CLINICAL INVESTIGATION

Clinical and experimental data on the use of laquinimod for the treatment of multiple sclerosis

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Laquinimod is a synthetic quinoline 3-carboxamide derivative with immunomodulatory properties. Treatment with laquinimod ameliorated disease activity in experimental autoimmune encephalomyelitis, a mouse model for multiple sclerosis. In the past, an immunosuppressive mechanism of action was initially assumed. With increasing knowledge on its effects, the immunomodulatory and possibly also neuroprotective properties of laquinimod came into the focus. Data from a Phase IIb trial in relapsing–remitting multiple sclerosis suggests this compound has clinical efficacy on MRI parameters of inflammation. In the recently published Phase III ALLEGRO trial, laquinimod 0.6 mg/day showed a reduction of relapse rates and even more pronounced effects on disability progression compared with placebo. Data from the additional Phase III BRAVO trial are awaited with great interest. In all published studies, laquinimod was well tolerated and no severe side effects were reported. In summary, laquinimod has the potential to add to our therapeutic armamentarium as a new oral immunomodulator in multiple sclerosis.

Keywords: experimental autoimmune encephalomyelitis • immunomodulation • laquinimod • multiple sclerosis • oral • roquinimex

Multiple sclerosis (MS) is a chronic inflammatory disease of the CNS and is the most common cause of neurological disability in young adults. It is widely assumed that MS is an autoimmune disease where inflammatory infiltration leads to demyelination and axonal damage in the CNS [1]. A better understanding of the immunopathological processes of the disease led to new therapeutic approaches. Although new treatment options have entered the therapeutic stage in recent years, existing therapies are only partially effective and early treatment is still crucial to limit progression of disability. Approved immunomodulatory or immunosuppressive drugs for relapsing–remitting MS need parenteral application and include IFN β-1a (which is administered either subcutaneously [sc.] or intramuscularly [im.]), IFN β-1a and glatiramer acetate for baseline therapy (sc.), as well as mitoxantrone and natalizumab for escalation therapy (intravenous application). For over a decade, these injectable disease-modifying drugs have dominated the treatment of MS. Here, patient compliance is a major concern for physicians treating MS. With licensing of fingolimod – an orally available immunodulator – for the treatment of MS, a new era of therapy has been initiated. At present, more than 100 therapeutic studies in MS are ongoing and a large proportion of these include formulations that are enterally incorporated. Indeed, up to 22% of the general population suffer from a needle phobia, which may prevent self-injections as treatment for MS. In relapsing–remitting MS patients, rates of treatment interruption range between 6 to 13% within the first 6 months [2–5]. A long-term follow-up study over 4 years reported that up to 46% of parenteral immunomodulatory therapies

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were at least temporarily discontinued [6]. While oral compounds are not generally characterized by better adherence rates compared with sc. and im. therapy, many patients with a fear or weariness of injections would prefer the use of oral drugs and thus in recent years drug companies have increased their effort to design new orally available compounds.

With increasing knowledge on the cellular and molecular pathomechanisms of MS, the focus in the development of new therapeutic compounds has shifted from unselective immunosuppression towards more selective, target-specific treatments [7]. There is still an unmet need for new high-efficacy drugs with good safety and tolerability profiles. Meanwhile, a new generation of MS therapies is emerging, many of which are in or have just finished Phase III trials, including laquinimod.

Pharmacologic & pharmacokinetic properties of the compound

Laquinimod (internal compound abbreviation ABR-215062, correct IUPAC name: 5-chloro-*N*-ethyl-1,2-dihydro-4-hydroxy-1-methyl-2-oxo-*N*-phenyl-3-quinolinecarboxamide) has a molecular weight of 357 Da. It is a synthetic compound that was synthesized at Active Biotech Research AB and licensed to TEVA Pharmaceutical Industries Ltd in 2004 as an immunomodulatory agent for studies in the treatment of relapsing–remitting MS [8]. Efficacy had already been demonstrated in many animal models of autoimmune diseases, including Guillain–Barré–Strohl syndrome, rheumatoid arthritis, systemic lupus erythematosus, autoimmune colitis and MS [9–13]. Thus, laquinimod seems to affect crucial common inflammatory pathways in autoimmunity. The efficacy of laquinimod is believed to be at least partially based on a shift of the T-helper (Th) cell balance from a 'Th1' reaction, with secretion of proinflammatory cytokines (e.g., IL-2, IL-6, TNF-α or IFN-γ), towards a 'Th2' response, with an increase in regulatory cytokines (e.g., IL-4 and IL-10) [14]. So far, the definite mechanism of laquinimod contributing to these immunomodulatory effects is only partially elucidated.

First studies on the pharmacokinetic characteristics of laquinimod were performed in preclinical trials in rats, mice, rabbits and dogs [15]. Laquinimod is characterized by a high oral bioavailability, small volume of distribution and low total clearance rate, and thus reaches its highest plasma concentration within 1 h after oral administration. Laquinimod is present in the CNS at 10–13% relative to blood levels. In humans, the maximum serum level (C_{max}) of laquinimod is below 5 µM after administration of 0.05–2.4 mg of the drug. Little fluctuation is observed between the minimum serum level (C_{min}) and C_{max} once steady state is reached. Laquinimod is metabolized by CYP3A4 in liver microsomes. After metabolization, four hydroxylated and two alkylated products are metabolically inactive and exuded primarily through the urine. Less than 5% of laquinimod is eliminated unchanged in urine and faces. Strong specific inhibitors of CYP3A4 enzymes such as ketoconazole and nutritives, such as grapefruit juice, can interfere with the elimination of laquinimod [16]. However, laquinimod is only a low-affinity substrate of CYP3A4, which reduces the risk of competitive inhibition of other substrates. Consequently, other CYP enzyme inhibitors including steroids and erythromycin only showed inhibitory effects *in vitro*. *In vivo*, erythromycin and steroids do not reach relevant plasma concentrations to induce CYP3A4 for the drug–drug interaction [15]. With the current dosing protocol of laquinimod, the serum plasma level, which is necessary to competitively inhibit another common substrate, etinyl estradiol (an important compound in contraceptives), is 30-times above the expected C_{max}.

Studies on laquinimod in experimental MS models

Experimental autoimmune encephalomyelitis (EAE) is a rodent model that reflects many aspects of MS [17]. This autoimmune disease can be elicited in susceptible mouse or rat strains by active immunization with myelin antigen or the adoptive transfer of encephalitogenic T cells and shares many histopathological and clinical features with MS [18]. Primary studies in EAE were carried out with roquinimex (Linomide®), the historical precursor compound of laquinimod. Roquinimex displayed its efficacy in a series of animal models for different autoimmune diseases, including experimental autoimmune neuritis (EAN) [19–23]. Roquinimex treatment also resulted in an ameliorated disease course in both acute and chronic EAE models in SJL mice as well as in outbred rats [24,25]. Roquinimex induced the expression of Th2 cytokines, which may play a role in modulation of T-cell autoimmunity [13]. Moreover, roquinimex had been evaluated in several clinical trials with relapsing–remitting and secondary progressive MS patients and demonstrated disease modifying effects with a reduction of MRI activity as a primary measure of disease activity in a clinical Phase II trial [26]. However, this trial had to be stopped due to severe side effects, including pleuritis and adverse cardiac events, such as pericarditis and myocardial infarction, which even lead to some fatalities [26,27].

As a consequence, laquinimod was developed as a derivative of roquinimex without these severe side

effects. While laquinimod is structurally related to its precursor compound, it is pharmacologically and structurally distinct. Laquinimod has been shown to inhibit acute EAE in the Lewis rat via a reduction of inflammatory infiltration [28]. It is also effective in murine EAE in a preventive, as well as therapeutic, setting [29]. In this study in mice, laquinimod was approximately 20-times more potent than the immunomodulator roquinimex, with its efficacy clearly being strain dependent. Furthermore, beneficial effects were reported in various other experimental autoimmune inflammatory disease models, including EAN in the Lewis rats. Daily sc. administration of laquinimod reduced the incidence of EAN dose dependently and improved clinical signs of the disease. Moreover, laquinimod inhibited antigen-specific T-cell responses towards the peripheral myelin protein epitope P0 peptide 180–199. Histological analyses of peripheral nerves displayed less demyelination and inflammation after laquinimod treatment [14]. The analysis of cytokine profiles revealed a Th1 to Th2/Th3 cytokine shift including an increased expression of IL-4, IL-10 and TGF-β, while more recent studies in healthy humans also point at effects on the proinflammatory 'Th17' cytokine IL-17 [28]. Effects on antigen presentation may also be mediated via the NF-κB pathway [30]. In EAE, the effect of laquinimod has been shown to be independent of endogenous IFN-β [31]. Moreover, preventive and therapeutic treatment with laquinimod reduced inflammation, clinical signs and demyelination in myelin oligodendrocyte glycoprotein (MOG)-induced EAE via interaction with the migratory capacity of T cells. In this study, laquinimod treatment resulted in a reduction of VLA-4 mediated adhesiveness and proinflammatory cytokines, such as IL-17. Within lesions, treated animals presented similar axonal densities, but less acute axonal damage in comparison with control animals [32]. In a very recent study on sera from patients participating in a laquinimod Phase II trial over 9 months (LAQ/5062), laquinimod treatment led to an increase in expression of the neurotrophic factor BDNF of up to 11-times compared with placebo, thus indicating a possible additive neuroprotective mechanism of action [32–34]. To date, the exact mechanism of laquinimod action in MS patients is still unclear. Some evidence from EAE models points to effects not only on T cells, but also on monocytes, which was recently well reproduced [32,35].

Clinical studies

To date, eight Phase I studies with healthy volunteers and MS patients have been completed. In healthy individuals, laquinimod at dosages of 0.1–1.2 mg/day was well tolerated. After 1–2 weeks of treatment with 2.4 mg/day, an elevation of inflammatory markers was observed in some individuals. The first proof-of-concept study in MS patients was performed in more than 20 centers in the Netherlands, Russia, Sweden and the UK. This multicenter, double-blinded, placebo-controlled, parallel-group Phase II trial investigated the effects of laquinimod at dosages of 0.1 and 0.3 mg/day versus placebo over 24 weeks. The study encompassed 209 patients with relapsing–remitting MS or 'secondary-progressive' MS with an expanded disability status scale of 0–5.5 and evidence of disease activity on cerebral MRI (cMRI) or clinical relapses in the previous 1–2 years.

The primary outcome measure was the cumulative number of gadolinium (Gd)-enhancing lesions (indicating acute inflammation) on a cMRI at week 24. At the end of the study, Gd-enhancing lesions were significantly reduced (by 44%) in the 0.3-mg/day group compared with the placebo group, whereas the difference between the 0.1-mg group and placebo was not significant [36]. Other secondary outcome measures, including the number and volume of Gd-enhancing lesions, as well as the relapse rate, displayed a positive trend towards the 0.3 mg/day group, yet this study was not powered to detect further statistically significant differences. cMRI scans obtained 8 weeks after discontinuation of the study medication revealed an increase in disease activity in both treatment groups, which might be interpreted as a trend suggestive of a rebound effect.

In a further Phase II trial, a multicenter, double-blinded, placebo-controlled, parallel-group study funded by TEVA Pharmaceuticals Industries Ltd investigated the effects of laquinimod in the treatment of relapsing–remitting MS (LAQ/5062) [37]. The study enrolled 306 patients in two treatment arms (0.3 and 0.6 mg/day) and a placebo arm. The primary outcome measure was the cumulative number of Gd-enhancing lesions in the last four cMRI scans adjusted to the baseline scan during treatment (weeks 24, 28, 32 and 36). In comparison with the placebo group, treatment with 0.6 mg laquinimod resulted in a significant reduction of over 40% in mean cumulative number of Gd-enhancing lesions per scan. Significant beneficial effects in the 0.6-mg/day group were also observed for almost all secondary study measures and exploratory outcomes. The cumulative number of new lesions on T2-weighted images and the cumulative number of new hypointense T1 lesions were significantly reduced by 44 and 51%, respectively, compared with placebo. By contrast, effects on the annualized relapse rate in subjects treated with 0.6 mg were not significant, yet the study was not powered for this end point. Primary or secondary outcome variables in the 0.3-mg/day group were

not significantly different to the placebo group. Polman and colleagues reported statistically significant effects on contrast-enhancing lesions for the 0.3-mg/day dose when a triple dose of Gd was used, which increases the sensitivity for the detection of Gd-enhancing lesions in MRI [36]. Importantly, safety in the Phase II trials was very good and no signs of irreversible side effects were observed. A total of 95% of the subjects completed the Phase II trials and proinflammatory side effects, such as serositis, thrombophlebitis, myocardial infarction or pulmonary embolism, as with the precursor roquinimex were observed. In the study, a dose-dependent elevation of liver enzymes was observed, which was reversible in all cases. While one case of Budd–Chiari syndrome occurred in a patient with an initially unidentified heterozygous Leiden V mutation after 1 month of laquinmod exposure, a general increase in thrombotic events has not yet been found until now.

Subjects enrolled in the LAQ/5062 trial were offered to continue the study in an open-label extension for a further 36 weeks. In this trial, the placebo group was re-randomized to laquinimod at 0.3 or 0.6 mg/day and there was a significant reduction in the mean number of Gd-enhancing lesions in subjects switching from placebo to verum. In patients already enrolled in one of the verum arms, the effect of laquinimod on Gd-enhancing lesions observed in the first phase of the study was maintained during the extension phase [38]. Moreover, patients initially enrolled into the placebo-arm and then consecutively switched to one of the verum arms (0.3- or 0.6-mg laquinimod) during the extension phase, showed similar proportions of inactive scans as in patients initially enrolled in verum arms of the LAQ/5062 study.

In view of the results from the LAQ/5062 trial, the sponsor decided to pursue the 0.6-mg/day dosage in further studies. Thus, two pivotal Phase III trials were designed for the definite assessment of laquinimod, with the aim of licensing the compound for the treatment of relapsing–remitting MS [39]. The ALLEGRO study is a 2-year, multicenter, double-blinded, placebo-controlled Phase III trial to evaluate the efficacy, safety and tolerability of laquinimod at 0.6 mg/day in relapsing–remitting MS. Enrollment was already completed in November 2008 and the study is currently in its extension phase. The primary outcome measure was the number of confirmed relapses during the study period. The secondary outcome measures consisted of time to sustained expanded disability status scale progression and MRI outcome measures at 12 and 24 months. The ALLEGRO trial was completed in December 2010 and results were recently published in March 2012. In this study, a significant reduction of the annualized relapse rate has been shown: 62.9% of the patients in the laquinimod-treated group were relapse-free in comparison with 52.2% in the placebo group. Interestingly, the significant effects on disability progression were even more pronounced (11.1 vs 15.7% reduction in the risk of confirmed disability progression). Consistent with the clinical efficacy of laquinimod, MRI analysis also demonstrated a significantly reduced inflammatory disease activity. The mean cumulative numbers of Gd-enhancing lesions and new or enlarging T2 lesions were significantly reduced in patients receiving laquinimod. Most importantly, laquinimod was very well tolerated and there were no concerns related to neoplasia or opportunistic infections. In some cases, elevated liver enzymes were reported; however, these elevations were only transient and resolved after discontinuation [39].

A second Phase III trial, named BRAVO, is a 2-year, multinational, multicenter, double-blinded, parallel-group and placebo-controlled study that was also initiated in 2008. This trial was designed to compare the efficacy and safety of oral treatment with laquinimod at 0.6 mg/day to a placebo group in patients with relapsing–remitting MS. The study also included a reference arm with im. IFN β-1a. Enrollment of patients at 154 sites in the USA, Europe, Israel and South Africa was completed in June 2009. The primary outcome measure is relapse rate over 24 months of treatment with accumulation of disability being another variable. After finalization of the BRAVO study, publication of the data is now awaited with great interest.

In addition to more clinical studies in MS at higher dosages, laquinimod is also under evaluation in further Phase II trials for Crohn's disease and systemic lupus erythematosus.

Future perspective

Despite the progress in pharmacotherapy of MS in the last two decades, there is still an unmet need for effective and at the same time safe new treatment options

Executive summary

- Efficacy of laquinimod has been shown in many animal models of autoimmune diseases.
- Laquinimod is a possible oral treatment option for multiple sclerosis and has demonstrated its efficacy in clinical Phase IIb and III trials. At present, publication of data from two Phase III trials are awaited with great interest.
- In all published studies, laquinimod was well tolerated and no severe side effects were reported.

to better serve our MS patients. With its beneficial immunomodulatory mechanism of action and very good safety profile, laquinimod has the potential to become a new oral treatment option, as an alternative to today's injectable 'disease-modifying' drugs. To date, the positioning of laquinimod among the existing immomodulatory compounds and other future oral immunomodulators is still unclear. Importantly, laquinimod at 0.6 mg has already displayed a very good safety and tolerability profile in the Phase IIb and in a Phase III trial. Further data on its efficacy are awaited with great interest.

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The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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■■ **Most recently published data.**