Cladribine tablets in people with relapsing multiple sclerosis: A real-world multicentric study from southeast European MS centers

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ABSTRACT

Background: Cladribine is an oral disease-modifying drug authorized by the European Medicine Agency for the treatment of highly active relapsing multiple sclerosis (MS).

Objectives: To provide real-world evidence of cladribine's effectiveness and safety in people with MS (pwMS).

Methods: A retrospective observational multi-center, multi-national study of pwMS who were started on cladribine tablets in ten centers from five European countries.

Results: We identified 320 pwMS treated with cladribine tablets. The most common comorbidities were arterial hypertension and depression. Three patients had resolved hepatitis B infection, while eight had positive Quantiferon test prior to cladribine commencement. There were six pwMS who had malignant diseases, but all were non-active. During year 1, 91.6% pwMS did not have EDSS worsening, 86.9% were relapse-free and 72.9% did not have MRI activity. During the second year, 90.2% did not experience EDSS worsening, 86.5% were relapse-free and 75.5% did not have MRI activity. NEDA-3 was present in 58.0% pwMS in year 1 and in 54.2% in year 2. In a multivariable logistic regression model age positively predicted NEDA-3 in year 1. The most common adverse events were infections and skin-related adverse events. Lymphopenia was noted in 54.7% of pwMS at month 2 and in 35.0% at month 6. Two pwMS had a newly discovered malignant disease, one breast cancer, and one melanoma, during the first year of treatment.

Conclusion: Our real-world data on the effectiveness and safety of cladribine tablets are comparable to the pivotal study and other real-world data with no new safety signals.

1. Introduction

Cladribine is an oral disease-modifying drug authorized by the European Medicine Agency for the treatment of highly active relapsing multiple sclerosis (MS) in 2017 (European Medicines Agency, 2022). The approved dosing regimen is 3.5 mg per kilogram of body weight delivered over two consecutive years, with two treatment weeks separated by one month each year (European Medicines Agency, 2022; Giovannoni et al., 2010). Cladribine is a synthetic purine analog that interferes with DNA synthesis, providing semi-selective immune reconstitution that accounts for prolonged treatment effects after a short treatment dose (Baker et al., 2019). It targets lymphocytes, as highly active proliferating cells, which depend on two key enzymes involved in adenosine metabolism, the activating deoxycytidine kinase (DCK) and the inhibitory cytoplasmic $5'$ nucleotidases (5NT) (Baker et al., 2019). The semi-selectivity of cladribine can be explained by the relative expression of the DCK to 5NT ratio in different lymphocyte subsets (Baker et al., 2019). The highest expression of DCK with low 5NT is found in B cell subsets, followed by CD4 T subsets, while CD8 T subsets, NK cells, and plasma cells have a lower DCK to 5NT ratio (Baker et al., 2019). Neutrophils and monocytes show high 5NT expression and low DCK, so the absolute numbers of monocytes and neutrophils are maintained following cladribine treatment (Baker et al., 2019). The therapeutic efficacy is thought to be caused by a marked and long-term depletion of memory B cell subsets, coupled with a less pronounced, but likewise long-term, depletion of CD4 T subsets (Baker et al., 2019). The population of CD4 T regulatory cells, CD8 T suppressor cells, and regulatory B cells recover rapidly, so repopulating pathogenic cells re-emerge into a regulatory environment, which appears to lead to the re-establishment of immune tolerance (Baker et al., 2019). A limited influence on cells of the innate immune system and modest effects on CD8 and plasma cells contributes to cladribine's safety as these cells allow for immediate and durable protection from infection (Baker et al., 2019).

The efficacy and safety of oral cladribine in people with relapsing multiple sclerosis (pwMS) were investigated in the CLARITY Phase III study, followed by the 2-year CLARITY Extension study (Giovannoni et al., 2010; Giovannoni et al., 2018). Treatment with cladribine tablets significantly reduced relapse rates, the risk of disability progression, and the accumulation of new T2 hyperintense lesions on brain MRI compared to placebo (Giovannoni et al., 2010). Although randomized controlled trials provide the highest level of a drug's efficacy and safety, they are fraught with several limitations. Firstly, the drug is investigated on a highly selected group of patients that may not reflect the characteristic of pwMS encountered in everyday clinical practice. Secondly, the drug is studied on a small number of pwMS with a limited follow-up which may not reveal the true extent of the drug's long-term efficacy which is especially important with an immune reconstitution type of MS treatment where the durable effect of the drug on disease activity is expected. In recent years, long-term data from Italian pwMS who participated in cladribine tablets randomized clinical trials, along with real-world data cohorts from Germany, Finland, Australia, Italy, and Canada, have been published, which is necessary for the understanding of cladribine's role in a real-world clinical setting (Patti et al., 2020; Pfeuffer et al., 2022; Rauma et al., 2022; Lizak et al., 2021; Bose et al., 2021; Petracca et al., 2022). In this study, we report real-world data on cladribine use in five southeast European countries – Croatia, Slovenia, Serbia, Montenegro, and Hungary.

2. Patients and methods

2.1. Patients

This was a retrospective observational multi-center study. The study population consisted of pwMS who were started on cladribine tablets in ten centers from five European countries: University Hospital Center Zagreb, University Hospital Center Sisters of Charity, University Hospital Dubrava, General Hospital Virovitica, National Memorial Hospital "dr. Juraj Njavro" Vukovar, and General Hospital Bjelovar in Croatia; University Medical Centre Ljubljana in Slovenia, University Hospital Szeged in Hungary, University Clinical Centre of Serbia in Serbia, and Clinical Center of Montenegro in Montenegro. The inclusion criteria were: 1) start of treatment with cladribine tablets after the reimbursement approval in participating countries in the period from July 2018 to April 2022, 2) complete at least a 1st-year course of treatment (a total dose of 1.75 mg per kg body weight over the two months), 3) relapsing-remitting multiple sclerosis and 4) minimal data set consisting of the number of relapses and EDSS available for the year prior of treatment start. Cladribine tablets were administered according to the summary of product characteristics in a dose of 3.5 mg/kg over two years.

2.2. Methods

Electronic data records were analyzed for all participants. Demographic and disease-related data such as age, sex, disease duration, and previous disease-modifying therapy (DMT) exposure were collected. Data on clinical and radiological activity 12 months prior to cladribine initiation was collected with a follow-up of up to 24 months. Baseline EDSS (Expanded Disability Status Scale) was defined as the last recorded EDSS within one year before treatment initiation and the baseline number of relapses and MRI activity was defined as the number of relapses and MRI activity during that year. Clinical disease activity was defined as a presence of a relapse defined as an occurrence of new or worsening neurological symptoms attributable to MS that meets the following criteria: Symptoms must persist for >24 h and should not be attributable to confounding clinical factors (e.g., fever, infection, injury, adverse reactions to medications); Symptoms should be preceded by neurological stability for at least 30 days; Symptoms should be accompanied by new objective neurological worsening determined with a timely EDSS/ Functional Systems Score (FSS) assessment. Active MRI lesions were defined as the presence of new or enlarging and/or Gd + lesions on the follow-up MRI. NEDA-3 (no relapses, no EDSS progression, and no active lesions on brain MRI) was assessed at month 12 and month 24. All the data on disease activity was analyzed separately for the first (baseline to 12 months) and second (13–24 months) year of follow-up as the patients started cladribine treatment at various time points.

Ethical approval from the Ethical Committee of the University Hospital Center Zagreb was obtained for the study. Since this was a retrospective study, patient consent was not required according to the local legislation of each center.

2.3. Objectives

The primary objective of this study was to identify how many pwMS treated with cladribine did not have signs of clinical or radiological activity in years 1 and 2 of follow-up.

Secondary objectives were: (1) to identify how many patients with pwMS treated with cladribine achieved NEDA-3 at month 12 and month 24, (2) to identify how many pwMS treated with cladribine were relapse-free during the entire follow-up, (3) to identify predictors of achieving NEDA-3, (4) to identify predictors of being relapse-free, and (5) to evaluate the safety of the treatment.

2.4. Statistical analysis

Statistical analysis was performed with the software IBM SPSS v25. The Kolmogorov-Smirnov test was applied to test whether the data have a normal distribution. Differences between repetitive measures were determined with the Wilcoxon signed ranks test. Univariable logistic regression analysis was used to determine which variables were statistical predictors of achieving NEDA-3 at 12 and 24 months. Variables that

Table 1
Demographic and clinical characteristics of the study cohort.

	n = 320
Age years (SD)	41.6 ± 10.6
Gender n (%) female	241 (75.3)
Disease duration years (min-max)	6.63 (0.12–41.0)
EDSS median (min-max)	3.0 (0–7.5)
Previous DMT n (%)	
No	85 (26.6)
Yes	235 (74.4)
MET*	189 (59.1)
Any interferon	91 (48.1)
Glatiramer acetate	66 (34.9)
Teriflunomide	35 (18.5)
Dimethyl fumarate	50 (26.5)
Azathioprine	1 (0.5)
HET*	46 (14.4)
Alemtuzumab	13 (28.3)
Natalizumab	13 (28.3)
Fingolimod	23 (50.0)
Ozanimod	2 (4.3)
Ocrelizumab	1 (2.2)
Mitoxantrone	2 (4.3)
Daclizumab	2 (4.3)
Number of previous DMTs n (%)	
1	147 (62.6)
2	64 (27.2)
3	23 (9.8)
4	1 (0.4)
Relapses at baseline[a] n (%)	**n = 317**
No	50 (15.8)
1	146 (46.1)
2	95 (30.0)
3	25 (7.9)
4	1 (0.3)
Active lesions on MRI at baseline[a] n (%)	**n = 296**
No	106 (35.8)
1	46 (15.5)
2	18 (6.1)
≥3	126 (42.6)
Gd + lesions on MRI at baseline[a] n (%)	**n = 245**
No	149 (60.8)
1	54 (22.0)
2	14 (5.7)
≥3	28 (11.4)

EDSS-expanded disability status scale. DMT-disease modifying therapies. MET-moderate efficacy therapies. HET-high efficacy therapies. Gd-gadolinium.

* Single pwMS could have been on several previous DMTs, therefore the numbers cannot be summed up.

[a] During 12 months prior to starting cladribine tablets.

achieved statistical significance ($p < 0.20$) were included in the multivariable logistic regression model. Survival analysis was performed in the form of Cox regression model to define which variables are statistically significant predictors of relapse occurrence. Statistical significance was set at 0.05.

3. Results

3.1. Baseline characteristics of the studied cohort

We identified 320 pwMS treated with cladribine tablets. Demographic and clinical characteristics are presented in Table 1. There were 75.3% (241) females, the mean age was 41.6 ± 10.6 years and the median disease duration was 6.63 (0.12–41.0) years. The median EDSS at baseline was 3.0 (0–7.5) and 26.6% were treatment naïve prior to cladribine commencement. Of the 74.4% previously treated with DMT, 59.1% received drugs with moderate efficacy, while 14.4% were treated with high efficacy therapies. Details on DMTs used and the number of previous DMTs used are provided in Table 1. In most instances, the switch to cladribine tablets was made due to clinical or MRI disease activity. Only pwMS who were switched from natalizumab (positive JCV

titers), daclizumab (drug withdrawal), ocrelizumab (adverse event), or mitoxantrone (cardiac safety), were switched due to safety reasons. In the 12 months prior to cladribine initiation, 84.2% experienced relapses, and 64.2% had active lesions on brain MRI. The mean annualized relapse rate (ARR) at baseline was 1.31 ± 0.84.

Data on comorbidities were available for 97 pwMS and are presented in the supplementary table (Table S1). The most common comorbidities were arterial hypertension and depression. Three patients had resolved hepatitis B infection, while eight had positive Quantiferon test prior to cladribine commencement. There were six pwMS who had malignant diseases, but all were non-active.

3.2. 12-month follow-up

Data for the 12-month follow-up was available for 245 pwMS (Fig. 1). After 12 months 91.6% did not have EDSS worsening, 86.9% were relapse-free and 72.9% did not have MRI activity. The mean ARR at month 12 was 0.13 ± 0.35. NEDA-3 was present in 58.0% pwMS. According to univariable logistic regression analysis, older age was a predictor of achieving NEDA-3 at 12 months (exp(B) 1.066, 95%CI 1.033–1.101, $p < 0.001$) (Table 2). In a multivariable logistic regression model older age positively predicted NEDA-3 at 12 months of follow-up and EDSS >3 at the baseline was a statistically significant negative predictor of NEDA-3 at 12 months.

3.3. 24-month follow-up

Data for the 24-month follow-up was available for 129 pwMS (Fig. 1). At month 24 (during the second year of follow-up), 90.2% did not experience EDSS worsening, 86.5% were relapse-free, and 75.5% did not have MRI activity. The mean ARR at month 24 was 0.13 ± 0.34. NEDA-3 was present in 54.2% at month 24 (months 13 to 24). According to univariable logistic regression analysis, at 24-months older age (exp (B) 1.047, 95%CI 1.004–1.091, $p = 0.031$) and longer disease duration (exp(B) 1.075, 95%CI 1.006–1.149, $p = 0.032$) were predictors of NEDA-3 (Table 2). In the multivariable analysis, those values were not statistically significant. Age was also a predictor of being relapse-free during both years of follow-up according to Cox regression analysis (exp(B) 0.950, 95% CI 0.921–0.979, $p = 0.001$).

3.4. Adverse events and lymphopenia

Data regarding adverse events were available for 114 pwMS in the first year of treatment and 86 pwMS in the second year of treatment (Table 3). The most common adverse events were infections, mainly urinary tract infections, and oral herpes simplex. The second most common were skin adverse events such as rash and hair loss. Two pwMS had a newly discovered malignant disease, one breast cancer, and one melanoma, during the first year of treatment.

Lymphocyte levels were available for 261 (81.6%) pwMS prior to starting cladribine, 181 (56.6%) two months after, and 183 (57.2%) six months after cladribine initiation. 253 pwMS had normal lymphocyte levels prior to starting cladribine, while five had grade 1 lymphopenia and 3 grade 2 lymphopenia. Two months after cladribine initiation 45.3% had normal lymphocyte levels, 22.7% had grade 1 lymphopenia, and 25.4% and 6.6% had lymphopenia grade 2 and 3, respectively. At month 6 normal lymphocyte levels were observed in 65%, grade 1 lymphopenia in 19.1% while 12.6% and 3.3% of pwMS had grade 2 and 3 lymphopenia, respectively. Cases of grade 4 lymphopenia were not recorded during the follow-up period. Compared to levels before cladribine start, levels of lymphocytes were statistically significantly lower at month 2 (1.660 (0.790–4.600)/μL ± vs. 0.952 (0.210–3.500)/μL, $p < 0.001$) and month 6 (1.630 (0.790–4.600)/μL vs. 1.100 (0.400–3.080)/ μL, p < 0.001) when compared to levels before cladribine start. As well, levels of lymphocytes were significantly lower at month 2 when compared to month 6 levels (0.925 (0.210–3.500)/μL vs. 1.090
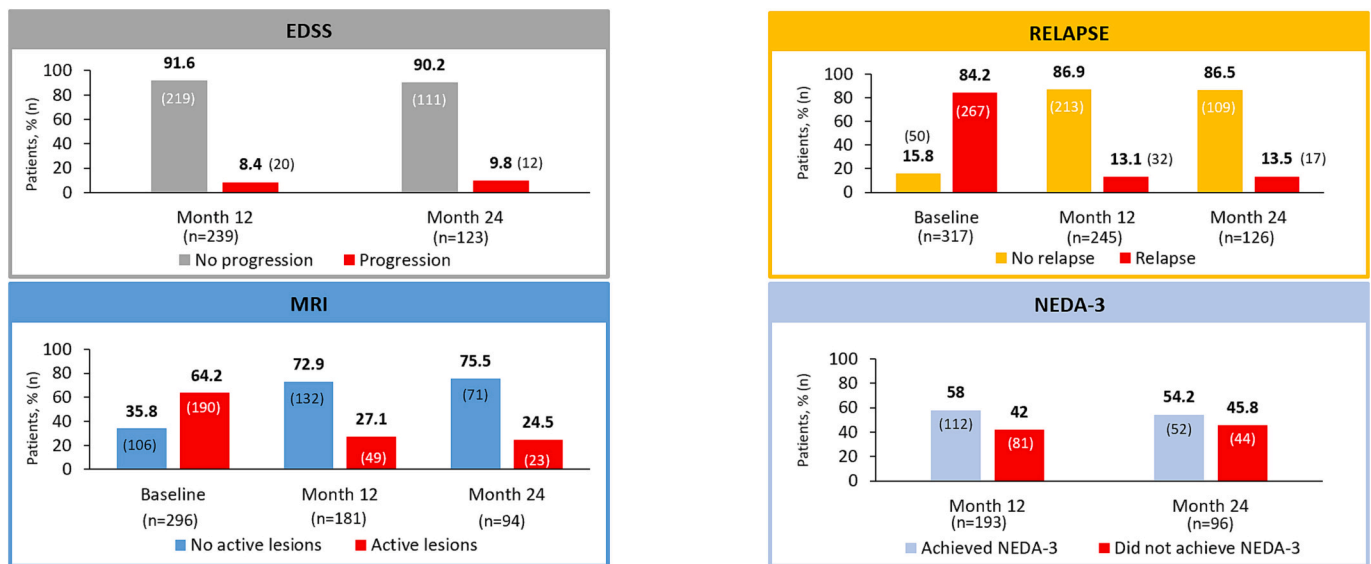
Fig. 1. Disease activity in the first (at 12 months) and second year of follow-up (months 13 to 24). EDSS-expanded disability status scale. NEDA-3-no evidence of disease activity (absence of relapses, EDSS progression, and MRI activity).

(0.400–3.080)/μL, p < 0.001) (Fig. 2).

4. Discussion

This study provides results on the use of cladribine tablets in a real-world population of pwMS in five European countries. The initial efficacy and safety data on cladribine tablets in pwMS was provided by the randomized placebo-controlled CLARITY study (Giovannoni et al., 2010). In the 3.5 mg/kg dose of the cladribine arm there were 68.8% females with a mean age of 37.9 years, mean disease duration of 7.9 years, and mean EDSS score of 2.8. In the current study, the cohort was older (mean age 41.6 years), with a higher female-to-male ratio (75.3% females), shorter disease duration (median 6.63) years, and a similar EDSS (median 3.0). In our study, most of the patients (74.4%) have been previously treated with DMT while in the CLARITY study, only 26.1% of patients were previously treated.

In the CLARITY study, 79.7% of the patients treated with cladribine did not experience relapses after 96 weeks (Giovannoni et al., 2010). A recently published nationwide registry study in Finland demonstrated that 83.6% remained relapse-free after 12 months of follow-up (Rauma et al., 2022). This is in line with the results of our study where 86.9% and 86.5% of pwMS did not experience relapses in the first and second year, respectively, after cladribine treatment initiation. A lower rate of relapse-free patients, 65% two years after cladribine treatment, was reported in an Australian cohort of patients (Lizak et al., 2021). However, a limitation of that report is that due to the withdrawal of the commercially available cladribine 87 out of 90 patients only received the first year of treatment.

It has been demonstrated that cladribine treatment causes a substantial decrease in MRI activity as well. In the CLARITY trial cladribine treatment led to a relative reduction of 73.4% in T2 active lesions on brain MRI compared to placebo (Giovannoni et al., 2010). A significant reduction in the occurrence of new or enlarging T2-hyperintense MRI lesions was found in a real-world study from two tertiary centers in Germany, as well (Pfeuffer et al., 2022). In the current study, 72.9% and 75.5% of pwMS did not have active lesions on MRI at 12 and 24 months, respectively, compared to 35.8% on baseline.

EDSS worsening was rare in our cohort and was present in 8.4% and 9.8% of patients at month 12 and month 24, respectively. This was consistent with the results from the CLARITY study as well as other real-world studies. In the CLARITY trial 3-month sustained EDSS progression

was present in 14.3% of patients 96 weeks after cladribine treatment (Giovannoni et al., 2010). In a real-world study from Italy, EDSS worsening was recorded in 12.7% of patients after a median follow-up of 22 months (Petracca et al., 2022). In the German cohort, EDSS remained stable in the majority of patients after 36 months of follow-up (Pfeuffer et al., 2022).

NEDA-3 was achieved by 58.0% and 54.2% of pwMS in our cohort in the first and second year of treatment, respectively. These results are in the range of those recorded in real-world and phase III studies. In the Italian real-world cohort NEDA-3 was observed in 64% of patients after a median of 22 months while in a post-hoc analysis of the CLARITY study NEDA-3 was present in 44% of patients (Petracca et al., 2022; Giovannoni et al., 2011).

In the current cohort we analyzed possible predictors of future disease activity after cladribine treatment. Age was a predictor of being relapse free during both years of follow-up (exp(B) 0.947, 95% CI 0,919–0.976, p < 0.001). Similar results have been found in a single-center real-world study in Canada where older age at treatment was associated.

with lower risk of developing a new clinical relapse (Bose et al., 2021). Age being a predictor of being relapse-free is not surprising as it has been demonstrated that relapses are age dependent in pwMS. In a retrospective study with a mean follow-up of 20.6 years the relapse rate started declining in patients aged over 40 and decreased by 17% every 5 years (Tremlett et al., 2008). As our cohort had a mean age of 41.6 years this may have been reflected in relapse occurrence. As well, age was a predictor of NEDA-3 in our cohort. Univariable logistic regression analysis revealed age to be a predictor of achieving NEDA-3 at 12 months (exp(B) 1.066, 95%CI 1.033–1.101, p < 0.001). At 24-months age (exp(B) 1.047, 95%CI 1.004–1.091, p = 0.031) and disease duration (exp(B) 1.075, 95%CI 1.006–1.149, p = 0.032) were predictors of NEDA-3. In a multivariable logistic regression model age positively predicted NEDA-3 and EDSS>3 at baseline negatively predicted NEDA-3 at 12 months of follow-up. In contrast, the analysis of the Italian and German real-world cohorts did not find age to be a predictor of either NEDA-3 or relapses at the end of the follow-up (Pfeuffer et al., 2022; Petracca et al., 2022). As well, EDSS at baseline was not associated with the time to loss of NEDA-3 in the German cohort (Pfeuffer et al., 2022). We did not find an effect of previous DMT exposure on future relapses or NEDA-3. This is consistent with findings from the post hoc analysis of the CLARITY trial as well as real-world data where previous DMT

Table 2
Results of the univariable and multivariable logistic regression model for predicting NEDA-3 at month 12 (during the first year of follow-up), and month 24 (13–24 months of follow-up); and results of the Cox regression analysis for the relapse occurrence during the entire follow up.

	Univariable logistic regression			Multivariable logistic regression		
	Exp (B)	95% CI for Exp (B)	p value	Exp (B)	95% CI for Exp (B)	p value
NEDA-3 at month 12						
Age	1.066	1.033–1.101	**<0.001**	1.079	1.041–1.119	**<0.001**
Sex[a]	0.609	0312–1.190	0.147	0.624	0.306–1.271	0.194
Disease duration	1.028	0.986–1.072	0.192	1.001	0.953–1.053	0.954
Disease duration >5 years	0.994	0.554–1.786	0.985			
EDSS on baseline	0.948	0.799–1.125	0.543			
EDSS >3 on baseline	0.629	0.351–1.126	0.119	0.403	0.205–0.791	**0.008**
Number of relapses in year prior to baseline	0.825	0.592–1.150	0.256			
Relapse in year prior to baseline	1.484	0.706–3.121	0.298			
Active lesions on MRI at baseline[b]						
1	0.538	0.208–1.394	0.202			
2	0.875	0.221–3.460	0.849			
≥3	0.826	0.416–1.642	0.586			
Previous DMT	0.714		0.321			
Type of previous DMT[c]						
MET	0.778	0.391–1.549	0.475			
HET	0.527	0.212–1.311	0.169			
NEDA-3 at month 24						
Age	1.047	1.004–1.091	**0.031**	1.031	0.984–1.081	0.198
Sex[a]	1.018	0.413- 2.511	0.969			
Disease duration	1.075	1.006–1.149	**0.032**	1.041	0.964–1.123	0.307
Disease duration >5 years	2.280	0.959–5.420	0.062			
EDSS on baseline	0.927	0.740–1.160	0.507			
EDSS >3 on baseline	0.970	0.432–2.177	0.941			
Number of relapses in year prior to baseline	0.811	0.483–1.361	0.428			
Relapse in year prior to baseline	0.940	0.236–3.739	0.930			
Active lesions on MRI at baseline[b]						
1	3.667	0.636–21.147	0.146			
2	0.917	0.152–5.531	0.924			
≥3	0.955	0.354–2.574	0.927			
Previous DMT	2.411	0.855–6.797	0.096	1.885	0.614–5.601	0.273
Type of previous DMT[c]						
MET	3.158	1.065–9.361	0.038			
HET	1.319	0.380–4.577	0.663			
	Univariable Cox regression analysis			**Multivariable Cox regression analysis**		
	HR	95% CI for HR	P value	HR	95% CI for HR	P value
Relapse occurrence						
Age	0.947	0.919–0.976	**<0.001**	0.950	0.921–0.979	**0.001**
Sex[a]	0.730	0.390-1.367	0.326			
Disease duration	0.982	0.937–1.029	0.439			
Disease duration >5 years	0.987	0.532–1.833	0.986			
EDSS on baseline	0.956	0.799–1.143	0.619			
EDSS >3 on baseline	1.058	0.575–1.949	0.856			
Number of relapses in year prior to baseline	1.379	0.952–1.999	0.090	1.271	0.874–1.850	0.210
Relapse in year prior to baseline	0.868	0.385–1.960	0.734			
Active lesions on MRI at baseline[b]						
1	1.657	0.708–3.876	0.244			
2	0.360	0.047–2.758	0.326			
≥3	0.655	0.308–1.396	0.273			
Previous DMT	1.383	0.642–2.982	0.408			
Type of previous DMT[c]						
MET	1.287	0.580–2.853	0.535			
HET	1.702	0.671–4.313	0.263			

NEDA-3-no evidence of disease activity (absence of relapses, EDSS progression and MRI activity). EDSS-expanded disability status scale. DMT-disease modifying therapy. MET-moderate efficacy therapies. HET-high efficacy therapies.

[a] Male sex is reference category.

[b] No active lesions is reference category.

[c] No DMT is reference category.

exposure was not associated with cladribine treatment outcomes (Petracca et al., 2022; Rammohan et al., 2012).

Reports on the long-term efficacy of cladribine in pwMS are limited and mainly come from follow-up of patients who participated in the initial randomized controlled trials. The CLARITY extension trial demonstrated that up to 75% of pwMS did not experience relapses after four years (Giovannoni et al., 2018). In the CLARINET-MS study long-term efficacy of cladribine tablets was established by following patients who participated in the initial phase III studies using data from the Italian MS registry (Patti et al., 2020). After 36 and 60 months 66.2%

and 57.2% remained relapse-free, respectively, and the probability of being progression-free at 60 months after the last cladribine dose was 63.7%. A study from Israel showed that 68.9% (42/61) of the pwMS treated with cladribine tablets were relapse-free in year-3, and 82.9% (29/35) were relapse-free in year-4 (Magalashvili et al., 2022). A longer follow-up of our patient population, as well as populations from other cohort studies, is necessary to corroborate these results. This is of importance as cladribine tablets are registered for the treatment of highly active relapsing MS due to its profound effect on disease activity during the first two years of treatment with an expected efficacy of up to

Table 3
Adverse events recorded in the first and second years of cladribine treatment.

	year 1	year 2
Infections		
Urinary tract infections	7	
Oral herpes simplex	6	
COVID-19	4	1
Respiratory infection	3	
Herpes zoster	1	
Salmonellosis	1	
Breast abscess	1	
Penile warts	1	
Skin adverse events		
Hair loss	10	3
Rash	8	1
Gastric adverse events		
Nausea	3	1
Epigastric pain	3	
Hepatic lesion	2	
Diarrhea	1	
Cholecystitis	1	
Malignancies		
Breast cancer	1	
Melanoma	1	
Other		
Headache	4	1
Fatigue	2	
Hyperthyroidism	1	1
Anemia	1	1
Tachycardia	1	
Spontaneous abortion	1	
Musculoskeletal pain	1	
Intracerebral hematoma		1
Bleeding gums		1

four years post-treatment, and possibly beyond, due to its immune reconstitution properties (Giovannoni and Mathews, 2022). There is an expert consensus that selected patients who exhibit minimal disease activity in the period post cladribine treatment may receive additional courses although definite trials are missing (Habek et al., 2022; Meuth et al., 2022). As there is uncertainty on the exact approach to pwMS treated with cladribine tablets beyond four years, real-world studies are essential to provide data on the effectiveness of cladribine outside the confines of randomized controlled trials. This will only be revealed by long-term follow-up of real-world cohorts, like the one reported, which will aid in the development of future guidelines as well as coverage decisions (Real-World Evidence, 2022).

In our cohort, there were six pwMS who had non-active malignancies, one case of each of the following: cervical carcinoma, ovarian carcinoma, ovarian teratoma, testicular cancer, non-Hodgkin lymphoma, and breast cancer (Table S1). Although the pivotal cladribine tablet trials revealed a possible increase of malignancy risk in cladribine-treated pwMS, leading to the initial rejection of market authorization of cladribine in Europe, further meta-analysis and larger follow-up studies demonstrated no evidence of heightened cancer risk in pwMS treated with cladribine tablets (Pakpoor et al., 2015; Cook et al., 2019). As cladribine is administered as a short oral course with benefits that continue well beyond the dosing period it is suitable for pwMS with clinically and/or radiologically active disease and a positive history of malignant diseases as it does not cause prolonged immunosuppression, and therefore a possible increase in cancer risk, unlike continuously administered therapies such as B-cell monoclonal antibodies and sphingosine 1-phosphate (S1P) receptor modulators (Deeks, 2018). As well, because of its immune reconstitution properties, cladribine can be administered to pwMS with resolved infections that may become active with the administration of continuous immunosuppressive therapies, such as hepatitis B reactivation in ocrelizumab or fingolimod-treated pwMS (Ciardi et al., 2018; Lu et al., 2020). In the current cohort, there were three pwMS with resolved hepatitis B infection. Prior to cladribine initiation, all the patients were negative for hepatitis B DNA

with two patients administering prophylactic therapy with lamivudine for up to 6 months after cladribine start, and one having regular HBV reactivation monitoring and no prophylactic therapy. None experienced reactivation of the infection. Six patients had latent tuberculosis with a positive QuantiFERON test and negative sputum analysis prior to cladribine start. Our protocol consisted of prophylactic therapy with isoniazid for 6 months with cladribine commencement after two weeks of isoniazid treatment.

We did not detect any cases of grade 4 lymphopenia in the pwMS with available data. In our cohort with 54.7% of pwMS had lymphopenia at month 2 and 35% at month 6. Grade 4 lymphopenia was present in a small number of pwMS in the initial CLARITY study, 0.7 vs. 0% of placebo recipients, and was more common in pwMS who had grade 3 lymphopenia when the drug was re-administered (Deeks, 2018). Similar to our cohort, the Finnish registry study did not record any grade 4 lymphopenia cases (Rauma et al., 2022). However, the authors reported 74.7% of patients with lymphopenia at any point during follow-up with higher grades (grade 2 and 3) being present in 57.2% of pwMS compared to 32% at month 2 and 15.9% at month 6 in our cohort (Rauma et al., 2022).

An integrated analysis of the safety of cladribine tablets revealed that the most common adverse event after lymphopenia was infections (Cook et al., 2019). The main infection that occurred more commonly in cladribine-treated patients than in the control group was herpes zoster, however, no systemic infections were noted (Cook et al., 2019). In our cohort, the most common infection was urinary tract infections followed by oral herpes and COVID-19 (Table 3). After infections, the second most common adverse event was skin problems, such as skin rash and hair loss. In a real-world study in Germany, 32% of cladribine-treated pwMS developed at least one skin reaction including skin rash, pruritus, mucositis, and hair thinning, however, the majority of patients continued cladribine treatment (Rolfes et al., 2021). The underlying mechanism of these changes may be toxicity or immune-mediated skin changes (Rolfes et al., 2021). As they are rarely considered in the safety management of pwMS treated with cladribine tablets real-world studies are important to raise awareness of this potential cladribine-related side-effect.

This study has several limitations. Firstly, typical biases of a retrospective multicentric observational study (possible selection bias, heterogeneity in the clinical data collected between the different centers, heterogeneity in the population recruited, and in the clinical and neuroradiological follow-up protocols) may influence the results. Secondly, there was no systematic re-baselining of the patient's MRI after the start of treatment with cladribine tablets, which may have led to an overestimation of the radiological activity on follow-up. Thirdly, since patients were followed in different centers ascertainment bias is possible. However, we provide real-world cladribine effectiveness and safety data on a large multi-centric, multi-national cohort from Southeast European countries, from which there is very limited data on the treatment of MS. Furthermore, the use of the cladribine tablets in a very diverse patient population with many comorbidities and previous DMTs used adds a significant safety data to the currently existing literature.

In conclusion, the results of this study confirm the effectiveness of cladribine tablets in treating relapsing MS demonstrating that the majority of patients achieve NEDA-3 in the first and second year after cladribine initiation with age being a statistically significant positive predictor of achieving NEDA-3 at 12 months of follow-up. Longer follow-up is necessary to establish the durability of cladribine's effectiveness.
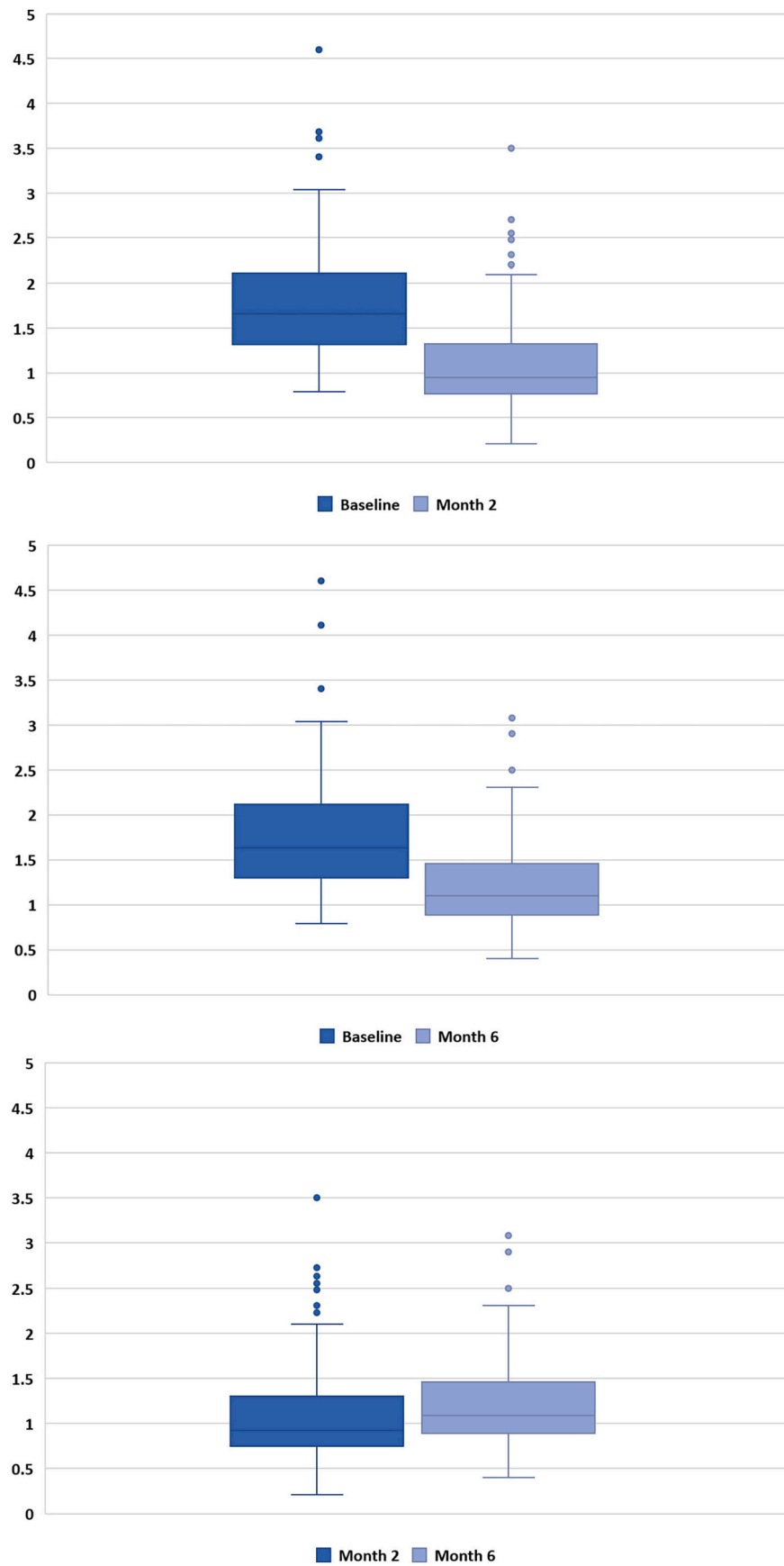
Author statements

Fig. 2. Lymphocyte values before, 2, and 6 months after the start of the treatment. Lymphocyte values are expressed per μL with a comparison of values at baseline and month 2 on the upper, baseline and month 6 in the middle, and month 2 and month 6 on the lower graph.

CR participated as a clinical investigator and/or received consultation and/or speaker fees from: Biogen, Merck, Novartis, Sanofi Genzyme, Roche, Teva.

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Declaration of Competing Interest

None.

Data availability

Data from this study not published within this article are available upon reasonable request for any qualified investigator.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jneuroim.2023.578164.

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