determine whether the higher dose of 1.2 mg laquinimod, which is being tested in the Phase III trial, CONCERTO (<http://clinicaltrials.gov/NCT01707992>), will have greater efficacy than 0.6 mg and lead to its widespread approval. It is becoming increasingly clear that laquinimod exerts its effects through modulation of innate immunity, and that its treatment is associated with potential neuroprotection. In future studies, it will be important to elucidate the molecular targets of laquinimod and determine if laquinimod influences other aspects of autoimmune disease, including humoral responses. Given its unique mode of action and its effects on disability progression, laquinimod may become an important therapy for different phases of MS.

## Acknowledgments and disclosures

Support for this work was provided to S.S. Zamvil by the National Institutes of Health (NIH; R01 AI-073737 and R01 NS-063008), the NMSS (RG 3913 and RG 4768), the Guthy Jackson Charitable Foundation, the Dana Foundation, the Maisin Foundation and Teva Pharmaceuticals, Inc.

M. Varrin-Doyer is a fellow of the National Multiple Sclerosis Society (NMSS). U. Schulze-Toppfhoff received fellowship support from the NMSS and the Deutsche Forschungsgemeinschaft (DFG; SCHU 2587/1-1).

SSZ has received personal compensation serving as a consultant or serving on data safety monitoring boards for Biogen-Idec, Teva, EMD Merck Serono, Genzyme, Novartis, Eli Lilly and Bayhill Therapeutics.

## References

- Aharoni, R., Saada, R., Eilam, R., Hayardeny, L., Sela, M., Arnon, R., 2012. Oral treatment with laquinimod augments regulatory T-cells and brain-derived neurotrophic factor expression and reduces injury in the CNS of mice with experimental autoimmune encephalomyelitis. *J. Neuroimmunol.* 251, 14–24.
- Bomback, A.S., Appel, G.B., 2010. Updates on the treatment of lupus nephritis. *J. Am. Soc. Nephrol.* 21, 2028–2035.
- Bruck, W., Wegner, C., 2011. Insight into the mechanism of laquinimod action. *J. Neurol. Sci.* 306, 173–179.
- Bruck, W., Zamvil, S.S., 2012. Laquinimod, a once-daily oral drug in development for the treatment of relapsing–remitting multiple sclerosis. *Expert. Rev. Clin. Pharmacol.* 5, 245–256.
- Bruck, W., Pfortner, R., Pham, T., Zhang, J., Hayardeny, L., et al., 2012. Reduced astrocytic NF-kappaB activation by laquinimod protects from cuprizone-induced demyelination. *Acta Neuropathol.* 124, 411–424.
- Bruck, W., Gold, R., Lund, B.T., Oreja-Guevara, C., Pratt, A., et al., 2013. Therapeutic decisions in multiple sclerosis: moving beyond efficacy. *JAMA Neurol.* 70, 1315–1324.
- Brunmark, C., Runstrom, A., Ohlsson, L., Sparre, B., Brodin, T., et al., 2002. The new orally active immunoregulator laquinimod (ABR-215062) effectively inhibits development and relapses of experimental autoimmune encephalomyelitis. *J. Neuroimmunol.* 130, 163–172.
- Comi, G., Pulizzi, A., Rovaris, M., Abramsky, O., Arbizu, T., et al., 2008. 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* 371, 2085–2092.
- Comi, G., Abramsky, O., Arbizu, T., Boyko, A., Gold, R., et al., 2010. Oral laquinimod in patients with relapsing–remitting multiple sclerosis: 36-week double-blind active extension of the multi-centre, randomized, double-blind, parallel-group placebo-controlled study. *Mult. Scler.* 16, 1360–1366.
- Comi, G., Jeffery, D., Kappos, L., Montalban, X., Boyko, A., et al., 2012. Placebo-controlled trial of oral laquinimod for multiple sclerosis. *N. Engl. J. Med.* 366, 1000–1009.
- Comi, G., Vollmer, T., Cutter, G., Sasso, N., Ladhani, D., Gorfine, T., 2013. Disease progression in relapse-free patients treated with laquinimod. *Mult. Scler.* 19 (11 Suppl.), 74–558 (P1036).
- Dudziak, D., Kamphorst, A.O., Heidkamp, G.F., Buchholz, V.R., Trumppfeller, C., et al., 2007. Differential antigen processing by dendritic cell subsets in vivo. *Science* 315, 107–111.
- Filippi, M., Rocca, M.A., Pagani, E., De Stefano, N., Jeffery, D., et al., 2013. Placebo-controlled trial of oral laquinimod in multiple sclerosis: MRI evidence of an effect on brain tissue damage. *J. Neurol. Neurosurg. Psychiatry* [Epub ahead of print] PubMed PMID: 24029546.
- Gurevich, M., Gritzman, T., Orbach, R., Tuller, T., Feldman, A., Achiron, A., 2010. Laquinimod suppress antigen presentation in relapsing–remitting multiple sclerosis: in-vitro high-throughput gene expression study. *J. Neuroimmunol.* 221, 87–94.
- Hauser, S.L., Waubant, E., Arnold, D.L., Vollmer, T., Antel, J., et al., 2008. B-cell depletion with rituximab in relapsing–remitting multiple sclerosis. *N. Engl. J. Med.* 358, 676–688.
- Jolivel, V., Luessi, F., Masri, J., Kraus, S.H., Hubo, M., et al., 2013. Modulation of dendritic cell properties by laquinimod as a mechanism for modulating multiple sclerosis. *Brain* 136, 1048–1066.
- Jonsson, S., Andersson, G., Fex, T., Fristedt, T., Hedlund, G., et al., 2004. Synthesis and biological evaluation of new 1,2-dihydro-4-hydroxy-2-oxo-3-quinolinecarboxamides for treatment of autoimmune disorders: structure-activity relationship. *J. Med. Chem.* 47, 2075–2088.
- Kalb, R., 2005. The protean actions of neurotrophins and their receptors on the life and death of neurons. *Trends Neurosci.* 28, 5–11.
- Kuchroo, V.K., Prabhu Das, M., Brown, J.A., Ranger, A.M., Zamvil, S.S., et al., 1995. B7-1 and B7-2 costimulatory molecules activate differentially the Th1/Th2 developmental pathways: application to autoimmune disease therapy. *Cell* 80, 707–718.
- Kuchroo, V.K., Anderson, A.C., Waldner, H., Munder, M., Bettelli, E., Nicholson, L.B., 2002. T cell response in experimental autoimmune encephalomyelitis (EAE): role of self and cross-reactive antigens in shaping, tuning, and regulating the autopathogenic T cell repertoire. *Annu. Rev. Immunol.* 20, 101–123.
- Linker, R.A., Lee, D.H., Demir, S., Wiese, S., Kruse, N., et al., 2010. Functional role of brain-derived neurotrophic factor in neuroprotective autoimmunity: therapeutic implications in a model of multiple sclerosis. *Brain* 133, 2248–2263.

- Lund, B.T., Kelland, E.E., Hayardeny, L., Barilan, O., Gilmore, W., Weiner, L.P., 2013. Assessment of changes in immune measures of multiple sclerosis patients treated with laquinimod. *J. Neuroimmunol.* 263, 108–115.
- Magliozzi, R., Howell, O., Vora, A., Serafini, B., Nicholas, R., et al., 2007. Meningeal B-cell follicles in secondary progressive multiple sclerosis associate with early onset of disease and severe cortical pathology. *Brain* 130, 1089–1104.
- Molnarfi, N., Schulze-Toppf, U., Weber, M.S., Patarroyo, J.C., Prod'homme, T., et al., 2013. MHC class II-dependent B cell APC function is required for induction of CNS autoimmunity independent of myelin-specific antibodies. *J. Exp. Med.* 210, 2921–2937.
- Noseworthy, J.H., Wolinsky, J.S., Lublin, F.D., Whitaker, J.N., Linde, A., et al., 2000. Linomide in relapsing and secondary progressive MS: part I: trial design and clinical results. North American Linomide Investigators. *Neurology* 54, 1726–1733.
- Polman, C., Barkhof, F., Sandberg-Wollheim, M., Linde, A., Norder, O., Nederman, T., 2005. Treatment with laquinimod reduces development of active MRI lesions in relapsing MS. *Neurology* 64, 987–991.
- Schulze-Toppf, U., Shetty, A., Varrin-Doyer, M., Molnarfi, N., Sagan, S.A., et al., 2012. Laquinimod, a quinoline-3-carboxamide, induces type II myeloid cells that modulate central nervous system autoimmunity. *PLoS ONE* 7, e33797.
- Steinman, L., Zamvil, S.S., 2005. Virtues and pitfalls of EAE for the development of therapies for multiple sclerosis. *Trends Immunol.* 26, 565–571.
- Thone, J., Ellrichmann, G., Seubert, S., Peruga, I., Lee, D.H., et al., 2012. Modulation of autoimmune demyelination by laquinimod via induction of brain-derived neurotrophic factor. *Am. J. Pathol.* 180, 267–274.
- Toubi, E., Nussbaum, S., Staun-Ram, E., Snir, A., Melamed, D., et al., 2012. Laquinimod modulates B cells and their regulatory effects on T cells in multiple sclerosis. *J. Neuroimmunol.* 251, 45–54.
- Vollmer, T., Soelberg Sorensen, P., Arnold, D.L., on behalf of the BRAVO Study Group, 2011. A placebo-controlled and active comparator phase III trial (BRAVO) for relapsing-remitting multiple sclerosis. *Mult. Scler. J.* 17, S507–S508.
- Vollmer, T., Montalban, X., Comi, G., Ziemssen, T., Boyko, A., Vermersch, P., Sasson, N., Gorfine, T., Knappertz, V., Filippi, M., 2013. Multicentre, randomized, placebo controlled study to evaluate the efficacy, safety and tolerability of two doses of oral laquinimod. *Mult. Scler.* 19 (11 Suppl.), 74–558 (P1054).
- Vollmer, T.L., Sorensen, P.S., Selmaj, K., Zipp, F., Havrdova, E., et al., 2014. A randomized placebo-controlled phase III trial of oral laquinimod for multiple sclerosis. *J. Neurol.* (Feb 18 Epub ahead of print PubMed PMID: 24535134).
- Weber, M.S., Prod'homme, T., Youssef, S., Dunn, S.E., Rundle, C.D., et al., 2007. Type II monocytes modulate T cell-mediated central nervous system autoimmune disease. *Nat. Med.* 13, 935–943.
- Wegner, C., Stadelmann, C., Pfortner, R., Raymond, E., Feigelson, S., et al., 2010. Laquinimod interferes with migratory capacity of T cells and reduces IL-17 levels, inflammatory demyelination and acute axonal damage in mice with experimental autoimmune encephalomyelitis. *J. Neuroimmunol.* 227, 133–143.
- Wolinsky, J.S., Narayana, P.A., Noseworthy, J.H., Lublin, F.D., Whitaker, J.N., et al., 2000. Linomide in relapsing and secondary progressive MS: part II: MRI results. MRI Analysis Center of the University of Texas-Houston, Health Science Center, and the North American Linomide Investigators. *Neurology* 54, 1734–1741.
- Yang, J.S., Xu, L.Y., Xiao, B.G., Hedlund, G., Link, H., 2004. 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. *J. Neuroimmunol.* 156, 3–9.
- Zamvil, S.S., Steinman, L., 1990. The T lymphocyte in experimental allergic encephalomyelitis. *Annu. Rev. Immunol.* 8, 579–621.
- Zou, L.P., Abbas, N., Volkmann, I., Nennesmo, I., Levi, M., et al., 2002. Suppression of experimental autoimmune neuritis by ABR-215062 is associated with altered Th1/Th2 balance and inhibited migration of inflammatory cells into the peripheral nerve tissue. *Neuropharmacology* 42, 731–739.