REVIEW ARTICLE

Magnetic Resonance Imaging Evidence Supporting the Efficacy of Cladribine Tablets in the Treatment of Relapsing-Remitting Multiple Sclerosis

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Abstract

Numerous therapies are currently available to modify the disease course of multiple sclerosis (MS). Magnetic resonance imaging (MRI) plays a pivotal role in assessing treatment response by providing insights into disease activity and clinical progression. Integrating MRI findings with clinical and laboratory data enables a comprehensive assessment of the disease course. Among available MS treatments, cladribine is emerging as a promising option due to its role as a selective immune reconstitution therapy, with a notable impact on B cells and a lesser effect on T cells. This work emphasizes the assessment of MRI's contribution to MS treatment, particularly focusing on the influence of cladribine tablets on imaging outcomes, encompassing data from pivotal and real-world studies. The evidence highlights that cladribine, compared with placebo, not only exhibits a reduction in inflammatory imaging markers, such as T1-Gd+, T2 and combined unique active (CUA) lesions, but also mitigates the effect on brain volume loss, particularly within grey matter. Importantly, cladribine reveals early action by reducing CUA lesions within the first months of treatment, regardless of a patient's initial conditions. The selective mechanism of action, and sustained efficacy beyond year 2, combined with its early onset of action, collectively position cladribine tablets as a pivotal component in the therapeutic paradigm for MS. Overall, MRI, along with clinical measures, has played a substantial role in showcasing the effectiveness of cladribine in addressing both the inflammatory and neurodegenerative aspects of MS.

1 Introduction

Multiple sclerosis (MS) is an immune-mediated chronic inflammatory and neurodegenerative disease of the central nervous system (CNS) characterized by demyelination and axonal damage [1]. MS affects a total of 2.8 million people

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Key Points

Treatment with cladribine tablets effectively lowers the levels of inflammatory imaging markers, including T1-Gd+ lesions, new or enlarging T2 lesions, and combined unique active lesions.

Cladribine tablets rapidly reduce magnetic resonance imaging activity, regardless of the patient's disease status or treatment history.

Treatment with cladribine tablets significantly reduces brain volume loss, particularly grey matter loss, compared with placebo.

worldwide, making it the most common cause of disability in young adults [2]. The precise etiology of MS still requires clarification, but it appears to be multifactorial. Indeed, the interplay between genetic factors, including

genes associated with immune system function, and environmental factors, such as obesity, vitamin D deficiency and infectious agents such as Epstein–Barr virus [3], is thought to play a key role in MS occurrence [4]. In addition to the complex etiology, diagnosis and treatment of MS are also complicated by the heterogeneity of symptoms, which may include sensory and motor disturbances, vision impairment and gait disorders, as well as cognitive deficits [1].

Pharmacological approaches for treating MS include platform therapies such as injectables and oral therapies, and pulsed therapies including biologicals and oral agents that work through immune reconstitution and provide long periods of drug-free disease control [5–9]. Among immune reconstitution therapies (IRT), cladribine tablets constituted the first oral therapy with an infrequent dosing schedule. Cladribine tablets are administered at a cumulative dose of 3.5 mg/kg body weight over the first 2 years (1.75 mg/kg/ year), followed by a drug-free interval in years 3 and 4 [10].

Cladribine is a synthetic chlorinated deoxyadenosine analog that is incorporated into the DNA of dividing cells, causing DNA damage and subsequent cell death through apoptosis. It is biologically active in selected cell types characterized by a high intracellular ratio of deoxycytidine kinase to 5′-nucleotidases [11]. Due to this enzymatic configuration, cladribine tablets cause selective depletion of lymphocytes, with a less pronounced effect on T cells and a large reduction of B cells [12, 13]. Subsequently, naïve B cells recover rapidly toward baseline levels, while memory B cells remain reduced until month 24 [13]. These dynamics of cellular depletion and repopulation explain the early onset of action and sustained efficacy over time of cladribine tablets [14, 15], as well as the maintenance of immune competence. Instead, only minimal effects are observed on the innate immune system [16, 17].

In terms of efficacy, direct comparison studies between cladribine tablets and other disease-modifying therapies (DMTs) have not been carried out. However, using data from randomized controlled trials (RCTs), propensity score-matching analyses showed the superiority of cladribine tablets in reducing relapse rates compared with several platform and second-line therapies [18, 19]. Regarding the control of disability assessed by the Expanded Disability Status Scale (EDSS), a recent prospective study showed comparable efficacy between cladribine tablets and ocrelizumab [20]. However, in all comparative analyses to date, magnetic resonance imaging (MRI) measurements were not reported.

Monitoring response to cladribine tablets and other DMTs is important and should include sensitive imaging readouts, in addition to clinical signs of inflammatory activity and measures of disability worsening [21–23]. In this regard, recent progresses in MRI have improved the diagnosis and monitoring of CNS damage, enabling better assessment of clinical and subclinical disease activity, progression, and response to therapy [24]. A range of MRI techniques are available, and the choice must be tailored to the individual patient and available resources. The most commonly used MRI sequences are T1-weighted, with or without gadolinium enhancement (T1-Gd), and T2-weighted with fluid-attenuated inversion recovery (FLAIR) [25]. In particular, T1-Gd imaging detects new or recently active lesions and is indicative of blood–brain barrier breakdown. T2-weighted imaging can reveal new or enlarging lesions, and its sensitivity is improved by using the FLAIR sequence, which suppresses the T2-hyperintense signal from cerebrospinal fluid [25, 26]. Evaluation of these lesion-related MRI markers provides objective measures of inflammatory status and correlates with clinical outcomes, such as relapses [27]. By contrast, to assess the diffuse brain damage occurring in MS, potentially due to neurodegeneration, it is essential to include measures of atrophy alongside evaluating focal brain lesions, as these measures are closely associated with the accumulation of disability [28].

To our knowledge, the impact of cladribine tablets treatment on MRI measures has not been systematically reviewed. While previous published manuscripts provided a general overview of the clinical efficacy data of cladribine tablets, including MRI findings [29, 30], our review focuses specifically on analyzing data from clinical trials and real-world studies to highlight the effect of cladribine tablets on MRI outcomes in relapsing-remitting (RR)-MS patients.

2 Literature Search Methodology

A comprehensive literature search was conducted in the PubMed database for studies published between 2010 and September 2023 on RRMS patients, specifically focusing on the effect of cladribine tablets treatment on MRI outcomes. General search terms such as 'cladribine tablets', 'cladribine tablets and MRI', and 'multiple sclerosis' were used. Additionally, posters presented at the most recent international congresses were included. Case reports were excluded from the review. In terms of real-world analyses, only studies reporting MRI data were considered for inclusion.

3 Magnetic Resonance Imaging (MRI) Evidence of the Efficacy of Cladribine Tablets from the Randomized, Double-Blind, Controlled, Phase III CLARITY Study

The efficacy and safety of cladribine tablets have been investigated in two large, phase III, randomized, double-blind, placebo-controlled studies, specifically (1) the CLARITY

and CLARITY Extension studies, performed on patients with RRMS, and (2) the ORACLE-MS study, conducted in patients with early MS who were at high risk of converting to clinically definite MS (CDMS) [31–33].

In the CLARITY study, 1326 adults with stable RRMS were randomized to receive cladribine tablets with a cumulative dose of either 3.5 or 5.25 mg/kg over 2 years, or placebo. Patients who received cladribine tablets had significantly fewer relapses, less disability worsening and better MRI outcomes, compared with patients who received placebo [31]. As assessed by MRI, the short-course treatment with cladribine resulted in the suppression of active inflammatory lesions. Specifically, a significant relative reduction in T1-Gd+ lesions, new or enlarging T2 lesions, and combined unique active (CUA) lesions of 85.7%, 73.4%, and 74.4%, respectively, was found [31]. Similar findings were observed for both the cladribine tablets dose groups versus placebo [31] (Table 1).

In the landscape of highly effective DMTs, achieving the disease activity-free state or no evidence of disease activity (NEDA), defined by (1) no relapse, (2) no change in EDSS score, and (3) no new MRI lesions, represents one of the key therapeutic goals in MS therapy. Considering this, a post-hoc analysis of data from the CLARITY study assessed the effects of cladribine on this composite outcome. A significant difference in freedom from disease activity between cladribine and placebo emerged as early as 24 weeks from treatment start, and more than 80% of cladribine-treated patients remained free from disease activity at 48 weeks and up to 96 weeks [34]. The efficacy of cladribine tablets observed from 24 weeks and persisting up to 96 weeks was also found specifically across all MRI activity outcomes (T1-Gd+, active T2, and CUA lesions), as demonstrated in a post-hoc analysis conducted by Comi et al. [35]. The analysis revealed a significant increase in patients free from T1-Gd+ lesions at 96 weeks in the active arms, with percentages reaching 87.2% and 91.4% in the 3.5 and 5.25 mg/kg groups, respectively, compared with a slight increase in the placebo arm [35] (Table 1). Additionally, focusing on a more stringent definition of MRI activity, indicated by the measurement of CUA lesions, approximately 60% of patients taking cladribine tablets remained free from new lesions during the 2 years of treatment, a rate more than double that achieved in the placebo arm, indicating a favorable response in a significant proportion of treated patients [35] (Table 1).

The timeline of treatment effects on MRI measures paralleled a similar pattern in clinical outcomes. Notably, the annualized relapse rate was reduced as early as 4 weeks and becomes significant at 24 weeks, indicating an early onset of cladribine effect that persists throughout the 96-week study [31, 35]. This substantial reduction in inflammatory lesions over 96 weeks aligns with the established mechanism of action of cladribine tablets, which causes a prompt and sustained reduction in lymphocytes [11].

The favorable effects of cladribine tablets on clinical and MRI responses were also sustained in a CLARITY subgroup of patients with high disease activity (HDA), defined as (1) patients with two or more relapses during the year prior to study entry, regardless of treatment status, or (2) patients who, during the year prior to study entry, experienced one or more relapses and one or more T1-Gd+ lesions or nine or more T2 lesions while receiving other DMTs [36]. This subgroup of patients was examined in a subsequent post-hoc analysis showing that cladribine was equally effective in both treatment-naïve and previously treated patients (Table 1) [37].

4 CLARITY EXTENSION Study: The Efficacy of Cladribine Tablets Persists Beyond the Period of Active Treatment

Patients who completed the CLARITY study were eligible to enter the CLARITY Extension study, which investigated the long-term safety, tolerability, and efficacy of a second cladribine tablets treatment regimen, or placebo [33]. After a median treatment gap of 40.3 weeks, patients who had received either dose of cladribine tablets in CLARITY were randomly assigned to receive either cladribine tablets 3.5 mg/kg (CC group) or placebo (CP group) in the CLARITY Extension. All patients who had received placebo in CLARITY were assigned to receive cladribine tablets 3.5 mg/kg (PC group) in CLARITY Extension to compare the effect of early versus late treatment.

The findings from the extension of CLARITY demonstrated that the clinical benefits of cladribine tablets resulted in a durable clinical response, providing a 'treatment-free' period for at least 2 additional years without the need for further treatments [33]. Indeed, no incremental benefit emerged from additional courses [33]. This long-lasting effect of cladribine tablets is also maintained on MRI outcomes, with the majority of patients in each treatment group remaining free from T1-Gd+ lesions, even without retreatment after 2 years [38]. Yet, evidence of MRI activity has been identified in a small subset of patients treated with cladribine in CLARITY, who later received a placebo in the Extension phase. This MRI activity was also associated with a prolonged treatment gap between CLARITY and its Extension [38].

In addition to the long-lasting effectiveness, the Extension study showed that no cases of clinical or MRI rebound were recorded in patients who received placebo in the Extension study [38]. This is an important aspect because the patient is not vulnerable to disease reactivation if treatment must

Table 1 Summary of MRI results from the phase III CLARITY study

Study	Outcomes	Lesion type/parameter	Placebo	CladT 3.5	CladT 5.25
CLARITY [31]	Absolute number of lesions and (relative reduction vs. placebo %) Placebo = 437 CladT 3.5 = 433 CladT 5.25 = 456	T1 Gd+	0.91	0.12 (85.7)	0.11 (87.9)
		Active T2	1.43	0.38 (73.4)	0.33 (76.9)
		Combined unique	1.72	0.43 (74.4)	0.38 (77.9)
CLARITY [34] Post-hoc analysis	Percentage and (number of patients free of indicated lesions), % (n) Placebo = 424 CladT 3.5 = 422 CladT 5.25 = 443	T1 Gd+	47.4 (201)	87.2 (368)	91.4 (405)
		Active T2	27.6 (117)	61.8 (261)	62.8 (278)
		T1 Gd+ and active T2 lesion	25.5 (108)	60.0 (253)	61.2 (271)
CLARITY [35] MRI outcomes	Relative reduction vs. placebo (lesion/patient/ scan, %) CladT 3.5 = 433 CladT 5.25 = 456	T1 Gd+		85.7	87.9
		Active T2		73.4	76.9
		Combined unique		74.4	77.9
		T1 hypointense		2.9	8.2
		Change from baseline in T2 lesion volume, mL		24.0	41.2
CLARITY [37] Post-hoc analysis	Mean lesion/scan in HRA+DTA patients (n)[a]	New T1 Gd+ DMT-naïve	1.19	0.13	
		New T1 Gd+ Prior-DMT	1.28	0.09	
		Active T2 DMT-naïve	1.84	0.40	
		Active T2 Prior-DMT	1.56	0.38	
		Combined unique DMT-naïve	2.24	0.44	
		Combined unique Prior-DMT	2.07	0.46	
		New T1 hypointense DMT-naïve	0.70	0.15	
		New T1 hypointense Prior-DMT	0.58	0.07	

CladT 3.5 cladribine tablets 3.5 mg/kg, *CladT 5.25* cladribine tablets 5.25 mg/kg, *DMT* disease-modifying treatment, *Gd+* gadolinium-enhancing, *HRA+DAT* patients with high relapse activity (two or more relapses during the year prior to study entry) regardless of DMT status *PLUS* patients with one or more relapses during the year prior to study entry while receiving other DMTs, *AND* (one or more T1-Gd+ lesions *OR* nine or more T2 lesions), *MRI* magnetic resonance imaging

[a] Number of patients for each group: DMT-naïve, placebo = 93; DMT-naïve, CladT 3.5 = 94; prior DMT, placebo = 56; prior DMT, CladT 3.5 = 46

be interrupted, mainly due to the mechanism of action of the molecule.

5 ORACLE-MS Study: Evidence on the Efficacy of Cladribine Tablets in Patients with Early Multiple Sclerosis

The 2-year ORACLE-MS study was a double-blind, randomized, placebo-controlled, multicenter, phase III trial that investigated the impact of cladribine tablets on the conversion to CDMS in patients showing early signs of the disease after a first demyelinating event.

Patients were randomly assigned to receive cladribine tablets at 3.5 mg/kg, 5.25 mg/kg, or placebo [32]. Cladribine

tablets significantly delayed conversion to clinically defined MS according to Poser et al. [39] and the 2005 McDonald criteria [40] at both doses compared with placebo. Notably, the diagnostic criteria have changed since the study began. Using the updated 2017 McDonald criteria on the ORACLE-MS group is expected to reveal a greater number of patients with RRMS.

MRI data indicated that cladribine tablets 3.5 mg/kg resulted in lowering median numbers of new or persisting T1-Gd+ lesions, new or enlarging T2 lesions, and CUA lesions compared with placebo [32]. This aligns with findings in the CLARITY study [31], supporting the notion that oral cladribine tablets were able to mitigate neuroinflammation during the early phase of the disease. A post-hoc analysis of ORACLE-MS data demonstrated a trend towards

reduced T1-Gd+ lesions as early as week 13 with cladribine tablets 3.5 mg/kg compared with placebo [41]. These early clinical improvements induced by cladribine tablets in the CLARITY and ORACLE-MS studies correspond substantially with the rapid reduction of B cells (CD19+) and other lymphocyte subsets implicated in MS pathology and observed soon after treatment initiation [17].

Subgroup analysis where patients were stratified based on age, baseline clinical characteristics, and presence of lesions showed that cladribine tablets reduced the risk of MS conversion across all subgroups compared with placebo [42]. Notably, no discernible subgroups were identified exhibiting a heightened treatment benefit compared with others. Overall, this declined rate of MS conversion in patients treated with cladribine tablets aligned with the observed reduction in cumulative numbers of T1-Gd+, new/enlarging T2, and CUA lesions, independently of clinical characteristics, including baseline lesion burden [42]. Collectively, these results provide support for the early use of cladribine tablets to mitigate later disease worsening. This is further demonstrated by several studies of real-word cohorts showing that early use of cladribine tablets can change the disease course of MS [43–46], also considering its mechanism of action [29].

6 Effect of Cladribine Tablets on Brain Volume Loss

Brain volume loss (BVL) occurs at a faster rate in patients with MS compared with age-matched healthy individuals, and this phenomenon begins early in the course of the disease [47]. BVL reflects irreversible tissue damage and neurodegeneration, and thus it is closely associated with and serves as a predictor of disability worsening [47, 48]. The rate of brain atrophy in MS can be influenced by treatment, and BVL has been included in the composite outcome NEDA-4, with the aim of improving treatment strategies and patient outcomes [49]; however, its application to routine clinical monitoring can be hindered by the presence of lesions and technical issues in some settings [50, 51]. Despite these limitations, the importance of including atrophy measures in clinical trials to monitor MS worsening has been increasingly recognized.

In an exploratory analysis of data from the CLARITY study, cladribine tablets decreased whole-brain atrophy compared with placebo, and this was associated with a lower risk of disability worsening after 2 years of follow-up [52]. Brain volume was measured using the Structural Image Evaluation Normalization of Atrophy (SIENA) software [53] on T1-weighted MRI scans acquired between months 6 and 24, to avoid the confounding effect of pseudoatrophy in the first 6 months of treatment. Patients with complete datasets ($n = 1025$) had received cladribine tablets 3.5 mg/kg ($n = 336$), cladribine tablets 5.25 mg/kg ($n = 351$), or placebo ($n = 338$). From months 6–24, mean percentage brain volume change and annualized mean percentage brain volume change were significantly reduced in patients treated with cladribine tablets 3.5 mg/kg by $-0.56 \pm 0.68\%$ ($p = 0.010$) and 5.25 mg/kg by $-0.57 \pm 0.72\%$ ($p = 0.019$), compared with those treated with placebo ($-0.70 \pm 0.79\%$) (Table 2) [52]. There was also a significant correlation between annualized BVL and cumulative probability of disability worsening (hazard ratio [HR] 0.67, 95% confidence interval [CI] 0.571–0.787; $p < 0.001$) [52]. These results showed that treatment with cladribine tablets was able to reduce diffuse brain tissue damage and, consequently, the neurodegeneration associated with clinical worsening of MS.

There is compelling evidence that both global and regional (mainly grey matter regions such as the cortex and thalamus) BVL is relevant in MS pathology and correlates closely with disability progression [47, 50]. For this reason, measures of brain atrophy are increasingly used as secondary or even primary endpoints in MS clinical trials targeting disease progression [48, 54–57].

In a recent study, we compared the percentage changes in grey and white matter volumes among patients treated with cladribine tablets at a dose of 3.5 mg/kg and those who received a placebo in the CLARITY study [58]. Using scans

Table 2 Change in brain volume from months 6–24 in the pivotal phase III CLARITY trial of cladribine tablets in patients with RMS (adapted from De Stefano et al. [52])

Endpoint	CladT 3.5 mg/kg [$n = 336$]	p vs. placebo	CladT 5.25 mg/kg [$n = 351$]	p vs. placebo	Placebo [$n = 338$]
Brain volume change, mean ± SD %	-0.77 ± 0.94	**0.02**	-0.77 ± 0.95	**0.02**	-0.95 ± 1.06
Annualized brain volume change, mean ± SD %	-0.56 ± 0.68	**0.01**	-0.57 ± 0.72	**0.02**	-0.70 ± 0.79

Significant p-values are highlighted in bold

CladT cladribine tablets, *RMS* relapsing multiple sclerosis, *SD* standard deviation

acquired at 0, 6, 12 and 24 months [59], volume changes over the initial 6 months were assessed to examine pseudoatrophy in both white and grey matter compartments, while changes between the 6- and 24-month time points were assessed to gauge the effects of cladribine tablets treatment on these same brain compartments. Notably, during the first 6 months of treatment, pseudoatrophy was observed in both grey and white matter, with a more pronounced impact on grey matter than white matter (Table 3) [58]. Between months 6 and 24, patients who received cladribine tablets had less grey matter volume loss compared with those who received placebo (− 0.90 vs. − 1.27; $p = 0.026$); however, white matter loss was not significantly different from placebo during this period (Table 3) [58]. Thus, in addition to previous data on whole-brain atrophy, these results highlight the beneficial effect of cladribine tablets in reducing the loss of grey matter, which is known to be impacted most in the MS course [60]. This effect on grey matter may help to explain recent data showing that treatment with cladribine tablets can stabilize cognitive function in both treatment-naïve and previously DMT-exposed patients [61]. In this regard, as part of the CLARIFY-MS study, no correlation between brain volume changes and various parameters of the Brief International Cognitive Assessment (BICAMS) test was found after 2 years of cladribine treatment [61]. This lack of correlation is most likely because the change in the entire brain volume, and not specific brain areas, is examined in this analysis. However, preliminary data showed that treatment with cladribine may allow for the prevention of additional atrophy in brain regions such as the corpus callosum, thalamus and brainstem [62, 63]. Along with these brain areas, it will be useful to extend the analysis of cladribine's protective effect on cortical atrophy, given its involvement in cognitive impairment in MS [64].

Further correlation analysis between MRI measures and cognitive tests or biomarkers (i.e., neurofilament and immunological cellular subsets) will be needed to identify markers predicting the individual treatment response to cladribine and monitoring disease progression.

7 MAGNIFY-MS Study: A Look at the Onset of Action of Cladribine Tablets by MRI Measurements

A rapid onset of efficacy is crucial to minimizing neurological damage and the accumulation of irreversible disability [65], regardless of treatment history. Clinical trials generally examine efficacy over a period of years, and little emerges from these studies about the rapidity of action of the various DMTs, particularly regarding MRI outcomes. This is also the case with cladribine tablets. The first follow-up scans in the CLARITY study were scheduled after 6 months of treatment, when they already showed a significant effect of cladribine tablets in the reduction of brain lesions, compared with placebo [31]. As reported above, an early effect of cladribine tablets was also observed in a post-hoc analysis of the ORACLE-MS study, where the first follow-up MRI was scheduled at week 13 [41]. Thus, the question of when the treatment effect of cladribine tablets starts remained unanswered.

The phase IV MAGNIFY-MS study assessed cladribine tablets' effects on MRI starting 1 month after the first administration. This 2-year, single-arm study characterizing the onset of cladribine tablets' activity in patients with highly active RRMS used early and frequent MRI monitoring [14]. The study population included 270 predominantly female (67%) adults (mean age 37.7 ± 9.75 years) treated with cladribine tablets 3.5 mg/kg. The outcome for the primary analysis was the change in CUA MRI lesions (i.e., T1-Gd+ lesions and new or enlarging active T2 lesions), comparing the pretreatment period (Period B: screening visit to baseline) with the first 6 months assessed in three overlapping study periods (Period 1: month 1 to month 6 visit; Period 2:

Table 3 Changes in grey matter and white matter volumes during the CLARITY study in patients with RMS receiving cladribine tablets 3.5 mg/kg or placebo (adapted from Cortese et al. [58])

Endpoint	0–6 months			6–24 months		
	CladT [$n = 267$]	Placebo [$n = 265$]	Change	CladT [$n = 336$]	Placebo [$n = 338$]	Change
Grey matter volume change, mean ± SD %	− 0.53 ± 0.10	− 0.25 ± 0.09	Δ52%, **$p = 0.045$**	− 0.90 ± 0.13	− 1.27 ± 0.14	Δ29%, **$p = 0.026$**
White matter volume change, mean ± SD %	− 0.49 ± 0.07	− 0.34 ± 0.07	Δ31%, $p = 0.137$	− 0.32 ± 0.16	− 0.40 ± 0.13	Δ21%, $p = 0.52$

Significant p values are highlighted in bold

CladT cladribine tablets, RMS, relapsing multiple sclerosis

month 2 to month 6 visit; and Period 3: month 3 to month 6 visit), analyzed in patients who had received one or more doses of cladribine tablets (Fig. 1a) [14].

MRI findings in this study identified the onset of the disease-modifying activity 30 days after initiating cladribine tablets, based on a reduction in highly sensitive T1-Gd+ lesions and CUA lesions. A reduction in active T2 lesions was detectable from the data acquired at 60 days. The response was more pronounced in subsequent months and sustained at month 6, the latest time point considered in the initial assessment [14]. Importantly, the early onset of action of cladribine tablets on CUA lesions was observed in patients irrespective of disease activity (patients with high relapse activity vs. non-high relapse activity), prior DMT treatment (naïve or previously DMT-exposed patients) or baseline CUA lesion count (Fig. 1b) [14].

The onset of cladribine tablets' effect on MRI corresponds to reported changes in peripheral blood lymphocyte subsets, including a rapid decrease in CD19+ B cells within the first 2 months of treatment [66, 67]. This was confirmed and refined in a MAGNIFY-MS substudy that, by analyzing immunological cell subpopulations, showed the large and long-term effect of cladribine tablets on the suppression of memory B-cell populations [12, 13].

The sustained action of cladribine tablets also extends to MRI outcomes. Indeed, the ability of cladribine tablets to reduce the lesion load was maintained during the 2-year follow-up, with the proportion of patients free of CUA lesions increasing from 47% at baseline to 86.2% by study end (24 months). Similar results were found for the proportion of patients free of T1-Gd+ or active T2 lesions over the same time frame [15].

These MRI findings were also supported by an annualized relapse rate of 0.11 (95% CI 0.09–0.15), with 71% of patients not experiencing a qualifying relapse during the study period [68].

Cladribine tablets were also able to regulate the serum neurofilament (sNfL) levels, a marker with a key role in both monitoring treatment response and capturing long-term disease outcomes [69, 70]. Data from the MAGNIFY-MS study and real-world cohorts showed a significant reduction of sNfL as early as 6 months after the initiation of treatment with cladribine tablets that was maintained for up to 2 years [13, 71, 72]. Beyond that, further analyses of MAGNIFY-MS data have investigated the association between changes in sNfL levels and MRI outcomes in patients stratified as (1) no, (2) residual, or (3) resolved CUA lesions. It was found that cladribine tablets significantly reduce neuroaxonal damage in patients with no or resolved CUA lesions after a 2-year treatment course, but not in those showing residual lesions [73]. These data also highlighted that sNfL

Fig. 1 Rapid action of cladribine tablets in reducing CUA lesions. **a** Periods used for analysis and MRI assessments. **b** Change in CUA lesion counts (mean ± standard deviation) in the indicated periods for subgroups of interest. Values are expressed as least squares means. *CUA* combined unique active lesions, *DMT* disease-modifying therapy, *HRA* high relapse activity, defined as two or more relapses in the previous year, *MRI* magnetic resonance imaging. Figure adapted from De Stefano et al. [14]
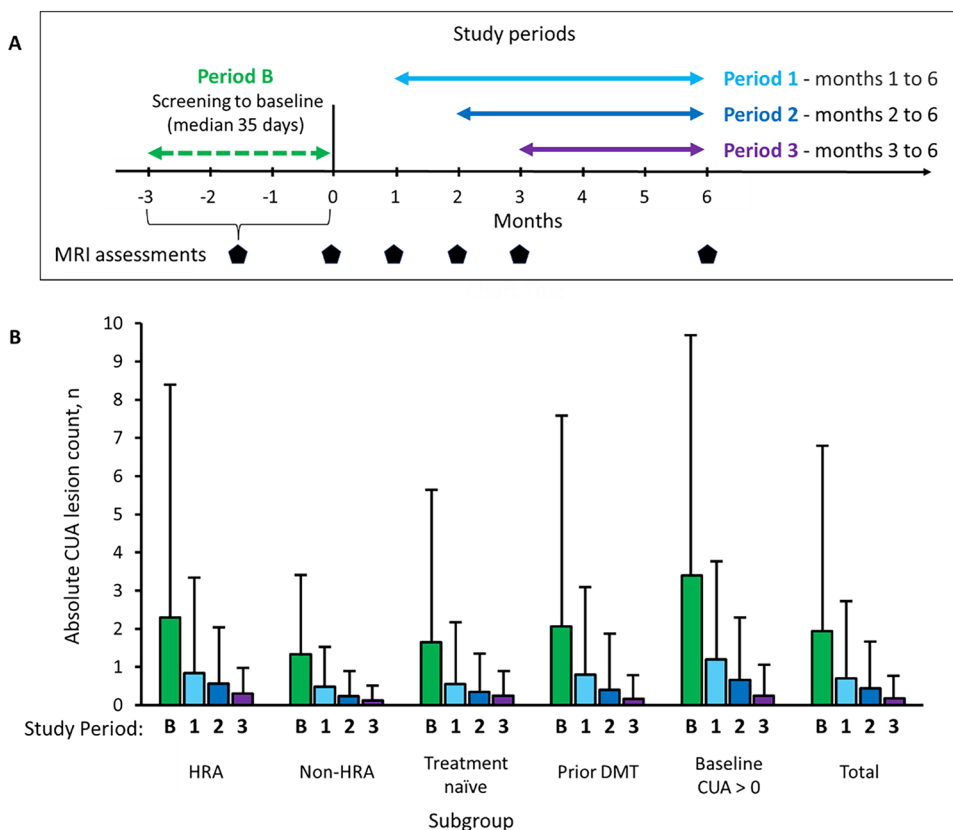
levels at baseline have limited potential as a biomarker for on-treatment MRI outcomes. Indeed, having high sNfL z-scores at baseline is not predictive of persistent activity at MRI after 2 years of treatment, measured by the evaluation of CUA lesions [73].

Overall, the results that emerged from the MAGNIFY-MS study corroborate and supersede those of pivotal studies [31–33]. In addition to showing an early onset of action, these findings underpin the long-term effect of cladribine tablets on MRI activity, without continuous immunosuppression in MS patients, distinguishing cladribine tablets from other high-efficacy DMTs.

8 Efficacy of Cladribine Tablets in the Real-World Setting

Randomized clinical trials are conducted in selected populations under controlled conditions that may not reflect routine clinical practice. Clinical trial enrolment is often restricted by age, EDSS score, disease activity and pretreatment, with a tendency to overrepresent treatment-naïve patients. Moreover, RCTs have short observation periods that fail to capture the durability of effects and long-term safety. On the contrary, real-world observational data of MS cohorts are more representative of routine clinical practice and provide valuable insight into long-term effectiveness and safety [74]. Several observational real-world cohort studies have assessed the efficacy of cladribine tablets in patients with relapsing forms of MS (RMS) using clinical and MRI assessments [44–46, 75–77].

An Italian real-world study examined a monocentric cohort consisting of 114 RRMS patients, of whom 50% were treatment-naïve. After completing 2 years of treatment with cladribine tablets, 90.9% of patients were free from clinical relapse, 96.2% showed no disability worsening, and 76.7% had no MRI activity, corresponding to achievement of NEDA-3 in 74.9% of patients [45] (Table 4). The authors defined a worsening of disability as an increase in EDSS equal to 1.5 points (if baseline EDSS = 0), 1.0 point (if baseline EDSS = 1.0–5.5) and 0.5 points (if baseline EDSS ≥ 6.0) at two independent clinical assessment 6 months apart. The highest percentage of NEDA-3 assessed in this study, compared for example with a Danish real-world cohort in which only 24% of patients achieved NEDA-3 at 24 months [76], may be due to the inclusion of patients with shorter disease duration and fewer previous DMTs in the Italian study [45], and may support the greater efficacy of cladribine tablets when placed early in the treatment algorithm.

However, some patients may not fully respond to treatment with cladribine tablets; therefore, identifying clinical or MRI markers that could predict treatment response would aid in selecting the most suitable patients for cladribine tablets treatment. In this context, Zanetta et al. also evaluated predictors of treatment response in an Italian cohort, reporting that a higher number of Gd+ lesions at baseline increased the risk of first clinical relapse and MRI activity

Table 4 Percentage of NEDA-3 in MS patients treated with cladribine tablets in real-world cohorts

Study	Patient population	% NEDA-3
Zanetta et al., 2023 [45]	114 RRMS patients: 57 naïve, 57 switch	74.9 % at 24 months: 90.9% of patients were free from clinical relapses 76.7% of patients had no MRI activity 96.2 % of patients had no disability worsening
Arena et al., 2023 [46]	217 RRMS patients: 50 naïve, 167 switch	35.5 % at 24 months: 88% of patients were free from clinical relapses 48% of patients had no MRI activity 80% of patients had no disability worsening
Petracca et al., 2022 [75]	243 RRMS patients: 71 naïve, 172 switch	64 % at 22 months: 88.5% of patients were free from clinical relapses 77.4% of patients had no MRI activity 87.3% of patients had no disability worsening
Pfeuffer et al., 2022 [44][a]	270 RMS patients: 97 naïve, 173 switch	74.4% of patients were free from clinical relapses 61.5% of patients had no new or enlarging T2 lesions[b] 75.9% of patients had no disability worsening

DMT disease-modifying treatment, Gd+, Gadolinium-enhancing; *MRI* magnetic resonance imaging, *MS* multiple sclerosis, *NEDA* no evidence of disease activity, *PIRA* progression independent of relapse activity, *RRMS* relapsing-remitting multiple sclerosis

[a]In the study by Pfeuffer et al. [44], Fig. 2d reports the proportion of patients with persistent NEDA-3, stratified according to previous treatment, whereas the value of NEDA-3 achieved by the entire cohort of patients, regardless of previous DMT, is not reported

[b]Only the proportion of patients without new or enlarging T2-hyperintense MRI lesions is reported; no data are reported on T1 Gd+ lesions. PIRA was evaluated in 21 patients (7.7%)

during follow-up, and was the only significant risk factor for the loss of NEDA-3 [45], highlighting the importance of MRI measures for predicting not only radiological trends but also the disease course.

A prospective Italian real-world cohort included 217 patients with RMS; 23% were treatment-naïve and 77% had switched from a previous DMT, of which 74.9% were moderately active treatments and 23.4% were highly active treatments [46]. After the second year of treatment, 90% were EDSS- worsening free (defined as ≥1 point for an EDSS score ≤5.0, and as ≥0.5 point for an EDSS score >5.0 from baseline), 80% had not relapsed, and 48% were MRI activity-free (Table 4). This rate of MRI activity after 24 months conflicts with data from the pivotal trials, in which an 85–92% reduction in T1-Gd+ lesions was observed [34, 35, 41]. The authors suggested that, while the pivotal trials used standardized acquisition methods and central evaluation, in real-world studies, MRI data are acquired and evaluated in different MS centers. This study also showed that a higher percentage of patients switching from moderate efficacy therapies achieve NEDA-3 compared with those switching from high efficacy therapies, confirming that cladribine tablets are an effective treatment for MS, particularly in treatment-naïve patients and in those switched from moderate efficacy therapies [46].

Petracca et al. conducted another Italian, observational, retrospective study that included 243 patients with RRMS [75]. They collected data on NEDA-3 status and its individual components, which included clinical relapses, disability worsening evaluated as in the study by Zanetta et al., and MRI activity defined as Gd+ lesions and/or new T2 hyperintense lesions. Disease activity occurring in the first 6 months was not considered, to exclude possible rebound effects from previous DMTs, which had been received by 172 patients (71%). Evidence of MRI activity was recorded more frequently in patients switching from other therapies compared with treatment-naïve patients (30 total events between months 6 and 22). The presence of Gd+ lesions at baseline predicted the development of MRI activity at follow-up [75]. Regarding the effectiveness of cladribine tablets, the authors found a high percentage (64%) of patients with NEDA-3 after 22 months of treatment (Table 4), in agreement with previous data from post-hoc analysis of CLARITY [34]. The only factor affecting the likelihood of maintaining NEDA-3 was the number of prior treatments [75].
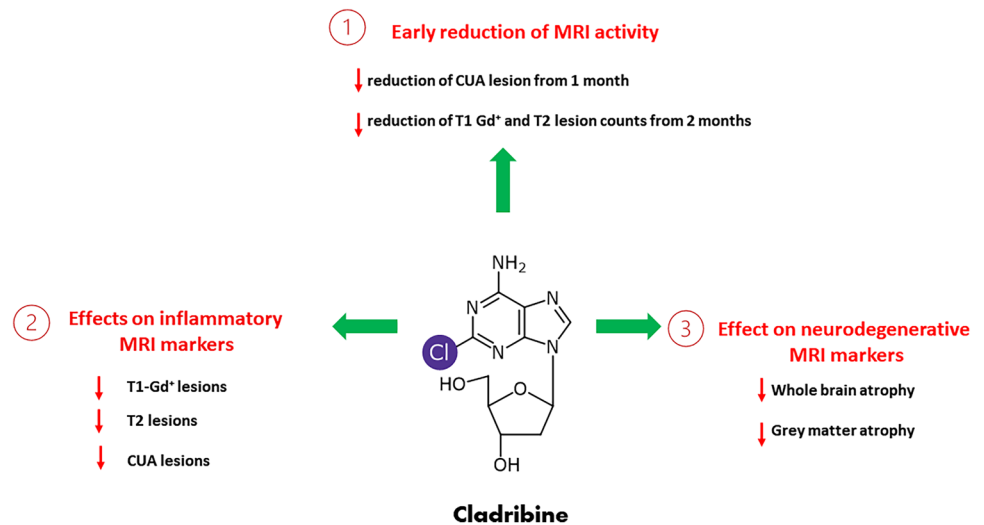
A study conducted by Pfeuffer et al. assessed the effect of previous DMT type on the risk of suboptimal response to cladribine tablets [44]. Analyzing a cohort of 270 cladribine-treated patients during a 36-month follow-up, the authors found a significant reduction of new or enlarging T2-hyperintense MRI lesions, compared with the 6-month period prior to starting cladribine tablets, accompanied by a significant reduction in relapse rate. The majority of patients did not experience worsening of disability, defined as an increase of EDSS equal to +1.5 points (if baseline = 0), +1.0 point (if baseline = 1.0–4.0) and 0.5 points (if baseline ≥4.5) at two independent clinical assessments 6 months apart. In line with the data discussed below, the authors found that cladribine tablets were more efficacious in treatment-naïve patients and patients treated with modest-efficacy drugs (i.e., β-interferon, glatiramer acetate, dimethyl fumarate and teriflunomide). In contrast, patients switching from natalizumab experienced persistent disease activity that occurred mainly within 6 months of initiation of cladribine tablets, likely due to a rebound phenomenon after natalizumab discontinuation. It is also important to consider that patients switching from natalizumab had a higher number of T2 lesions at baseline than patients who had switched from other DMTs [44]. Whether cladribine tablets may represent a potential exit strategy from natalizumab treatment is still an open question. Contrary to what was observed by Pfeuffer et al., a case series of 17 patients suggested that cladribine tablets represent a good exit strategy in patients who need to discontinue natalizumab due to progressive multifocal leukoencephalopathy risk [78].

Overall, real-world data from patient cohorts with diverse baseline characteristics corroborate the efficacy of cladribine tablets demonstrated in the pivotal studies. Further real-world analyses should investigate cladribine's effectiveness after the active treatment period and evaluate its durability over time. In a recent real-world cohort, it was observed that the efficacy of cladribine tablets persists beyond 2 years in terms of relapse rate, relapse-free status, and disability [79]. However, a limitation of this study is the absence of MRI data, preventing the evaluation of NEDA-3 status [79]. Nonetheless, these findings corroborate the observations from the CLARITY Extension study [33], providing real-world evidence for the sustained efficacy of cladribine tablets. Additionally, the long-lasting efficacy of cladribine tablets beyond year 4 was supported by results from the CLASSIC-MS study [80], which showed that patients treated with cladribine tablets had a lower risk of reaching EDSS-6 or -7 during a median follow-up of 10.9 years, compared with patients never exposed to active treatment [80].

The long-term effects of treatment with cladribine tablets and the management of patients after year 4 are currently topics of significant discussion among clinicians, as evidenced by numerous expert opinion reports [81–85]. These reports emphasize the importance of utilizing all available tools and techniques, including clinical, radiological, and biological markers, to identify and manage patients who may benefit from additional courses of cladribine tablets. Specifically, alongside evaluation of clinical and serum markers, MRI is highlighted as a key tool in guiding therapeutic decisions and identifying patients suitable for further treatment with cladribine tablets [81, 82, 84, 85].

Fig. 2 Multiple actions of cladribine tablets on MRI outcomes. *CUA* combined unique active lesions, *Gd+* gadolinium-enhancing, *MRI* magnetic resonance imaging.

① **Early reduction of MRI activity**

↓ reduction of CUA lesion from 1 month

↓ reduction of T1 Gd+ and T2 lesion counts from 2 months

② **Effects on inflammatory MRI markers**

↓ T1-Gd+ lesions

↓ T2 lesions

↓ CUA lesions

③ **Effect on neurodegenerative MRI markers**

↓ Whole brain atrophy

↓ Grey matter atrophy

Cladribine

9 Limitations

The studies included in the present review are not free of limitations. First, there is a discrepancy between the populations analyzed in clinical trials and real-world settings due to the stricter inclusion criteria applied in clinical trials compared with routine clinical practice. Second, caution is advised when directly comparing MRI efficacy outcomes across studies due to variations in assessment schedules and baseline patient characteristics. Furthermore, the interpretation of such comparisons is complicated by the heterogeneity in MRI protocols, including differences in resolution.

Finally, advanced MRI analysis, such as analysis of slowly expanding lesions or compartmentalized meningeal inflammation, as well as spinal cord volume analysis, were not conducted in either clinical trials or real-world studies discussed here.

10 Conclusions

Data from observational and real-world studies presented in this review underscore the efficacy of cladribine tablets in managing the disease course in RMS patients, aligning closely with observations from pivotal trials. Specifically focusing on the impact of cladribine tablets on MRI outcomes, the evidence indicates significant reduction in T1-Gd+, active T2, and CUA lesions, with noticeable effects emerging as early as the second month of treatment. This suggests an early and sustained onset of action lasting up to 24 months [14, 15].

A post-hoc analysis of the CLARITY study showed that subjects treated with cladribine tablets exhibited a markedly reduced annualized brain atrophy rate over a 2-year period when compared with the placebo group [52]. Furthermore, comprehensive investigations revealed that grey matter volume loss occurs at a reduced rate in RRMS patients treated with cladribine tablets compared with those receiving placebo [58]. These MRI findings significantly contributed to elucidating the efficacy of cladribine tablets in addressing both focal inflammation and diffuse tissue damage (Fig. 2) in MS patients. Ongoing analyses from MAGNIFY-MS will further evaluate long-term MRI activity to provide additional evidence on the effectiveness of cladribine in years 3 and 4.

Declarations

Conflict of interest Rosa Cortese was awarded a MAGNIMS-EC-TRIMS fellowship in 2019; she received speaker honoraria from Roche, Merck Serono S.p.A., Italy, an affiliate of Merck KGaA, and Sanofi, and travel support for conferences by Novartis. Nicola De Stefano has received honoraria from Biogen-Idec, Bristol Myers Squibb, Celgene, Genzyme, Immunic, Merck, Merck Serono S.p.A., Italy, an affiliate of Merck KGaA, Novartis, Roche, Sanofi and Teva for consulting services, speaking, and travel support. He serves on advisory boards for Biogen, Sanofi-Genzyme, Merck, Merck Serono S.p.A., Italy, an affiliate of Merck KGaA, Novartis, Teva, Roche, Celgene, and Immunic, and has received research grant support from the Italian MS Society. He is co-founder of Siena Imaging srl. Giovanna Testa and Francesco Assogna are employees of Merck Serono S.p.A., Italy, an affiliate of Merck KGaA.

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable

Availability of data and material Not applicable.

Code availability Not applicable.

Author contributions All authors conceived and contributed equally to the writing and revising of this manuscript.

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References

1. Reich DS, Lucchinetti CF, Calabresi PA. Multiple sclerosis. N Engl J Med. 2018;378:169–80.
2. Walton C, King R, Rechtman L, Kaye W, Leray E, Marrie RA, et al. Rising prevalence of multiple sclerosis worldwide: insights from the Atlas of MS, third edition. Mult Scler. 2020;26:1816–21.
3. Bjornevik K, Cortese M, Healy BC, Kuhle J, Mina MJ, Leng Y, et al. Longitudinal analysis reveals high prevalence of Epstein-Barr virus associated with multiple sclerosis. Science. 2022;375:296–301.
4. Olsson T, Barcellos LF, Alfredsson L. Interactions between genetic, lifestyle and environmental risk factors for multiple sclerosis. Nat Rev Neurol. 2017;13:25–36.
5. Giovannoni G. Disease-modifying treatments for early and advanced multiple sclerosis: a new treatment paradigm. Curr Opin Neurol. 2018;31:233–43.
6. Sorensen PS, Sellebjerg F. Pulsed immune reconstitution therapy in multiple sclerosis. Ther Adv Neurol Disord. 2019;12:1756286419836913.
7. Montalban X, Gold R, Thompson AJ, Otero-Romero S, Amato MP, Chandraratna D, et al. ECTRIMS/EAN Guideline on the pharmacological treatment of people with multiple sclerosis. Mult Scler. 2018;24:96–120.
8. McGinley MP, Goldschmidt CH, Rae-Grant AD. Diagnosis and treatment of multiple sclerosis: a review. JAMA. 2021;325:765–79.
9. Freeman L, Longbrake EE, Coyle PK, Hendin B, Vollmer T. High-efficacy therapies for treatment-naïve individuals with relapsing-remitting multiple sclerosis. CNS Drugs. 2022;36:1285–99.
10. European Medicines Agency. Mavenclad product information. 2022 [cited 2022 Oct 21]. https://www.ema.europa.eu/en/documents/product-information/mavenclad-epar-product-information_en.pdf.
11. Leist TP, Weissert R. Cladribine: mode of action and implications for treatment of multiple sclerosis. Clin Neuropharmacol. 2011;34:28–35.
12. Wiendl H, Schmierer K, Hodgkinson S, Derfuss T, Chan A, Sellebjerg F, et al. Specific patterns of immune cell dynamics may explain the early onset and prolonged efficacy of cladribine tablets: a MAGNIFY-MS substudy. Neurol Neuroimmunol Neuroinflamm. 2023;10: e200048.
13. Wiendl H, De Stefano N, Barkhof F, Montalban X, Achiron A, Derfuss T, et al. Blood biomarker dynamics in highly active relapsing multiple sclerosis patients treated with cladribine tablets: results of the 2-year MAGNIFY-MS study. Poster P008. Mult Scler. 2023;29:18–242.
14. De Stefano N, Barkhof F, Montalban X, Achiron A, Derfuss T, Chan A, et al. Early reduction of MRI activity during 6 months of treatment with cladribine tablets for highly active relapsing multiple sclerosis: MAGNIFY-MS. Neurol Neuroimmunol Neuroinflamm. 2022;9: e1187.
15. De Stefano N, Achiron A, Chan A, Derfuss T, Hodgkinson S, Leocani L, et al. Early onset of action and sustained efficacy of MRI outcomes during cladribine tablets treatment in highly active relapsing multiple sclerosis: results of the 2-year MAGNIFY-MS study. ECTRIMS 2022–P717. Mult Scler. 2022;28:130–691.
16. Comi G, Cook S, Giovannoni G, Rieckmann P, Sørensen PS, Vermersch P, et al. Effect of cladribine tablets on lymphocyte reduction and repopulation dynamics in patients with relapsing multiple sclerosis. Mult Scler Relat Disord. 2019;29:168–74.
17. Stuve O, Soelberg Soerensen P, Leist T, Giovannoni G, Hyvert Y, Damian D, et al. Effects of cladribine tablets on lymphocyte subsets in patients with multiple sclerosis: an extended analysis of surface markers. Ther Adv Neurol Disord. 2019;12:1756286419854986.
18. Signori A, Saccà F, Lanzillo R, Maniscalco GT, Signoriello E, Repice AM, et al. Cladribine vs other drugs in MS: merging randomized trial with real-life data. Neurol Neuroimmunol Neuroinflamm. 2020;7: e878.
19. Spelman T, Ozakbas S, Alroughani R, Terzi M, Hodgkinson S, Laureys G, et al. Comparative effectiveness of cladribine tablets versus other oral disease-modifying treatments for multiple sclerosis: results from MSBase registry. Mult Scler. 2023;29:221–35.
20. Alshawaf F, Ahmed S, Al-Hashel J, Alroughani R. Ocrelizumab and cladribine had similar EDSS stability and safety profile in patients with highly active multiple sclerosis (P8–3.011). Neurology. 2023;100:3255.
21. Gasperini C, Prosperini L, Tintoré M, Sormani MP, Filippi M, Rio J, et al. Unraveling treatment response in multiple sclerosis: a clinical and MRI challenge. Neurology. 2019;92:180–92.
22. Wattjes MP, Ciccarelli O, Reich DS, Banwell B, de Stefano N, Enzinger C, et al. 2021 MAGNIMS-CMSC-NAIMS consensus recommendations on the use of MRI in patients with multiple sclerosis. Lancet Neurol. 2021;20:653–70.
23. Calabresi PA, Kappos L, Giovannoni G, Plavina T, Koulinska I, Edwards MR, et al. Measuring treatment response to advance precision medicine for multiple sclerosis. Ann Clin Transl Neurol. 2021;8:2166–73.
24. Eshaghi A, Young AL, Wijeratne PA, Prados F, Arnold DL, Narayanan S, et al. Identifying multiple sclerosis subtypes using unsupervised machine learning and MRI data. Nat Commun. 2021;12:2078.
25. Wattjes MP, Steenwijk MD, Stangel M. MRI in the diagnosis and monitoring of multiple sclerosis: an update. Clin Neuroradiol. 2015;25(Suppl 2):157–65.
26. Oreja-Guevara C, ParadigMS Group. Overview of magnetic resonance imaging for management of relapsing-remitting multiple sclerosis in everyday practice. Eur J Neurol. 2015;22(Suppl 2):22–7.

27. Tintore M, Rovira À, Río J, Otero-Romero S, Arrambide G, Tur C, et al. Defining high, medium and low impact prognostic factors for developing multiple sclerosis. Brain. 2015;138:1863–74.

28. Tur C, Moccia M, Barkhof F, Chataway J, Sastre-Garriga J, Thompson AJ, et al. Assessing treatment outcomes in multiple sclerosis trials and in the clinical setting. Nat Rev Neurol. 2018;14:75–93.

29. Giovannoni G, Mathews J. Cladribine tablets for relapsing-remitting multiple sclerosis: a clinician's review. Neurol Ther. 2022;11:571–95.

30. Nabizadeh F, Mohamadi M, Rahmani S, Rajabi R, Afrashteh F, Najdaghi S, et al. Safety and efficacy of cladribine in multiple sclerosis: a systematic review and meta-analysis. Neurol Sci. 2023;44:3045–57.

31. Giovannoni G, Comi G, Cook S, Rammohan K, Rieckmann P, Soelberg Sørensen P, et al. A placebo-controlled trial of oral cladribine for relapsing multiple sclerosis. N Engl J Med. 2010;362:416–26.

32. Leist TP, Comi G, Cree BAC, Coyle PK, Freedman MS, Hartung H-P, et al. Effect of oral cladribine on time to conversion to clinically definite multiple sclerosis in patients with a first demyelinating event (ORACLE MS): a phase 3 randomised trial. Lancet Neurol. 2014;13:257–67.

33. Giovannoni G, Soelberg Sorensen P, Cook S, Rammohan K, Rieckmann P, Comi G, et al. Safety and efficacy of cladribine tablets in patients with relapsing-remitting multiple sclerosis: results from the randomized extension trial of the CLARITY study. Mult Scler. 2018;24:1594–604.

34. Giovannoni G, Cook S, Rammohan K, Rieckmann P, Sørensen PS, Vermersch P, et al. Sustained disease-activity-free status in patients with relapsing-remitting multiple sclerosis treated with cladribine tablets in the CLARITY study: a post-hoc and subgroup analysis. Lancet Neurol. 2011;10:329–37.

35. Comi G, Cook SD, Giovannoni G, Rammohan K, Rieckmann P, Sørensen PS, et al. MRI outcomes with cladribine tablets for multiple sclerosis in the CLARITY study. J Neurol. 2013;260:1136–46.

36. Giovannoni G, Soelberg Sorensen P, Cook S, Rammohan KW, Rieckmann P, Comi G, et al. Efficacy of cladribine tablets in high disease activity subgroups of patients with relapsing multiple sclerosis: a post hoc analysis of the CLARITY study. Mult Scler. 2019;25:819–27.

37. Vermersch P, Galazka A, Dangond F, Damian D, Wong SL, Jack D, et al. Efficacy of cladribine tablets in high disease activity patients with relapsing multiple sclerosis: post hoc analysis of subgroups with and without prior disease-modifying drug treatment. Curr Med Res Opin. 2021;37:459–64.

38. Comi G, Cook S, Rammohan K, Soelberg Sorensen P, Vermersch P, Adeniji AK, et al. Long-term effects of cladribine tablets on MRI activity outcomes in patients with relapsing-remitting multiple sclerosis: the CLARITY Extension study. Ther Adv Neurol Disord. 2018;11:1756285617753365.

39. Poser CM, Paty DW, Scheinberg L, McDonald WI, Davis FA, Ebers GC, et al. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. Ann Neurol. 1983;13:227–31.

40. Polman CH, Reingold SC, Edan G, Filippi M, Hartung H-P, Kappos L, et al. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria." Ann Neurol. 2005;58:840–6.

41. Freedman MS, Coyle PK, Comi G, L Scarberry S, Damian D, Hyvert Y, et al. Early MRI outcomes in participants with a first clinical demyelinating event at risk of multiple sclerosis in the ORACLE-MS study. Mult Scler J Exp Transl Clin. 2021;7:2055217321990852.

42. Cree BAC, Bowen JD, Hartung H-P, Vermersch P, Hughes B, Damian D, et al. Subgroup analysis of clinical and MRI outcomes in participants with a first clinical demyelinating event at risk of multiple sclerosis in the ORACLE-MS study. Mult Scler Relat Disord. 2021;49: 102695.

43. Rauma I, Viitala M, Kuusisto H, Atula S, Sipilä JOT, Ryytty M, et al. Finnish multiple sclerosis patients treated with cladribine tablets: a nationwide registry study. Mult Scler Relat Disord. 2022;61: 103755.

44. Pfeuffer S, Rolfes L, Hackert J, Kleinschnitz K, Ruck T, Wiendl H, et al. Effectiveness and safety of cladribine in MS: Real-world experience from two tertiary centres. Mult Scler. 2022;28:257–68.

45. Zanetta C, Rocca MA, Meani A, Martinelli V, Ferrè L, Moiola L, et al. Effectiveness and safety profile of cladribine in an Italian real-life cohort of relapsing-remitting multiple sclerosis patients: a monocentric longitudinal observational study. J Neurol. 2023;270:3553–64.

46. Arena S, Chisari CG, Toscano S, Bucello S, Grimaldi LM, Ragonese P, et al. REal-World effectIveNess of claDribine for patients with multiple sclerosis: a Sicilian multicentric experience (REWIND study). Curr Neuropharmacol. 2023. https://doi.org/10.2174/1570159X21666230322140711.

47. De Stefano N, Airas L, Grigoriadis N, Mattle HP, O'Riordan J, Oreja-Guevara C, et al. Clinical relevance of brain volume measures in multiple sclerosis. CNS Drugs. 2014;28:147–56.

48. Sormani MP, Arnold DL, De Stefano N. Treatment effect on brain atrophy correlates with treatment effect on disability in multiple sclerosis. Ann Neurol. 2014;75:43–9.

49. Kappos L, De Stefano N, Freedman MS, Cree BA, Radue E-W, Sprenger T, et al. Inclusion of brain volume loss in a revised measure of "no evidence of disease activity" (NEDA-4) in relapsing-remitting multiple sclerosis. Mult Scler. 2016;22:1297–305.

50. Sastre-Garriga J, Pareto D, Battaglini M, Rocca MA, Ciccarelli O, Enzinger C, et al. MAGNIMS consensus recommendations on the use of brain and spinal cord atrophy measures in clinical practice. Nat Rev Neurol. 2020;16:171–82.

51. Storelli L, Pagani E, Pantano P, Piervincenzi C, Tedeschi G, Gallo A, et al. Methods for brain atrophy MR quantification in multiple sclerosis: application to the multicenter INNI dataset. J Magn Reson Imaging. 2023;58:1221–31.

52. De Stefano N, Giorgio A, Battaglini M, De Leucio A, Hicking C, Dangond F, et al. Reduced brain atrophy rates are associated with lower risk of disability progression in patients with relapsing multiple sclerosis treated with cladribine tablets. Mult Scler. 2018;24:222–6.

53. Smith SM, Zhang Y, Jenkinson M, Chen J, Matthews PM, Federico A, et al. Accurate, robust, and automated longitudinal and cross-sectional brain change analysis. Neuroimage. 2002;17:479–89.

54. Chataway J, Schuerer N, Alsanousi A, Chan D, MacManus D, Hunter K, et al. Effect of high-dose simvastatin on brain atrophy and disability in secondary progressive multiple sclerosis (MS-STAT): a randomised, placebo-controlled, phase 2 trial. Lancet. 2014;383:2213–21.

55. Moccia M, de Stefano N, Barkhof F. Imaging outcome measures for progressive multiple sclerosis trials. Mult Scler. 2017;23:1614–26.

56. Sormani MP, Schiavetti I, Ponzano M, Colato E, De Stefano N. Treatment effect on brain atrophy correlates with treatment effect on cognition in multiple sclerosis. Ann Neurol. 2023;94:925–32.

57. Fox RJ, Coffey CS, Conwit R, Cudkowicz ME, Gleason T, Goodman A, et al. Phase 2 trial of ibudilast in progressive multiple sclerosis. N Engl J Med. 2018;379:846–55.

58. Cortese R, Battaglini M, Sormani MP, Luchetti L, Gentile G, Inderyas M, et al. Reduction in grey matter atrophy in patients

with relapsing multiple sclerosis following treatment with cladribine tablets. Eur J Neurol. 2023;30:179–86.

59. Battaglini M, Jenkinson M, De Stefano N, Alzheimer's Disease Neuroimaging Initiative. SIENA-XL for improving the assessment of gray and white matter volume changes on brain MRI. Hum Brain Mapp. 2018;39:1063–77.

60. Calabrese M, Magliozzi R, Ciccarelli O, Geurts JJG, Reynolds R, Martin R. Exploring the origins of grey matter damage in multiple sclerosis. Nat Rev Neurosci. 2015;16:147–58.

61. Langdon D, Brochet B, Havrdova EK, Lechner-Scott J, Montalban X, Patti F, et al. Stabilization of cognitive function in patients with highly active relapsing multiple sclerosis treated with cladribine tablets during the 2-year CLARIFY-MS study. Poster P090. Mult Scler. 2023;29:18–242.

62. Barrantes Cepas D, Noteboom S, Battaglini M, Colato E, Montalban X, De Stefano N, et al. Treatment with cladribine tablets is associated with slower regional atrophy: a retrospective study on atrophy patterns in multiple sclerosis [poster P187]. In: European committee for treatment and research in multiple sclerosis (ECTRIMS) 2023.

63. Raji A, Winkler G. Cladribine tablets in highly active MS monitored by global and regional brain volumetry [poster P1500]. In: European committee for treatment and research in multiple sclerosis (ECTRIMS) 2023.

64. Eijlers AJC, Dekker I, Steenwijk MD, Meijer KA, Hulst HE, Pouwels PJW, et al. Cortical atrophy accelerates as cognitive decline worsens in multiple sclerosis. Neurology. 2019;93(14):e1348–59.

65. Miller JR. The importance of early diagnosis of multiple sclerosis. J Manag Care Pharm. 2004;10:S4-11.

66. Baker D, Herrod SS, Alvarez-Gonzalez C, Zalewski L, Albor C, Schmierer K. Both cladribine and alemtuzumab may effect MS via B-cell depletion. Neurol Neuroimmunol Neuroinflamm. 2017;4: e360.

67. Ceronie B, Jacobs BM, Baker D, Dubuisson N, Mao Z, Ammoscato F, et al. Cladribine treatment of multiple sclerosis is associated with depletion of memory B cells. J Neurol. 2018;265:1199–209.

68. De Stefano N, Achiron A, Barkhof F, Chan A, Derfuss T, Hodgkinson S, et al. Early onset of action and sustained efficacy of MRI outcomes during cladribine tablets treatment in highly active relapsing multiple sclerosis: results of the 2-year MAGNIFY-MS study. Mult Scler Related Disord. 2023;71: 104322.

69. Benkert P, Meier S, Schaedelin S, Manouchehrinia A, Yaldizli Ö, Maceski A, et al. Serum neurofilament light chain for individual prognostication of disease activity in people with multiple sclerosis: a retrospective modelling and validation study. Lancet Neurol. 2022;21:246–57.

70. Thebault S, Abdoli M, Fereshtehnejad S-M, Tessier D, Tabard-Cossa V, Freedman MS. Serum neurofilament light chain predicts long term clinical outcomes in multiple sclerosis. Sci Rep. 2020;10:10381.

71. Paolicelli D, Ruggieri M, Manni A, Gargano CD, Carleo G, Palazzo C, et al. Real-life experience of the effects of cladribine tablets on lymphocyte subsets and serum neurofilament light chain levels in relapsing multiple sclerosis patients. Brain Sci. 2022;12:1595.

72. Seiberl M, Feige J, Hilpold P, Hitzl W, Machegger L, Buchmann A, et al. Serum neurofilament light chain as biomarker for cladribine-treated multiple sclerosis patients in a real-world setting. Int J Mol Sci. 2023;24:4067.

73. Schmierer K, Wiendl H, Barkhof F, Montalban X, Achiron A, Derfuss T, Chan A, et al. Reduction of neuroaxonal damage in patients with highly active relapsing MS treated with cladribine tablets. In: European committee for treatment and research in multiple sclerosis (ECTRIMS) 2023.

74. Kalincik T, Butzkueven H. Observational data: understanding the real MS world. Mult Scler. 2016;22:1642–8.

75. Petracca M, Ruggieri S, Barbuti E, Ianniello A, Fantozzi R, Maniscalco GT, et al. Predictors of cladribine effectiveness and safety in multiple sclerosis: a real-world, multicenter, 2-year follow-up study. Neurol Ther. 2022;11:1193–208.

76. Sorensen PS, Pontieri L, Joensen H, Heick A, Rasmussen PV, Schäfer J, et al. Real-world experience of cladribine treatment in relapsing-remitting multiple sclerosis: a Danish nationwide study. Mult Scler Relat Disord. 2023;70: 104491.

77. Santos M, Sequeira J, Abreu P, Guerreiro R, Santos M, Ferreira J, et al. Safety and effectiveness of cladribine in multiple sclerosis: real-world clinical experience from 5 tertiary hospitals in Portugal. Clin Neuropharmacol. 2023;46:105–11.

78. Möhn N, Skripuletz T, Sühs K-W, Menck S, Voß E, Stangel M. Therapy with cladribine is efficient and safe in patients previously treated with natalizumab. Ther Adv Neurol Disord. 2019;12:1756286419887596.

79. Magalashvili D, Mandel M, Dreyer-Alster S, Didikin M, Harari G, Flechter S, et al. Cladribine treatment for highly active multiple sclerosis: real-world clinical outcomes for years 3 and 4. J Neuroimmunol. 2022;372: 577966.

80. Giovannoni G, Boyko A, Correale J, Edan G, Freedman MS, Montalban X, et al. Long-term follow-up of patients with relapsing multiple sclerosis from the CLARITY/CLARITY extension cohort of CLASSIC-MS: An ambispective study. Mult Scler. 2023;29(6):719–30.

81. Oreja-Guevara C, Brownlee W, Celius EG, Centonze D, Giovannoni G, Hodgkinson S, et al. Expert opinion on the long-term use of cladribine tablets for multiple sclerosis: systematic literature review of real-world evidence. Mult Scler Relat Disord. 2023;69: 104459.

82. Meuth SG, Bayas A, Kallmann B, Linker R, Rieckmann P, Wattjes MP, et al. Long-term management of multiple sclerosis patients treated with cladribine tablets beyond year 4. Expert Opin Pharmacother. 2022;23:1503–10.

83. Meca-Lallana V, García Domínguez JM, López Ruiz R, Martín-Martínez J, Arés Luque A, Hernández Pérez MA, et al. Expert-agreed practical recommendations on the use of cladribine. Neurol Ther. 2022;11:1475–88.

84. Habek M, Drulovic J, Brecl Jakob G, Barbov I, Radulovic L, Rajda C, et al. Treatment with cladribine tablets beyond year 4: a position statement by Southeast European multiple sclerosis centers. Neurol Ther. 2023;12:25–37.

85. Centonze D, Amato MP, Brescia Morra V, Cocco E, De Stefano N, Gasperini C, et al. Multiple sclerosis patients treated with cladribine tablets: expert opinion on practical management after year 4. Ther Adv Neurol Disord. 2023;16:17562864231183220.