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Masterthesis

From anti-CD20 therapy to immune reconstitution: a retrospective study investigating the optimal timing for cladribine in multiple sclerosis

Alexandra Nuyts

Scriptie ingediend tot het behalen van de graad van master in de biomedische wetenschappen, afstudeerrichting klinische biomedische wetenschappen

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From anti-CD20 therapy to immune reconstitution: a retrospective study investigating the optimal timing for cladribine in multiple sclerosis. *

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*Running title: *Cladribine timing after anti-CD20 therapy in MS.*

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Keywords: multiple sclerosis, anti-CD20 therapy, cladribine, treatment interval, bridging therapy, NEDA-3

ABSTRACT

Anti-CD20 therapies are very effective at suppressing focal inflammation in multiple sclerosis (MS). However, they offer limited efficacy against progression independent of relapse activity (PIRA) due to pharmacokinetic barriers in the central nervous system (CNS). Cladribine, a small molecule that crosses the blood-brain barrier more effectively, offers a rational alternative. Nevertheless, the optimal duration of the treatment interval following discontinuation of anti-CD20 therapy and initiation of cladribine remains a subject of debate due to risks of cumulative immunosuppression or renewed disease activity. This single-center, retrospective, real-world study evaluated the effectiveness of cladribine in 43 persons diagnosed with MS (pwMS) who had switched from anti-CD20 therapy (primarily ocrelizumab), mainly due to functional decline (69.8%). PwMS were divided into three groups based on the length of their treatment interval. During the 24-month follow-up period following cladribine initiation, the majority of pwMS maintained no evidence of disease activity (NEDA-3 status) (58.3% to 63.2% per group). No statistically significant differences were observed among the three treatment interval groups in NEDA-3, confirmed disability worsening (CDW), magnetic resonance imaging (MRI) activity, or relapses. The data did show a stabilizing effect of bridging therapy on disease activity. Despite the small sample size, the results suggested that the timing of cladribine initiation, within the parameters studied, does not adversely affect therapeutic effectiveness in the first 24 months following switch. This supports the flexibility and safety of this treatment switch in clinical practice.

INTRODUCTION

Worldwide, more than 2 million people are affected by multiple sclerosis (MS), a chronic, immune-mediated neurodegenerative disorder of the central nervous system (CNS) (1, 2). MS arises when the immune system attacks the myelin sheath in the brain and spinal cord, leading to neurological dysfunction and progressive increase in disability. The disease typically manifests in young adulthood between 20 and 40 years of age, affects more women than men, and contributes to a significant functional, psychosocial, and socioeconomic burden (1-4). Symptoms vary widely depending on the location and extent of CNS damage and may include visual disturbances, fatigue, gait disturbances, and sensory or motor deficits, all of which significantly impact patients' quality of life (1, 2).

Clinically, MS encompasses a spectrum of phenotypes. Relapsing-remitting MS, the most common form affecting approximately 85% of patients, often presents initially as a clinically isolated syndrome (CIS) and may progress to secondary progressive MS (SPMS) over time (1, 2, 5). In contrast, primary progressive MS (PPMS) is characterized by insidious neurological progression from disease onset without distinct relapses (1, 6). Although this phenotype-based classification remains clinically useful, it does not fully capture the

mechanism underlying disability accumulation in MS. Traditionally, disability progression has been attributed to relapse-associated worsening (RAW). But growing evidence indicates that disability also progresses independently of clinical relapses, referred to as progression independent of relapse activity (PIRA). PIRA can be observed from early disease stages, including RRMS, and contributes substantially to long-term disability accumulation (5).

Despite the absence of a curative treatment, early and effective intervention is important, as patients experience ongoing inflammatory activity that can lead to irreversible neuroaxonal loss (1). In recent years, the therapeutic landscape has expanded substantially, with approximately 20 disease-modifying therapies (DMTs) now approved (2, 7). In addition to reducing relapse frequency, these DMTs have been shown to delay disability progression associated with both RAW and PIRA. These therapies modulate or suppress components of the immune system to reduce neuroinflammation and thereby improve long-term outcomes (1, 8).

Treatment strategies have gradually shifted from traditional injectables with modest efficacy, such as interferons and glatiramer acetate, as well as moderately effective oral DMTs (e.g., dimethyl fumarate and teriflunomide), toward the early use of high-efficacy therapies (HETs) (8). Evidence demonstrated that B cells play a central role in MS pathogenesis through antigen presentation, cytokine secretion, and coordination of autoreactive immune responses (3, 6, 8-10). CD20, a non-glycosylated surface protein expressed on most circulating B cells and, to a lesser extent, on a small subgroup of T cells, is therefore an effective and highly specific therapeutic target (9, 11). Based on the previous, B-cell-targeted therapies – particularly anti-CD20 monoclonal antibodies – are now a cornerstone of modern MS management.

The clinical development of anti-CD20 therapies began with rituximab (MabThera®), originally approved for B-cell lymphomas and later for rheumatoid arthritis and vasculitis, before being widely used off-label in MS (9, 12, 13). Early studies showed a rapid and significant reduction in magnetic resonance imaging (MRI)-detectable inflammatory activity and relapse rates, which was later confirmed in randomized controlled trials, proving the concept of B-cell depletion in MS (3, 10-12). Based on these finding, newer agents with improved designs, including ocrelizumab and ofatumumab, were developed to achieve completer and more sustained B-cell depletion, better tolerability, and – for ofatumumab – subcutaneous administration (11). In pivotal trials, ocrelizumab (Ocrevus®) significantly reduced annualized relapse rates and new MRI lesions in relapsing MS (RMS) (OPERA I and II) (14). Additionally, efficacy in PPMS was demonstrated by slowing disability progression and MRI lesion accumulation in the ORATORIO trial, leading to its approval as the first anti-CD20 therapy for PPMS (2, 6). Ofatumumab (Kesimpta®), the first fully human anti-CD20 antibody administered subcutaneously, also showed strong efficacy in the ASCLEPIOS I and II trials, reducing relapse rates and active MRI lesions in patients with RMS (9, 15). Together, these studies underscore the high effectiveness of anti-CD20 therapies and explain their widespread adoption as first-line HETs.

Although anti-CD20 therapies are among the most effective DMTs for MS, there remain challenges in a subgroup of patients. First, clinical studies show that a clinically relevant number of patients continue to exhibit residual disease activity. When treated with ofatumumab, approximately 10% of patients experienced a confirmed increase in disability within six months, and MRI activity was not completely suppressed (15). Similar findings have been reported for ocrelizumab, with around 10% of patients with RRMS and up to approximately 30% of patients with PPMS developing disability progression despite therapy (6, 14). In this clinical context, it may be necessary to switch to a treatment with a different mechanism of action. Second, in addition to suboptimal response, long-term treatment with anti-CD20 monoclonal antibodies may be associated with relevant safety issues such as infusion and injection-related reactions (IRR's) and an increased risk of infections (9, 16). While most infections are mild to moderate, serious and potentially life-threatening infections have also been described, including severe respiratory tract infections, hepatitis B virus reactivation, varicella-zoster virus infections, and, in rare cases, progressive multifocal leukoencephalopathy (PML) (9, 16). When safety issues warrant the stop of anti-CD20 monoclonal antibodies, switching to a

therapy with a different mode of action, such as cladribine, may represent a rational therapeutic strategy.

Cladribine (Mavenclad®), an immune reconstitution therapy (IRT), represents a well-established alternative for anti-CD20 therapies. Cladribine is a synthetic purine analog that induces rapid and selective lymphocyte depletion, targeting both B and T cells (17, 18). It reduces total B cell counts by ≥ 90% and T cell counts by approximately 50%, followed by a gradual reconstitution of the adaptive immune system (17, 19). In contrast to continuous therapies, cladribine is administered intermittently in two short annual treatment courses of up to 10 days per year, separated by one month (18, 19). This pulsed dosing regimen provides long-lasting therapeutic effects with minimal cumulative exposure (17). Evidence from the CLARITY Extension-study, complemented by real-world data, shows that most patients remain free from MS disease activity for several years following the initial two-year regimen (17, 19, 20). These durable effects, combined with its non-continuous dosing schedule, make cladribine an attractive option for patients in whom continuous B-cell depletion with anti-CD20 therapies is insufficient or poorly tolerated (21). While timely switching between therapies is crucial to minimize the risk of renewed disease activity, several uncertainties remain regarding how such transitions should be optimally managed. In particular, the appropriate transition period between anti-CD20 therapy and cladribine initiation, as well as the possible need for bridging therapies, such as cyclophosphamide (Endoxan®), remain subjects of ongoing debate.

Existing evidence on transitioning from anti-CD20 therapy to cladribine is limited and mainly grounded by expert opinion rather than empirical data. Current recommendations vary but generally suggest that cladribine should only be started after some degree of B-cell recovery has occurred, to prevent reduced effectiveness (19). At the same time, it is recognized that the reason for changing therapy plays a crucial role in this regard (19). When the switch is due to safety or tolerance issues, it may be appropriate to wait until both lymphocyte counts (≥ 800 cells/μL) and B cell populations have recovered (8, 19). In contrast, in patients switching due to a suboptimal response to anti-CD20 therapy, a longer washout period may be undesirable (19, 21). In these cases, earlier initiation of cladribine may be considered to prevent renewed inflammatory disease activity. This highlights a clinical dilemma in managing treatment transitions. Delaying cladribine initiation to permit immune reconstitution after anti-CD20 discontinuation may create a window for disease reactivation (8). Conversely, initiating cladribine too early, in the context of sustained B-cell depletion, may reduce its effectiveness and increase the risk of overlapping immunosuppressive effects, including lymphopenia and infections (8, 19).

Therefore, this retrospective real-world study aims to evaluate whether the timing of transition from anti-CD20 therapy to cladribine influences the effectiveness of cladribine in suppressing disease activity over 24 months of follow-up, and whether there is a need for a bridging therapy during this interval. Disease activity will be assessed by disability progression, MRI activity, and relapse rates. In addition, immune reconstitution will be explored by evaluating B cell ($CD19^+$) counts and absolute lymphocyte counts (ALC). By identifying potential patterns in the timing of this therapy transition and the role of bridging strategies, this study may provide evidence to support more informed clinical decision-making.

METHODS

Ethical considerations – This study was conducted in accordance with national legislation and the principles of the Declaration of Helsinki (2024). Given the retrospective design of the study, which relied on existing clinical data, it did not interfere with routine clinical care. Consequently, informed consent was not requested.

Study design – This study was designed as a single-center, retrospective, real-world cohort study conducted at the Noorderhart Rehabilitation & MS center in Pelt, Belgium. The study population consisted of persons diagnosed with MS (pwMS), irrespective of clinical subtype.

PwMS were eligible for inclusion if they had been treated with an anti-CD20 monoclonal antibody (rituximab, ocrelizumab, or ofatumumab), followed by subsequent treatment with cladribine. The use of bridging therapy between discontinuation of anti-CD20 treatment and initiation of cladribine did not affect eligibility for inclusion. To allow adequate assessment of cladribine

effectiveness, only pwMS with a minimum follow-up duration of 24 months after initiation of cladribine – corresponding to at least 12 months following completion of the second cladribine treatment cycle – were included. Follow-up duration was calculated from the first dose of cladribine until either a subsequent therapy switch or the patient's last documented visit. PwMS were excluded when:

1. They underwent autologous hematopoietic stem cell transplantation (aHSCT) between anti-CD20 therapy and initiation of cladribine; or
2. They did not complete the full treatment course of cladribine, having received only one treatment cycle instead of the required two cycles; or
3. They had insufficient follow-up data (e.g., missing clinical information to determine relapse activity, lack of scores to assess disability progression, or unavailable MRI images) or duration.

Identification of treatment intervals – To investigate the potential impact of the treatment interval duration, the cohort was stratified into three groups based on the time between the last anti-CD20 administration and the initiation of cladribine. The groups were defined as follows:

- Group 1 (Short interval): pwMS with an interval of less than 12 months.
- Group 2 (Intermediate interval): pwMS with an interval between 12 and 20 months.
- Group 3 (Long interval): pwMS with an interval exceeding 20 months.

These cut-offs were selected to ensure a balanced distribution for statistical comparison while reflecting distinct phases post-anti-CD20 therapy.

Data collection – Data were retrospectively collected from the iMed Electronic MS clinical database (version 7.0.3) and supplemented with information retrieved from the electronic patient dossier (clinical workstation (KWS – nexus health)). Prior to analysis, all available data were systematically reviewed and, where feasible, completed by an experienced neurologist to minimize missing values. The final dataset was locked on January 1, 2026.

Demographics and clinical characteristics of the cohort – Baseline was defined as the date of the last administration of anti-CD20 therapy. Baseline variables included patient demographics (age and sex), and clinical characteristics such as disease duration and disease course, treatment history, disability levels, relapse rates, and radiological disease activity.

The date of MS diagnosis was recorded to determine the disease duration at baseline. The MS disease course was defined as relapsing-remitting (RR), secondary progressive (SP), primary progressive (PP), or progressive-relapsing (PR). To characterize the treatment history of the cohort, the number of previous DMTs used prior to anti-CD20 therapy was recorded, as well as the specific duration of the anti-CD20 treatment itself.

Disability levels, as measured by the Expanded Disability Status Scale (EDSS), were extracted from clinical records (22). The baseline EDSS score was defined as the assessment closest to the last anti-CD20 administration, within a window of six months before and three months after baseline (17). Disease activity in the 12 months preceding baseline was evaluated by reviewing documented clinical relapses and radiological reports. Baseline radiological disease activity (DA) of the brain and spinal cord (SC) was evaluated using MRI scans obtained within 12 months prior to the last anti-CD20 administration. This baseline DA was defined as the presence of at least one new or enlarging T2-hyperintense lesion, and/or the presence of one or more gadolinium-enhancing (Gd+) T1 lesions on MRI scans compared to the previously available imaging (23, 24).

Outcome measures during the treatment interval – The treatment interval was defined as the time between the last administration of anti-CD20 therapy and initiation of cladribine. For this interval, data were collected on the reason for discontinuation of anti-CD20 therapy and the use of any bridging therapy. The occurrence of clinical relapses and MRI disease activity of the brain and SC during this interval was recorded to fully characterize the status at cladribine initiation. Clinical and laboratory parameters at the initiation of cladribine were extracted, including the EDSS score, $CD19^+$ B cell counts, and absolute lymphocyte counts (ALC).

Outcome measures after follow-up – Follow-up data were systematically collected after 24 months following initiation of cladribine, corresponding to at least 12 months after completion of the second cladribine treatment cycle. Variables assessed at this point included treatment discontinuation, the

occurrence of clinical relapses during the follow-up period, EDSS score, and MRI disease activity of the brain and SC. $CD19^+$ B cell counts and ALC were recorded at 12 and 24 months of follow-up to monitor the immunological response. For each individual, changes (Δ) in $CD19^+$ B cell counts and ALC were calculated as the difference between cladribine initiation and 12- and 24-month follow-up measurements.

To investigate the effectiveness of cladribine following anti-CD20 therapy, the following outcomes were assessed across the different treatment interval groups: (1) relapse activity, (2) confirmed disability worsening (CDW; yes/no), (3) MRI disease activity (DA; yes/no), and (4) no evidence of disease activity (NEDA-3). NEDA-3 consists of the combination of the first three components: absence of clinical relapses, absence of MRI activity, and absence of CDW (23). Consequently, any pwMS experiencing disability progression, either driven by RAW or PIRA, failed to achieve NEDA-3 status (25).

A clinical relapse was defined as the occurrence of new or worsening neurological symptoms lasting at least 24 hours, occurring in the absence of fever or infection, and separated from a previous relapse by at least 30 days (23). MRI activity during the follow-up period was defined using the same radiological criteria as for baseline disease activity. CDW was defined as an increase in EDSS of +1.5 points from a baseline of 0, +1.0 points from a baseline of 1 – 5.5, or +0.5 points from a baseline ≥ 6.0 (23, 24). PIRA was defined as disability progression occurring independently of, and in the absence of, clinical relapses or MRI activity. While PIRA was quantified using the same EDSS thresholds as CDW, it was strictly distinguished by the total absence of localized disease activity (24, 25). Additionally, PIRA could be identified by documented functional decline (e.g., worsening of gait or cognitive function) in the medical records that necessitated a treatment switch, even if the formal EDSS threshold for CDW was not reached (25).

Statistical analysis – Statistical analyses were performed using IBM SPSS Statistics (version 30.0) and GraphPad Prism (version 11.0.0). As this was a real-world data analysis, all pwMS meeting the inclusion criteria were included. PwMS were stratified according to treatment interval. All data were analyzed descriptively. Continuous variables were presented as mean ± standard deviation (SD) for normally distributed data, or as median with interquartile range [Q1-Q3] for non-normally distributed data. Categorical variables were presented as frequencies and percentages. Binary outcomes, including NEDA-3 status, CDW, and MRI DA, were compared using Fisher's exact test. Changes (Δ) in CD19+ B cell counts and ALC (continuous outcomes) were compared across groups using the Kruskal-Wallis test. All tests were two-tailed, and a p-value < 0.05 was considered statistically significant.

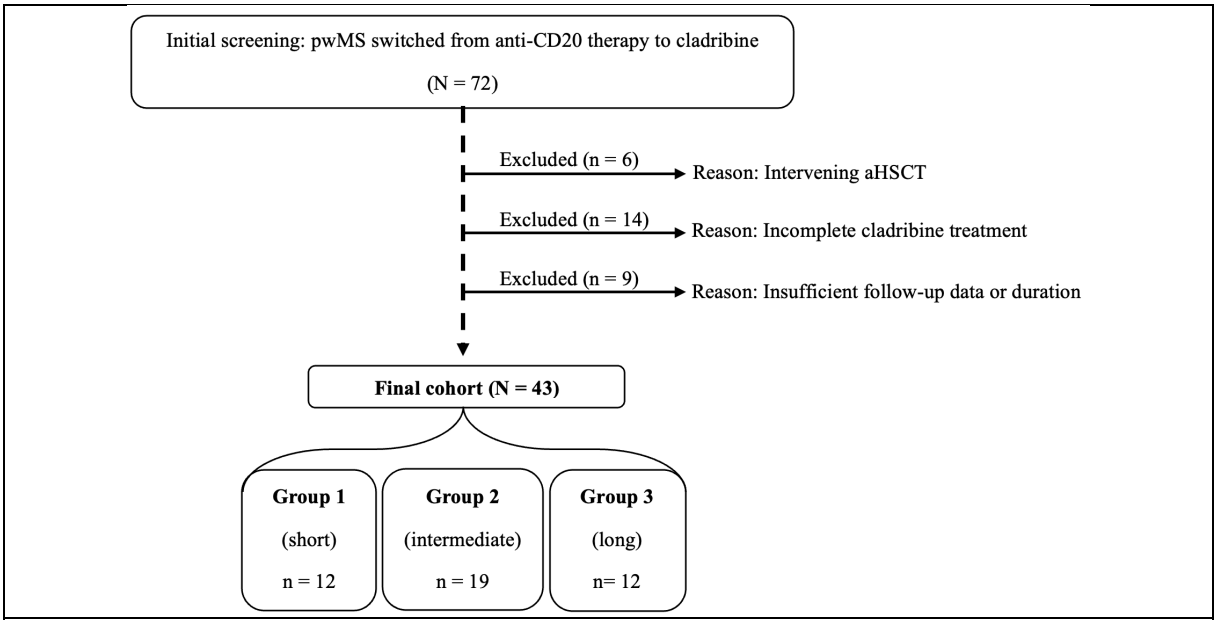

Figure 1. Flowchart of patient selection and treatment group stratification. *pwMS: persons diagnosed with multiple sclerosis, aHSCT: autologous hematopoietic stem cell transplantation.*

RESULTS

Cohort – Initial screening identified 72 pwMS who had transitioned from anti-CD20 therapy to cladribine. After applying the exclusion criteria, 29 pwMS were excluded, resulting in a final study cohort of 43 pwMS. These pwMS were stratified into three treatment interval groups: short interval (n = 12), intermediate interval (n = 19), and long interval (n = 12). A detailed overview of the selection process is presented in **Figure 1**.

Table 1. Demographics and clinical characteristics of the cohort.

Baseline characteristic	Total cohort (N = 43)
Female, n (%)	24 (55.8%)
Age, mean + SD (range)	54.8 ± 9.8 (31-72)
Years from diagnosis, median [Q1-Q3]	11 [5-20]
Months of follow-up, median [Q1-Q3]	39.6 [33.1-51.7]
Type of MS, n (%)	
RR	8 (18.6%)
SP	21 (48.8%)
PP	13 (30.2%)
PR	1 (2.3%)
Number of previous DMTs, n (%)	
Naïve	10 (23.3%)
1	7 (16.3%)
2	7 (16.3%)
3	9 (20.9%)
4	7 (16.3%)
5	3 (7.0%)
Disease activity 12m prior to baseline, n (%)	
≥ 1 relapse	6 (14.0%)
New/enlarging MRI lesions (brain & SC)	4 (9.3%)
Baseline EDSS, n (%)	
≤ 3.5	5 (11.6%)
4.0 – 5.5	13 (30.2%)
6.0 – 6.5	19 (44.2%)
≥ 7.0	3 (7.0%)
Last anti-CD20 therapy, n (%)	
Rituximab	13 (30.2%)
Ocrelizumab	28 (65.1%)
Ofatumumab	2 (4.7%)
Months of anti-CD20 therapy, mean + SD (range)	20.7 ± 13.1 (0.5-50.6)
Reason treatment switch, n (%)	
Functional decline	30 (69.8%)
Relapses	5 (11.6%)
MRI activity	1 (2.3%)
AE	6 (14.0%)
Patient choice	1 (2.3%)
Bridging therapy, n (%)	27 (62.8%)

MS: multiple sclerosis, RR: relapse-remitting, SP: secondary-progressive, PP: primary-progressive, PR: progressive-relapsing, DMT: disease modifying therapy, MRI: magnetic resonance imaging, SC: spinal cord, EDSS: Expanded Disability Status Scale, AE: adverse event

The cohort was predominantly female (55.8%), with a mean age of 54.8 ± 9.8 years, a median diagnosis duration of 11 [5-20] years, and a median follow-up duration of 39.6 [33.1-51.7] months. Most individuals had SPMS (n = 21, 48.8%) and an EDSS score of 6.0-6.5 (n = 19, 44.2%) at baseline. Ocrelizumab (n = 28, 65.1%) was the most used anti-CD20 therapy, with a mean treatment duration of 20.7 ± 13.1 months. The primary indication for treatment transition from anti-CD20 to cladribine was functional decline (n = 30, 69.8%). During the interval between these two treatments, 27 pwMS (62.8%) received bridging therapy. The detailed baseline characteristics are shown in **Table 1**.

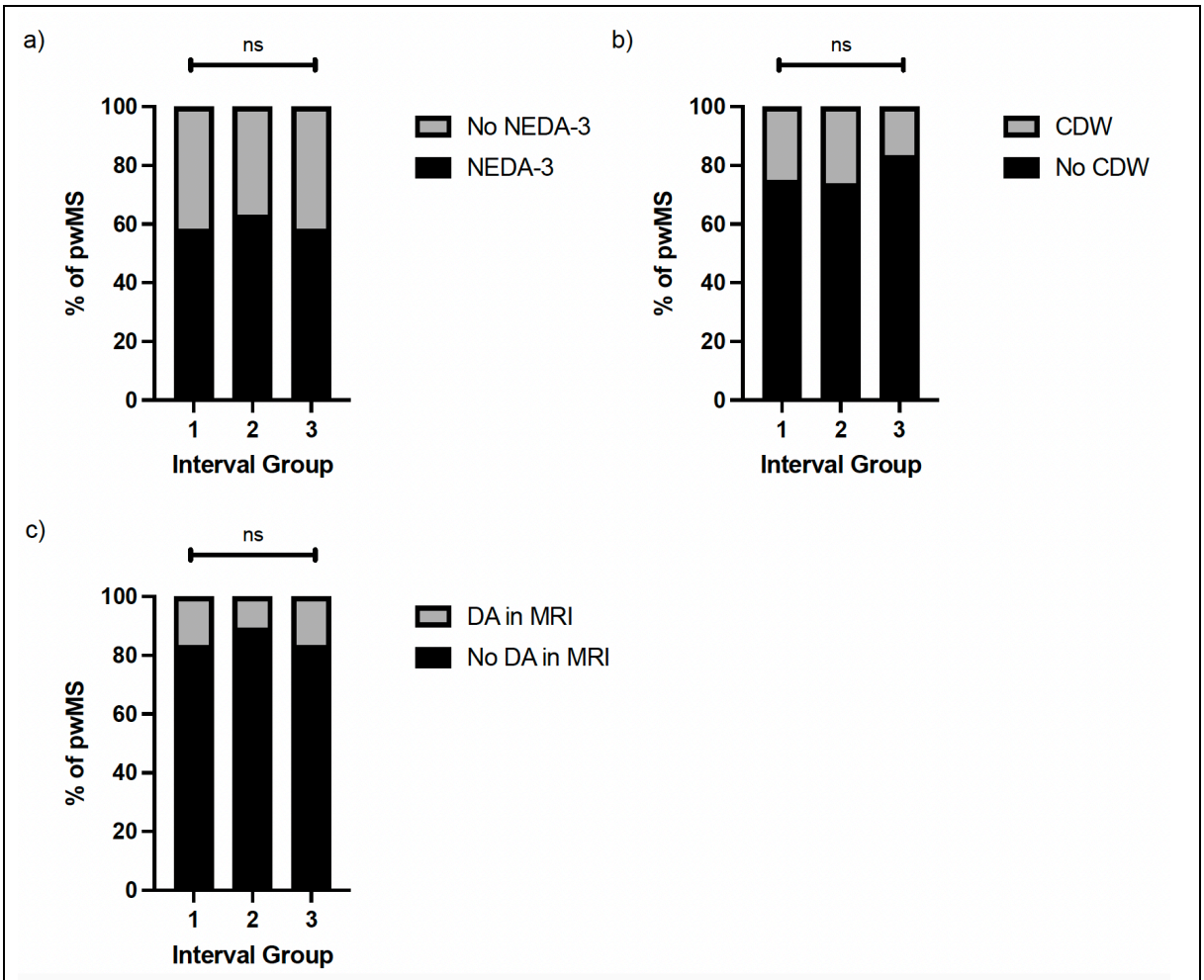

Figure 2. Clinical and radiological outcomes at 24 months follow-up after cladribine initiation. Comparison of NEDA-3 status, CDW, and MRI DA across the three interval groups (Group 1 (short interval): n = 12; Group 2 (intermediate interval): n = 19; Group 3 (long interval): n = 12). Outcomes were assessed from initiation of cladribine to 24 months of follow-up. The proportions of pwMS achieving NEDA-3 (**a**; $p = 1.000$), experiencing CDW (**b**; $p = 0.902$), and showing MRI activity (**c**; combined brain and SC; $p = 0.748$) are presented. Black bars represent individuals without disease activity (NEDA-3, no CDW or no MRI activity), whereas grey bars represent individuals with disease activity. Statistical comparisons between groups were performed using the Fisher's exact test; ns indicates no statistically significant difference ($p > 0.05$). *NEDA-3: no evidence of disease activity, CDW: confirmed disability worsening, MRI: magnetic resonance imaging, DA: disease activity, pwMS: persons diagnosed with multiple sclerosis, SC: spinal cord.*

Cladribine effectiveness following anti-CD20 therapy – The primary focus of this study is the evaluation of cladribine effectiveness at 24 months post-initiation in pwMS who switched from anti-CD20 therapy. Outcomes, including NEDA-3 status, CDW, MRI activity, and relapse activity, were assessed from the start of cladribine treatment up to 24 months of follow-up. The majority of pwMS maintained NEDA-3 status during the follow-up period.

The proportion of stable individuals was 58.3% in both Group 1 and Group 3, and 63.2% in Group 2 (**Fig 2a**). Statistical analysis revealed no significant differences between the three interval groups (p = 1.000), indicating consistent therapeutic effectiveness irrespective of the interval duration. Analysis of the individual NEDA-3 components at 24 months showed no significant differences in the distribution of CDW among the groups (p = 0.902, **Fig 2b**). The incidence was lowest in Group 3 (16.7%), compared with Group 1 (25.0%) and Group 2 (26.3%). Radiological activity, based on combined brain and SC MRI, was low and evenly distributed across the cohort. MRI activity was observed in two patients (16.7%) in both Group 1 and Group 3, and in two patients (10.5%) in Group 2, with no significant intergroup differences detected (p = 0.748, **Fig 2c**). Relapse activity was likewise limited, with no clustering observed in any specific treatment interval group. Two patients in Group 1 experienced a relapse, while one patient in Group 2 had a relapse. No relapses were reported in Group 3 over the entire 24-month period.

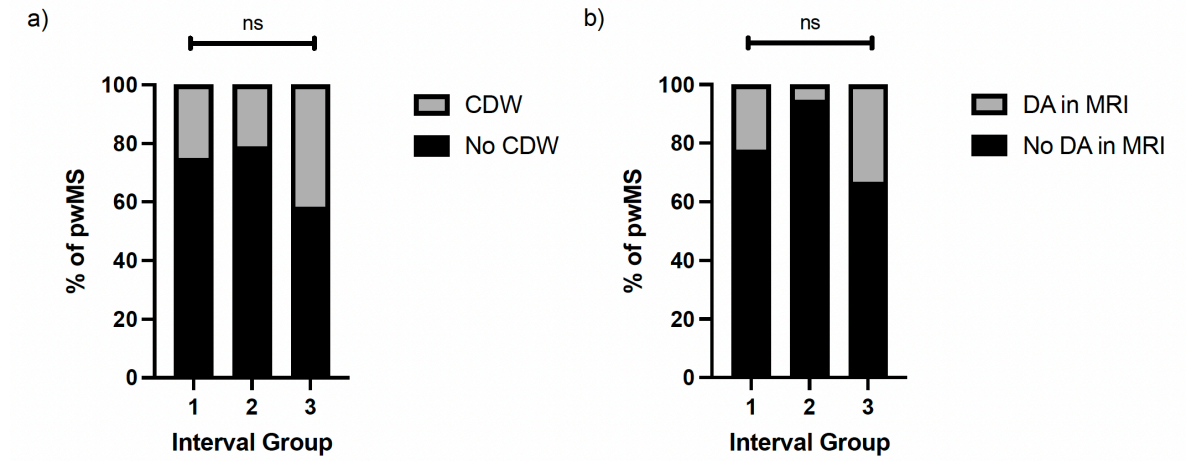

Figure 3. Clinical and radiological outcomes during the interval between anti-CD20 therapy discontinuation and cladribine initiation. Comparison of CDW and MRI DA across the three interval groups (N = 43; Group 1: n = 12; Group 2: n = 19; Group 3: n = 12). **(a)** Percentage of pwMS with CDW during the interval between the last anti-CD20 administration and cladribine initiation (p = 0.476). **(b)** Proportion of pwMS with MRI activity (combined brain and SC) during the same interval (N = 40; data unavailable for 3 pwMS in Group 3; p = 0.122). Black bars represent individuals without disease activity (no CDW or MRI activity), while grey bars represent individuals with disease activity. Statistical comparisons between groups were performed using the Fisher's exact test; ns indicates no statistically significant difference (p > 0.05). *CDW: confirmed disability worsening, MRI: magnetic resonance imaging, DA: disease activity, pwMS: people with multiple sclerosis, SC: spinal cord.*

Disease activity during treatment interval – During the interval between discontinuation of anti-CD20 therapy and cladribine initiation, most pwMS remained stable. Although Group 3 (long interval) exhibited a higher proportion of CDW (41.7%) compared to Group 1 (25.0%) and Group 2 (21.1%) during the interval, these differences were not statistically significant (p = 0.476; **Fig 3a**). Regarding radiological activity (combined brain and SC MRI), data were available for only 40 pwMS prior to cladribine initiation (**Fig 3b**). A clear numerical difference between the groups was observed, with only one individual (5.3%) in Group 2 (n = 19) exhibiting MRI activity, compared to 22.2% in Group 1 (n = 9) and 33.3% in Group 3 (n = 12). However, these differences did not reach statistical significance (p = 0.122). Relapse activity during the treatment interval was low and limited to three pwMS (one in Group 1, two in Group 2), with no relapses reported in Group 3. This distribution suggests that the occurrence of a relapse during the interval may have driven faster cladribine initiation.

Bridging therapy – The analysis of the impact of bridging therapy on clinical and radiological outcomes at 24 months follow-up after cladribine initiation across the different interval groups is presented in **Figure 4**. Overall, no statistically significant differences were observed between the bridging and non-bridging groups for absence of CDW (**Fig 4b**)

and absence of MRI disease activity (**Fig 4c**), regardless of interval duration. However, within interval groups 1 and 3, a non-significant numerical trend toward higher proportions of pwMS with favorable outcomes was observed in the bridging groups. Regarding NEDA-3 status (**Fig 4a**), a significant advantage of bridging therapy was demonstrated in Group 1 (short interval). Within this group, 100% of pwMS receiving bridging therapy achieved NEDA-3 status, compared to only 16.7% in the non-bridging group (p = 0.015). In the remaining interval groups, a similar tendency was observed, with pwMS receiving bridging therapy more frequently achieving NEDA-3 status. However, these results did not reach statistical significance.

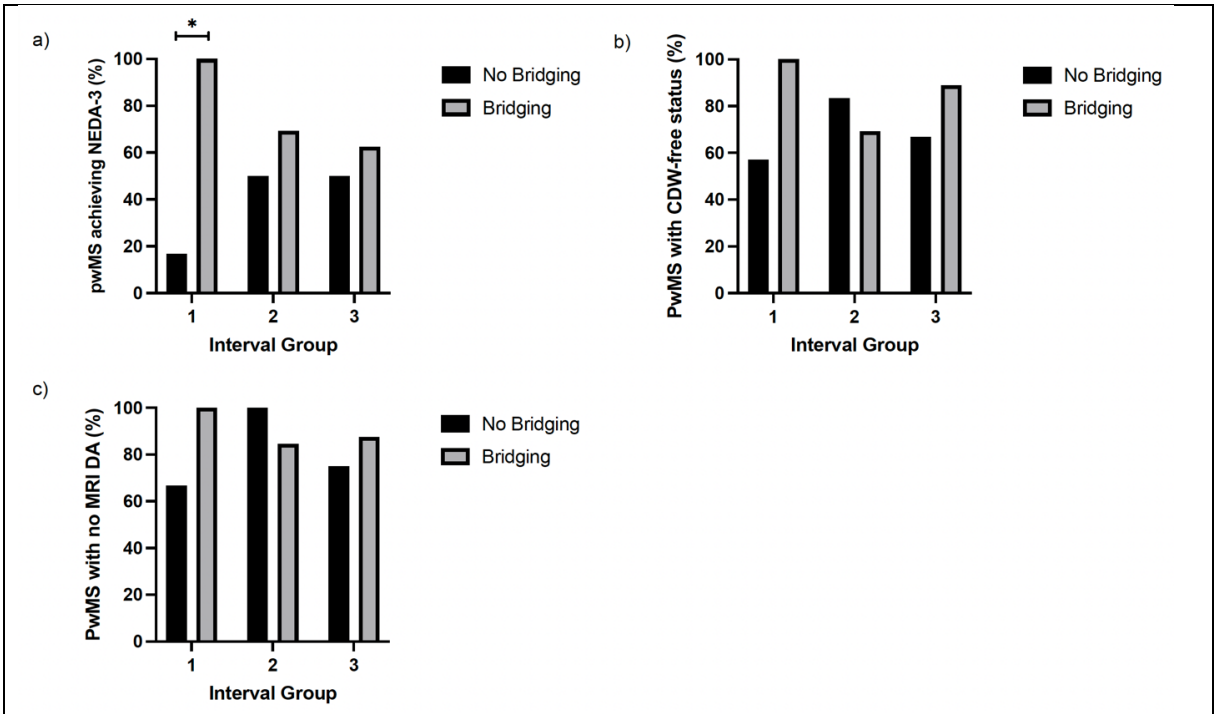

Figure 4. Impact of bridging therapy on clinical and radiological outcomes at 24-month follow-up after cladribine initiation, stratified by interval group. Percentage of pwMS achieving favorable outcomes 24 months post-cladribine initiation, comparing bridging therapy [grey bars] with no bridging [black bars] across the three interval groups (Group 1: n = 12; Group 2: n = 19; Group 3: n = 12). (**a**) Proportion of pwMS achieving NEDA-3 status, a significant difference was observed in interval group 1 (p = 0.015). (**b**) Proportion of pwMS remaining free from CDW and (**c**) showing no MRI disease activity (combined brain and SC). Statistical comparisons were performed using the Fisher exact test; * $p < 0.05$. *pwMS: people with multiple sclerosis, NEDA-3: no evidence of disease activity, CDW: clinical disability worsening, MRI: magnetic resonance imaging, SC: spinal cord.*

Immunological outcomes – To evaluate the immunological effects of cladribine, longitudinal changes in $CD19^+$ B-cell counts and ALC were analyzed following the initiation of cladribine. At 12 months post-cladribine initiation (N = 21), no statistically significant differences in $CD19^+$ B-cell counts were observed across the three interval groups (p = 0.153; **Fig. 5a**). Within the cohort, 13 pwMS (61.9%) exhibited a positive delta, indicating an increase in B-cell counts (repopulation), while seven individuals (33.3%) showed a negative delta, reflection ongoing depletion. This pattern remained consistent at the 24-month follow-up (N = 15), with no significant intergroup differences detected (p = 0.633; **Fig. 5b**), where 10 pwMS (66.7%) showed a positive delta and four (26.7%) showed a negative delta. Regarding ALC, no statistically significant differences between the groups were found at 12 months (p = 0.827; **Fig. 5c**). Notably, most pwMS (n = 17; 81%) demonstrated a negative delta value, confirming the expected sustained decrease in total lymphocytes across all groups. After 24 months, ALC results remained consistent across groups (p = 0.940), with 13 out of 15 individuals (86.7%) still exhibiting a negative delta (**Fig. 5d**).

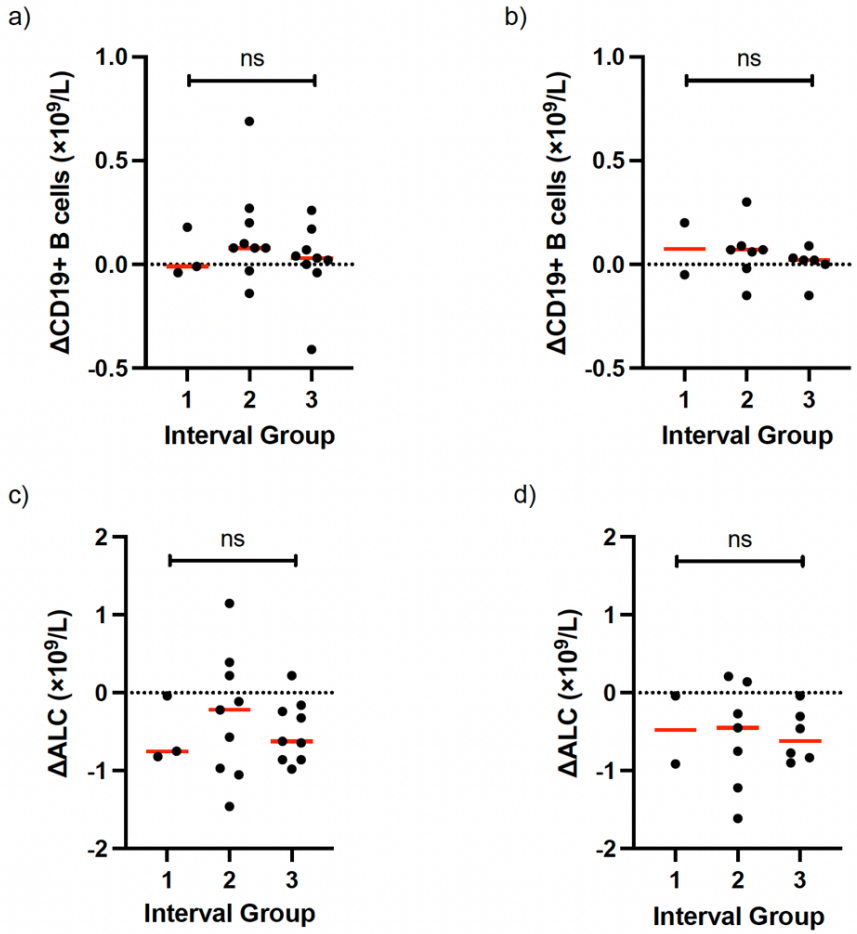

Figure 5. Longitudinal changes in CD19$^+$ B-cell and absolute lymphocyte counts (ALC) following cladribine initiation. The change (Δ) in cell counts relative to the initiation of cladribine for the three different interval groups. **(a)** Change in CD19$^+$ B-cell counts (×10^9/L) at 12 months (N = 21: Group 1: n = 3; Group 2: n = 9; Group 3: n = 9) and **(b)** at 24 months (N = 15: Group 1: n = 2; Group 2: n = 7; Group 3: n = 6). **(c)** Change in ALC (×10^9/L) at 12 months (N = 21) and **(d)** at 24 months (N = 15). Individual data are represented by black dots; horizontal red lines indicate the median value for each group. The dotted horizontal line at 0 represents no change from baseline. Statistical comparisons between interval groups were performed using the Kruskal-Wallis test; ns indicates no statistically significant difference ($p > 0.05$).

DISCUSSION

In this study, we investigated whether the interval between anti-CD20 and cladribine treatment affects clinical outcomes after 24 months, and whether there is a need for a so-called bridging therapy during treatment transition.

The most common reason for switching from anti-CD20 therapy to cladribine in this cohort (N = 43) is functional decline (69.8%), clinically defined as PIRA. Although anti-CD20 monoclonal antibodies are highly effective in suppressing focal inflammatory activity, such as relapses and MRI lesions, a substantial proportion of pwMS still experience disease progression. Our findings are consistent with existing research, reporting that 23.7% of people with RRMS on ocrelizumab developed confirmed disease progression, of which 87% is classified as PIRA (26).

The limited efficacy of anti-CD20 therapies, leading to this high percentage of PIRA, can be explained by the pharmacokinetic limitations of monoclonal antibodies in relation to the nature of inflammation in MS. Because monoclonal antibodies have a large molecular structure, they cross the blood-brain barrier (BBB) only to a limited extent. Studies show that the concentration of rituximab in cerebrospinal fluid (CSF) is 400 to 1,000 times lower than in serum, which may be insufficient to fully suppress inflammatory processes within the CNS (9, 27). In the progressive phase of MS, inflammation occurs largely within the CNS, independent of peripheral B-cell activity (26, 28). This so-called compartmentalized inflammation is associated with persistent, low-grade inflammation and neurodegeneration (26, 28). This can lead to clinical deterioration even when no relapses occur, and MRI imaging remains stable – a phenomenon known as “smouldering MS” - giving the clinical manifestation of PIRA (25).

A treatment switch to cladribine is a possible solution to tackle this smouldering disease activity and the resulting PIRA, given its potential to overcome therapeutic resistance. In contrast to anti-CD20 therapies, which primarily target peripheral B cells while largely sparing T cells, cladribine depletes both B- and T-lymphocytes by interfering with the DNA synthesis, with a particular predilection for memory B cells (29). Furthermore, as a small molecule, cladribine can cross the BBB more effectively. While cerebrospinal fluid concentrations are estimated at approximately 25% of plasma levels under physiological conditions, this penetration may be further enhanced in pwMS (29-31). Pathological disruption of the BBB, a hallmark across all stages of MS, facilitates greater CNS access for cladribine, enabling direct immunomodulatory effects within the CNS (31).

Although the pharmacological properties of cladribine provide a clear rationale for a therapeutic switch, the question of the optimal timing for initiation of cladribine remains challenging. In particular, the duration of the transition period between the last anti-CD20 administration and the start of cladribine is a subject of debate, due to potential risks of cumulative immunosuppression or recurrence of disease activity (8, 19). To address this question, we analysed different interval lengths to see whether there is an optimal timing for this switch. However, no significant differences were found in clinical outcome measures (NEDA-3, CDW, MRI-activity, and relapses) between a short (< 12 months), intermediate (12 – 20 months), or long treatment interval (> 20 months). These findings suggest that the timing of cladribine initiation, within the parameters studied, is not a determining factor for treatment effectiveness during the first 24 months after the switch.

A possible explanation for this sustained stability across all intervals is the prolonged biological effect of anti-CD20 therapies, which extend beyond their relatively short pharmacokinetic half-life of approximately 16-33 days, depending on the specific agent (32). Although circulating drug levels decline rapidly, B-cell depletion persists for a considerably longer period. B-cell reconstitution typically begins around six months after the last administration, with median total B-cell counts normalizing after 12 months (33, 34). However, memory B cells may remain below the lower limit of normal for up to 16 months or even longer (33). This prolonged effect is reflected in our interval data. Statistical analysis reveals no significant differences in MRI activity between the three interval groups, demonstrating that pwMS in the longest interval group did not experience significantly more radiological disease activity than those with a shorter interval. Furthermore, clinical relapse activity remained low across the entire cohort. In the longest interval groups, no relapses were reported during the transition

period. Together, these findings support the hypothesis that residual anti-CD20 effects continue to provide a prolonged, effective suppression of neuroinflammation. Crucially, across all interval groups during this transition period, the proportion of pwMS experiencing CDW was consistently higher than both relapse and MRI activity. This further underscores the focal mechanism of anti-CD20 therapies.

Beyond this residual suppression, the immunological environment created by anti-CD20 therapy may also influence the effectiveness of cladribine. The activity of cladribine is not only dependent on the number of lymphocytes but also on the proliferative state and enzymatic profile of the target cells. Cladribine is a prodrug that is phosphorylated into its active metabolite, cladribine triphosphate, by deoxycytidine kinase (DCK) (30, 31). The selective cytotoxicity of cladribine relies on a high ratio of DCK to the dephosphorylating enzyme 5'-nucleotidase (5'-NT), a profile characteristic of lymphocytes (30, 31). During DNA synthesis and -reparation, the incorporation of this active metabolite leads to DNA strand breaks and subsequent apoptosis (31). Consequently, cells in a proliferative state are particularly sensitive. Following anti-CD20-induced depletion, the emerging B cells have more activated and less mature phenotypes, characterized by increased expression of activation markers such as CD25 and CD69 (35, 36). While direct measurements of DCK activity in this specific post-anti-CD20 therapy population are limited, the rapid proliferation that these reconstituting cells may represent highly sensitive targets for cladribine. Based on these cellular dynamics, we may conclude that the absence of significant differences between the interval groups may be explained by a shift in cladribine's mode of action. In the group with an interval of a maximum of 12 months, cladribine likely acts "preventively" by targeting the limited but highly proliferative pool of B cells attempting to repopulate. In contrast, in the longer interval group, where B-cells have often normalized, cladribine may act more "reactively", inducing depletion in the re-emerged population. In both scenarios, the net result is similar, an effective depletion of the relevant pathogenetic B cells.

While the timing of the switch itself did not show a major impact, our second question investigated whether a bridging therapy during this transition interval could offer additional value. The analysis of bridging strategy demonstrated an overall consistent, but statistically diffuse, pattern. Across the overall cohort, an advantage is observed among pwMS who received bridging therapy, as they more frequently achieved favourable outcomes in terms of disability-free status, and the absence of relapses and MRI activity. The fact that this trend is observed across nearly all interval groups suggests that the additional effect of bridging during the transition phase may exert a stabilising influence on disease activity. The only statistically significant finding is observed for the NEDA-3 status within the group with the shortest interval. Although the observed differences (100% versus 16.7%) are clinically striking, their interpretation requires caution due to the limited sample size.

Finally, we evaluated whether a shorter treatment interval might carry negative clinical consequences, such as safety concerns. Reassuringly, a short interval (< 12 months) did not increase the risk of excessive immunosuppression. No significant differences in ALC or $CD19^+$ B cell counts are observed between the interval groups post-cladribine initiation. ALC dynamics are comparable across the groups, with more than 80% showing a decrease in total lymphocytes, consistent with the expected pharmacodynamic effect of cladribine. These findings suggest that immune reconstitution is primarily driven by the intrinsic mechanism of cladribine itself, with limited influence from the treatment interval. This observation is further supported by the real-world study of Konen et al. (2026), which reported a median interval of 213 days (approximately seven months) between anti-CD20 therapy discontinuation and cladribine initiation. This interval falls within our short-interval group and was shown to be both clinically feasible and safe, without the emergence of unexpected safety signals (24).

While this study provides valuable clinical insights, its conclusions should be interpreted with appropriate caution. The main limitations are its retrospective design and the small sample size (N = 43), which restricts the statistical power. In addition, data collection depended on the availability and completeness of medical records, which may result in missing data or information bias. Nevertheless, this study offers important first insights into the effect of the interval period on the effectiveness of cladribine after switching from anti-CD20

therapy. Moreover, observed trends suggest a potential stabilising benefit of bridging therapy during the transition phase. These findings contribute to a better understanding of optimal treatment timing and transitioning strategies. However, future research in larger, potentially prospective settings is necessary to validate these results and further support treatment guidelines for MS.

CONCLUSION

This retrospective real-world study provides initial insights into the impact of the interval between anti-CD20 therapy and cladribine on treatment outcomes in pwMS and suggests that, within the limits of this study, the duration of this interval does not have an impact on the effectiveness or safety of cladribine. In addition, findings point to a possible stabilizing effect of bridging therapy during the treatment interval, which requires further confirmation in larger, prospective studies.

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