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Laquinimod, an upcoming oral immunomodulatory agent for treatment of multiple sclerosis

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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 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 down-regulate 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.

Keywords

multiple sclerosis; experimental autoimmune encephalomyelitis; laquinimod; disease modifying therapy; immunomodulation

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. Laquinimod is a novel oral agent with immunomodulatory properties that is currently under evaluation for treatment of relapsing-

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remitting multiple sclerosis (RRMS) and other autoimmune diseases (Bomback & 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 (Noseworthy 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 phase III MS clinical trials, without evident immunosuppression or significant toxicities (Comi et al 2010; Comi et al 2008; 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 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 & 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 & 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 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 & 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 $CD4^+$ DC (Schulze-Topphoff et al 2012), potent APC for $CD4^+$ T-cell responses (Dudziak et al 2007). Laquinimod treatment also promoted 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 untreated 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 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 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 $CD1c^+$ DC, considered an equivalent of murine $CD8^-CD11b^+$ splenic DC. In addition, laquinimod-treated human monocyte-derived DC inhibited proliferation of $CD4^+$ 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 downregulated 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 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 production of CNS antigen-specific autoantibodies.

Through functional alterations induced in APC, laquinimod treatment modulated the differentiation and associated cytokine production of $CD4^+$ 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 $CD4^+$ 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-1α, MIP-1β 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 $CD1c^+$ 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 & 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 naïve 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 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-κB 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, results were briefly discussed and summarized by Polman and colleagues (Polman et al 2005). Those Phase I investigations provided safety and pharmacokinetic data that guided 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 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 detection of enhancing lesions. 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 (±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 numbers 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 ± 0.14 vs 2.12 ± 0.22 and 5.03 ± 0.08 vs 7.14 ± 0.07, respectively). Further, laquinimod-treated patients showed reduced brain atrophy, observed in both white and grey 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 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-β1a (Avonex®). RRMS patients (n = 1331) were randomized 1:1:1 to receive once-daily oral laquinimod (0.6 mg), placebo (once daily), or IFN-β1a (30 μg once weekly) (Vollmer et al 2014). 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 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 2011; Vollmer et al 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 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 (0.6mg and 1.2mg) or placebo in approximately 1,800 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, 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. 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.

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Table 1

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[#≠]	n.d.	n.d.	(Polman et al 2005)
	LAQ0.1 mg[#]	68/65				6.4±9.7 [−32%] n.s.			
	LAQ 0.3 mg[#]	74/69				5.2±9.9 [−44%] p = 0.0498			
Phase IIb	Placebo	102/91	Cumulative number of GdE lesions	0.77±1.25	n.d.	4.2±9.2[≈]	2.4±3.3[≈]	n.d.	(Comi et al 2008)
	LAQ 0.3 mg[≠]	98/92		0.76±1.02 n.s.		3.9±5.5 p > 0.1	2.5±3.2 p > 0.1		
	LAQ 0.6 mg[≠]	106/100		0.52±0.92 [−32%] p = 0.0978		2.6±5.3 [−40.4%[✧]] p = 0.0048	1.6±3.7 [−44%] p = 0.0013		
Phase III ALLEGRO	Placebo	556/427	Annualized relapse rate	0.39±0.03	15.7%[∞]	2.12±0.22[§]	7.14±0.07[§]	−1.30	(Comi et al 2012)
	LAQ 0.6 mg	550/437		0.30±0.02 [−23%] p = 0.002	11.1% [−36%] p = 0.01	1.33±0.14 [−37%] p < 0.001	5.03±0.08 [−30%] p < 0.001	−0.87 [−33%] p < 0.001	
Phase III BRAVO	Placebo	450/359	Annualized relapse rate	0.34±0.03	10%[¥]	2.34±0.25[§]	13.03±l.l[§]	−1.03	(Vollmer et al 2014)
	LAQ 0.6 mg	434/353		0.28±0.03 [−18%] p = 0.075	7% [−40.6%] p = 0.042	1.84+0.19 [−21.5%] p = 0.069	10.88±0.85 [−16.5%] p = 0.078	−0.75 [−28%] p < 0.001	
	IFNβ-la IM	447/378		0.26±0.02 [−26%] p = 0.007	8% [−28.3] p = 0.17	0.90±0.10 [−61%] p < 0.001	6.37±0.51 [−51%] p < 0.001	−1.14 [+11%] p = 0.14	

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;

[#] Per-protocol cohort;

[≠] Intention-to-treat cohort;

✧ Based on adjusted mean;

≈ Number of lesions per scan in the last four scans;

∝ Percentage of patients with confirmed expanded disability status scale (EDSS) progression at 3 months;

¥ Percentage of patients with confirmed EDSS progression at 6 months;

§ cumulative number of lesions at 12 and 24 months;

⌘ cumulative number of lesions at 24 weeks. p values were calculated in comparison with placebo group.