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Real-world experience of cladribine treatment in relapsing-remitting multiple sclerosis: A Danish nationwide study

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ABSTRACT

Background: Cladribine is a nucleoside analogue interfering with synthesis and repair of DNA. Treatment with cladribine leads to a preferential reduction in lymphocytes, resulting in profound depletion of B-cells with a rapid recovery of naïve B-cells, while T-cell show a lesser but long-lasting depletion It is approved for treatment of relapsing multiple sclerosis (MS). Cladribine tablets 3.5 mg/kg bodyweight are administered in two yearly treatment courses, each including two treatment series lasting 4 or 5 days, one at the start of the first month and the other at the start of the second month.

Objective: To describe treatment patterns of cladribine in a real-world setting.

Methods: Registry based observational cohort study with prospectively enrolled cases from December 2017 through June 2021. The data source is The Danish Multiple Sclerosis Registry, which is a near complete nationwide population-based registry. Outcomes were length of the treatment, preceding and following treatments, treatment response, and safety data.

Results: In total 268 patients had started therapy with cladribine tablets, 89 men and 179 women, with a median age of 40 years (interquartile range (IQR) 32–48. The disease course was relapsing-remitting MS in 97.8% of the patients, and at treatment start the median time from disease onset was 8.1 years (IQR 4.2–14.5) and EDSS 2.5 (IQR 1.5–3.5). Thirty-four patients (12.7%) were treatment naïve while 56 (20.9%) had received one previous disease-modifying therapy (DMT), 67 (25.0%) two, and 111 (41.4%) three or more previous DMTs.

In total, 214 (80.0%) patients had completed the full treatment of two courses of cladribine, while 54 (20.0%) had received only one course of cladribine tablets. The median follow-up time after cladribine initiation was 34.7 months (IQR 23.3–43.7). Compared with an annualized relapse rate (ARR) of 0.67 (95% CI [0.56, 0.79]) in the year prior to start of cladribine, ARR was reduced to 0.11 (95% CI [0.08, 0.15]) in year 0–2 after 3-month re-baseline with cladribine (84.8% reduction).

Adverse events, reported in 44 (16.4%) of the patients, were mild or moderate, and herpes zoster was only reported in 2 patients. In total, 30 (11.2%) patients discontinued cladribine treatment, of whom 7 (2.6%) discontinued because of adverse effects and 12 (4.5%) discontinued because of disease activity.

Conclusion: In this nationwide review of all Danish patients starting therapy with cladribine tablets in a real-world setting, cladribine treatment was safe, and the therapeutic response was as expected from previous clinical trials. A prolonged observation period is necessary to assess the long-term benefit and risk of cladribine.

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1. Introduction

Cladribine is a new highly effective disease-modifying treatment (DMT) approved for treatment of relapsing-remitting multiple sclerosis (RRMS) in Europe and for treatment of relapsing forms of MS in USA.

Cladribine (2-chloro-2′deoxy-β-D-adenosine) is a synthetic deoxyadenosine analogue with substitution of a hydrogen atom with chlorine, which makes the nucleoside analogue resistant to degradation by adenosine deaminase. Inside the cell, cladribine is activated through phosphorylation by the enzyme deoxy-cytidine kinase (DCK) and can be inactivated through de-phosphorylation by the enzyme, 5′-nucleotidase (5′-NTase) (Beutler, 1992; Leist and Weissert, 2011). The selective effect on lymphocytes is explained by a high concentration of DCK and a low concentration of 5′-NTase compared with other cells, resulting in intracellular accumulation of activated cladribine (Leist and Weissert, 2011) that is toxic, interfering with DNA repair (Brousil et al., 2006), and cladribine causes apoptosis through the caspase system (Genini et al., 2000).

Cladribine also depletes various innate immune cells, including NK cells and monocytes, although to a lesser extent than lymphocytes (Baker et al., 2017).

Cladribine can cross the blood brain barrier (BBB) and the concentration in the cerebrospinal fluid is 25% of that in plasma (Leist and Weissert, 2011). It is plausible that cladribine reduces disease activity in MS, at least in part, by depleting circulating memory B cells and thus reducing the influx of these cells into the CNS (Baker et al., 2017), and that cladribine may deplete CNS-resident immune cells in vivo as it has been shown that cladribine induced apoptosis of microglial cells, inhibited their proliferation and reduced the production of pro-inflammatory cytokines (Aybar et al., 2022).

Cladribine is administered orally in two courses, each consisting of two 4–5 days series: one at the start of the first month and the other starting in the second month, while the 2nd treatment courses are administered after one year. The total dose is 3.5 mg/kg bodyweight over 2 year (Giovannoni et al., 2010).

Cladribine belongs, together with alemtuzumab, to the therapies termed pulsed immunosuppressive therapies or pulsed immune reconstitution therapies that are thought to induce long-term qualitative beneficial changes in the adaptive immune system resulting in prolonged treatment effects after a short course of treatment (Sorensen and Sellebjerg, 2019).

In a placebo-controlled clinical trial (CLARITY), cladribine tablets 3.5 mg/kg significantly reduced the annualized relapse rate by 58% compared to placebo ($p<.001$), and significantly lowered the risk of 3-month and 6-month confirmed disability worsening (Giovannoni et al., 2010). The onset of action is fast with a significant reduction in magnetic resonance imaging (MRI) activity from one month and onwards, with full effect on MRI disease activity after three months (de Stefano et al., 2022). NEDA-3 after 2 years was achieved in 44% (Giovannoni et al., 2011b). In the CLARITY extension study, 75.1% of patients remained relapse-free in year 3 and 4 without further treatment (Giovannoni et al., 2018).

2. Material and methods

Only departments of neurology in public Danish hospitals are authorized to treat MS patients with DMTs, which are provided by the hospital free of charge for the patient. The patients are seen in the outpatient clinics 3 months after start of a DMT and then every 6-(12) months. It is mandatory for the hospital to report clinical data on all treated patients to the Danish Multiple Sclerosis Registry (DMSR) (Koch-Henriksen and Sorensen, 2000; Magyari et al., 2021), and since 1996, data on all patients treated with DMTs has been prospectively collected, comprising: year of disease onset; year of diagnosis; disease course; onset symptoms; dates of visits; relapses; EDSS scores; adverse events (AEs) along with their corresponding dates; MRI data; and cause and date of treatment discontinuation or switch of therapy. Hence, the nationwide population-based registry has a high completeness and density and contains information on all MS and clinically isolated syndrome (CIS) patients treated with DMT in Denmark.

The study was a nationwide, population-based longitudinal cohort study comprising prospectively enrolled adult patients (≥18 years) who started treatment with cladribine tablets between December 2017, when cladribine treatment was introduced in Denmark, and the end of April 2021. Patients were followed until termination from follow-up (e.g., death or emigration), initiation of a different DMT after cladribine, discontinuation, or date of data extraction from the registry (15th of June 2022), whichever came first.

Date of the first cladribine tablet dispensation was designed as baseline. However, all analyses regarding clinical endpoints were conducted using a re-baseline 90 days after the baseline date, to account for the fact that cladribine might not be working in the first months after initiation of therapy and carry-over activity from the previous treatment period could still be present.

The following endpoints during cladribine treatment were assessed: a) annualized relapse rate (ARR) from re-baseline after 1 and 2 years and over the entire follow-up period, b) first 24-week confirmed disability worsening (CDW), c) first signs of activity in magnetic resonance imaging (MRI) scans and d) NEDA-3 status (NEDA-3 defined as no relapses, no 24-week CDW, no new/enlarging T2 or gadolinium-enhancing lesions in MRI scans).

Relapses were defined as appearance of a new neurological deficit lasting more than 24 h in the absence of fever or infection, which occurred at least 30 days after the onset of a preceding event.

CDW was defined as an increase in EDSS score of 1.5 points for patients with a baseline EDSS score of 0, or 1 point for patients with a baseline EDSS score between 1 and 5.5, or 0.5 points for patients with a baseline EDSS 5.5 and above, confirmed after 24 weeks. MRI activity was defined as presence of new/enlarging T2 or gadolinium-enhancing lesions compared to re-baseline scan.

2.1. Analyses

All statistical analyses were performed in SAS Enterprise Guide version 7.15 (SAS Institute Inc.). A threshold of 0.05 was used for statistical significance. Clinical, radiological, and demographic characteristics at baseline are reported as frequencies and percentages for categorical variables and mean ± standard deviation (SD), median and interquartile range (IQR) for continuous variable.

2.1.1. ARRs analyses

We carried out negative binomial regression models to obtain point and 95% confidence interval (CI) estimates of ARRs 1 year prior to cladribine initiation, year 1 and 2 after re-baseline and over the entire follow-up period, as well as ARR reduction estimates. The analyses were performed for the entire population and stratified for the last therapy prior to cladribine, considering the following groups: (a) patients starting cladribine as initial therapy [=Naïve], (b) patients switching treatment to cladribine from a moderately effective DMT [=MeDMT], including IFN-beta, glatiramer acetate, teriflunomide or dimethyl fumarate, (c) patients switching treatment to cladribine from another highly effective DMT [=HeDMT], including natalizumab, fingolimod, ocrelizumab, rituximab, alemtuzumab or daclizumab.

Three separate multivariate negative binomial regression models were used to evaluate the impact of age at baseline, sex, previous treatment and disease activity status prior to baseline on the ARRs at year 1 and 2 after re-baseline and over the entire follow-up period, respectively, and to obtain adjusted ARRs estimates for the following subgroups of patients: (1) patients aged < 40 and ≥ 40 years, (2) men and women, (3) in Naïve, MeDMT and HeDMT patients and (4) patients with high and low/moderate level of disease activity prior to cladribine initiation. For the latter subgroup, highly active patients were defined as

having either ≥ 2 relapses in the year prior to baseline, or 1 relapse and baseline EDSS score of ≥3.0, or 1 relapse and significant signs of activity in the baseline MRI (≥ 9 total T2 lesions or ≥ 1 gadolinium enhancing lesions or ≥ 1 new/enlarging T2 lesions). To account for differences in exposure times between patients, the natural logarithm of the exposure period was included in all models as an offset term.

2.1.2. 24-week CDW analysis

The analysis of time to 24-week CDW was performed only on 176 patients (65.7% of total cohort) with an available baseline EDSS score (the last EDSS score recorded within the period −6/+3 months from cladribine initiation) and at least two EDSS scores during follow-up separated by the minimum confirmation period. We ran a Kaplan-Meier survival analysis on the entire cohort to obtain 6-month time-point survival estimates and median follow-up time, calculated via the reverse Kaplan-Meier method. We also ran a multivariable Cox regression to explore the influence of sex, age, previous treatment (HeDMT vs MeDMT) and disease activity status prior to baseline (all variables categorized as described previously) on the hazard of 24-week CDW. Proportionality assumption was checked using the empirical score process method and the variable "previous treatment" was found to violate the proportional hazard after 38 months of follow-up. We thus split the follow-up time at 38 months in two periods to obtain time-varying coefficients for this variable (prior to 38 months and after 38 months).

2.1.3. MRI activity

The analysis of time to first MRI activity was performed only in 165 patients (61.6% of total cohort) with an available baseline MRI scan (latest in the period −3/+3 months from cladribine initiation) and at least one scan after the re-baseline MRI 90 days after the baseline date. Kaplan-Meier survival curve for the entire population and a multivariable Cox regression with sex, age, previous treatment, and disease activity status prior to baseline were performed. All variables fulfilled the proportional hazard assumption.

2.1.4. No evidence of disease activity-3 (NEDA3)

NEDA-3 analysis was performed only on 113 patients (42.2% of total cohort) who fulfilled the requirements to be included both in the 24-week CDW and MRI activity analysis. Kaplan-Meier survival curve for the entire population and a multivariable Cox regression with sex, age, previous treatment, and disease activity status prior to baseline were performed. All variables fulfilled the proportional hazard assumption.

3. RESULTS

3.1. Study population

A total of 268 patients were included in the study of whom 66.8% were female and 97.8% relapsing-remitting MS (RRMS). Baseline characteristics: mean age of 40.6 ± 10.7 years, mean disease duration of 9.9 ± 7.6 years, mean EDSS 2.7 ± 1.8, mean number of relapses in the year prior to cladribine initiation 0.6 ± 0.8, and the mean number of T2 lesions at baseline (data from 171 patients available) was 11.7 ± 10.5 (Table 1).

Cladribine tablets was the first treatment in 34 (13%) patients, second treatment in 56 (20.9%), and third or more treatment in 178 (66.4%) patients (Table 1). Among previously treated patients, 111 (41.4%) switched from a MeDMT and 123 (45.9%) from another HeDMT (Table 2). The main reason for discontinuation of the previous treatment was disease activity (41.5%) followed by adverse events and JC virus in patients treated with natalizumab (Table 3). The most frequently recorded AEs leading to discontinuation of the previous disease-modifying treatment were: Gastrointestinal symptoms (7 patients), complications or reactions after drug administration (7 patients) and lymphopenia (3 patients all treated with dimethyl fumarate).

The mean follow-up time from cladribine initiation was 33.9 ± 11.1 months (median 34.7; IQR 23.3 – 43.7) and 199 patients (74%) were followed up for more than 24 months. Overall, 214 (80%) patients had completed two cycles of treatments, and 14 patients (5%) had received a 3rd cladribine course.

Table 1
Patient demographics and clinical characteristics at the time of cladribine initiation.

	Patients (*N* = 268)
Age, years, mean (SD), median (IQR)	40.6 (10.7), 40 (32 – 48)
Female, n (%)	179 (66.8)
Disease course, RRMS, n (%)	262 (97.8)
Disease duration from MS onset, years, mean (SD), median (IQR)	9.9 (7.6), 8.1 (4.2 – 14.5)
Disease duration from MS diagnosis, years, mean (SD), median (IQR)	7.7 (7.0), 5.2 (2.3 – 12.0)
Number of previous DMTs, n (%)	
None	34 (12.7)
1	56 (20.9)
2	67 (25.0)
≥ 3	111 (41.4)
EDSS, mean (SD), median (IQR) *	2.7 (1.8), 2.5 (1.5 – 3.5)
Number of relapses in the previous year, mean (SD), median (IQR)	0.6 (0.8), 0 (0 – 1)
Number of relapses in the previous year, n (%)	
0	141 (52.6)
1	93 (34.7)
≥ 2	34 (12.7)
Baseline MRI - number of T2 lesions, mean (SD), median (IQR) **	11.7 (10.5), 15 (0 – 25)
Baseline MRI - number of T2 lesions, n (%) **	
0–5	69 (25.7)
6–9	8 (3.0)
10–20	34 (12.7)
≥ 20	50 (18.7)
Uncountable	10 (3.7)
Baseline MRI - activity, n MRI with signs of disease activity (%) ***	79 (46.2)

SD = Standard Deviation, IQR = Interquartile range, MS = Multiple Sclerosis, EDSS = Expanded Disability Status Scale, MRI = Magnetic Resonance Imaging.
* Baseline EDSS (−180/+90 days of cladribine start) available for 249 patients (92.9%).
** Baseline MRI (cladribine start ± 90 days) available for 171 patients (63.8%).
*** Correction: MRI activity defined as presence of New/enlarging T2 lesions or Gd^+ lesions compared to previous MRI.

Table 2
Last disease-modifying treatment (DMT) prior to cladribine initiation.

Last DMT	N patients (%)	Treatment duration in months, median (IQR)
Natalizumab	55 (20.5)	24 (9 – 50)
Fingolimod	40 (14.9)	27 (16 – 54)
Teriflunomide	40 (14.9)	28 (12 – 40)
Dimethyl fumarate	35 (13.1)	16 (6 – 46)
Glatiramer acetate	24 (9.0)	8 (3 – 23)
Interferon-beta*	11 (4.1)	14 (6 – 26)
Daclizumab	9 (3.4)	8 (6 – 10)
Ocrelizumab	8 (3.0)	6 (1 – 11)
Alemtuzumab	8 (3.0)	58 (44 – 62)
Other	4 (1.5)	13 (8 – 27)
None (treatment naïve)	34 (12.7)	–
Total	268 (100)	–

* Includes Peginterferon-beta.

3.2. Effectiveness

3.2.1. Annualized relapse rate

After cladribine initiation, clinical relapses were recorded in 67

Table 3
Reasons for discontinuation of last DMT prior to cladribine in 234 previously treated patients.

Reason	N patients (%)
Disease activity	97 (41.5)
Adverse events	59 (25.2)
JCV antibodies	32 (13.7)
Other reasons*	26 (11.1)
Practical issues	10 (4.3)
Pregnancy/Pregnancy planning	7 (3.0)
Anti-drug antibodies	3 (1.3)
Total	234 (100)

* Includes contra-indication, lack of compliance, termination, progression, patient's decision.

patients (25% of total cohort). After excluding events happening before the re-baseline (90 days after cladribine initiation), 56 patients (21%) experienced a total of 70 relapses. Of these, 31 relapses were recorded in 28 patients between re-baseline and the second cladribine course, 36 relapses were recorded in 28 patients after the second cladribine course, and 3 relapses were recorded in 3 patients after the third cladribine course.

Overall, cladribine treatment reduced the ARR from 0.67 (95% CI [0.56, 0.79]) in the year prior to baseline to 0.11 [0.07, 0.15] in the first year after re-baseline (84.2% reduction); 0.11 [0.08, 0.15] in 2 years after re-baseline (83.4% reduction); and 0.10 [0.08, 0.13] when considering the whole follow-up period after re-baseline (84.8% reduction) (all p-values < 0.001; Fig. 1). The analysis stratified by patients' previous therapy further showed that a significant reduction in the ARR was obtained in all subgroups, although less pronounced in patients switching to cladribine from a HeDMT (Fig. 1).

The multivariable negative binomial regression models showed that switching from a HeDMT and having a high level of disease activity prior to baseline were risk factors associated with relapses for all the follow-up time periods considered, whereas being <40 years of age prior to baseline was a significant risk factor only when considering the whole follow-up period (Fig. 2).

3.2.2. 24-week CDW

Considering the last available follow-up, 15 out of 176 patients included in the analysis experienced a 24-week CDW (8.5%). This risk was comparable to the risk estimated in the Kaplan Maier (KM) survival analysis at the median follow-up time of 30 months (7.3%; 95% CI 3.0 – 11.4; Fig. 3), when KM estimates are more stable. Patients switching to cladribine from a HeDMT had a reduced hazard of disability worsening than patients switching from MeDMT or treatment naïve, but only before 38 months (HR 0.080; 95% CI 0.010 – 0.633, $p = .017$). None of the other variables included in the model had a significant effect on the hazard. However, given the low number of events recorded, these results must be interpreted cautiously.

3.2.3. MRI activity

Considering the last available follow-up, 59 out of 165 patients included in the analysis showed signs of radiological disease activity (35.8%). Again, this risk was comparable to the risk estimated in the KM survival analysis at the median follow-up time of 24 months after re-baseline (64.6% [95% CI 56.8 – 73.5] of the patients were free of MRI activity; Fig. 4). Patients <40 years at baseline had an increased hazard of having MRI activity compared to older patients (HR 2.521; 95% CI

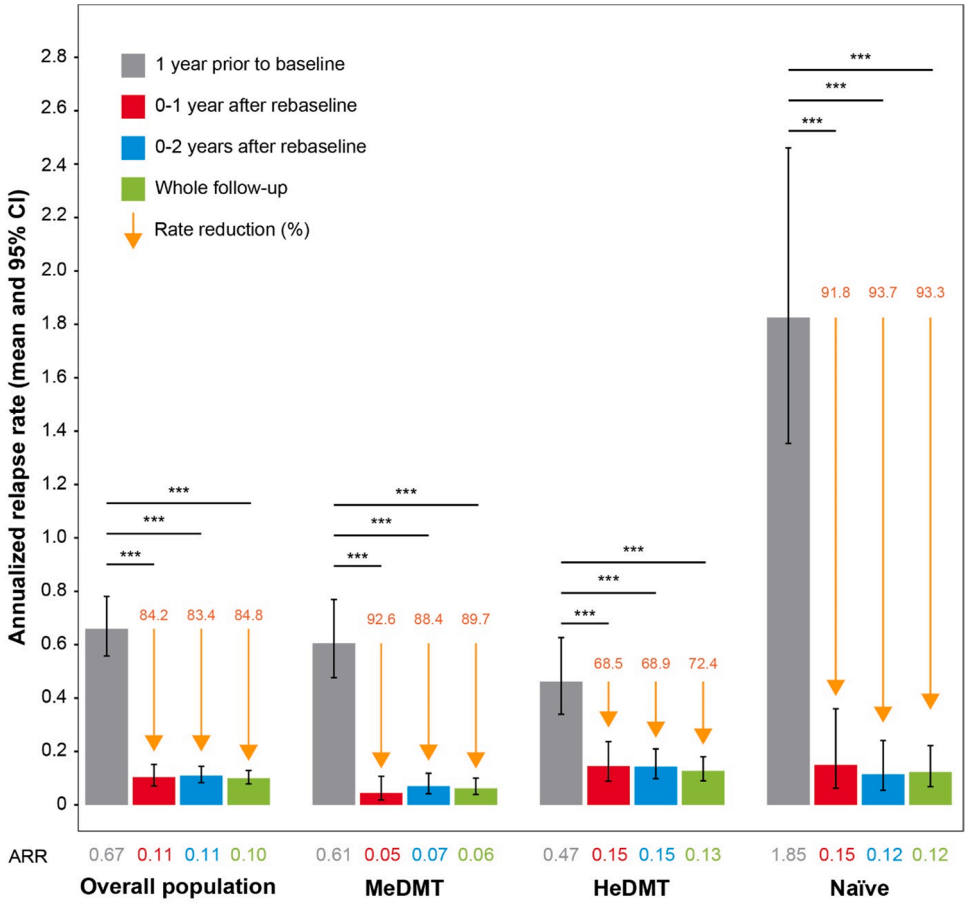

Fig. 1. Annualized relapse rate for different periods in the entire population and stratified by previous therapy (moderately effective (MeDMT), highly effective (HeDMT) or treatment naïve (Naïve).

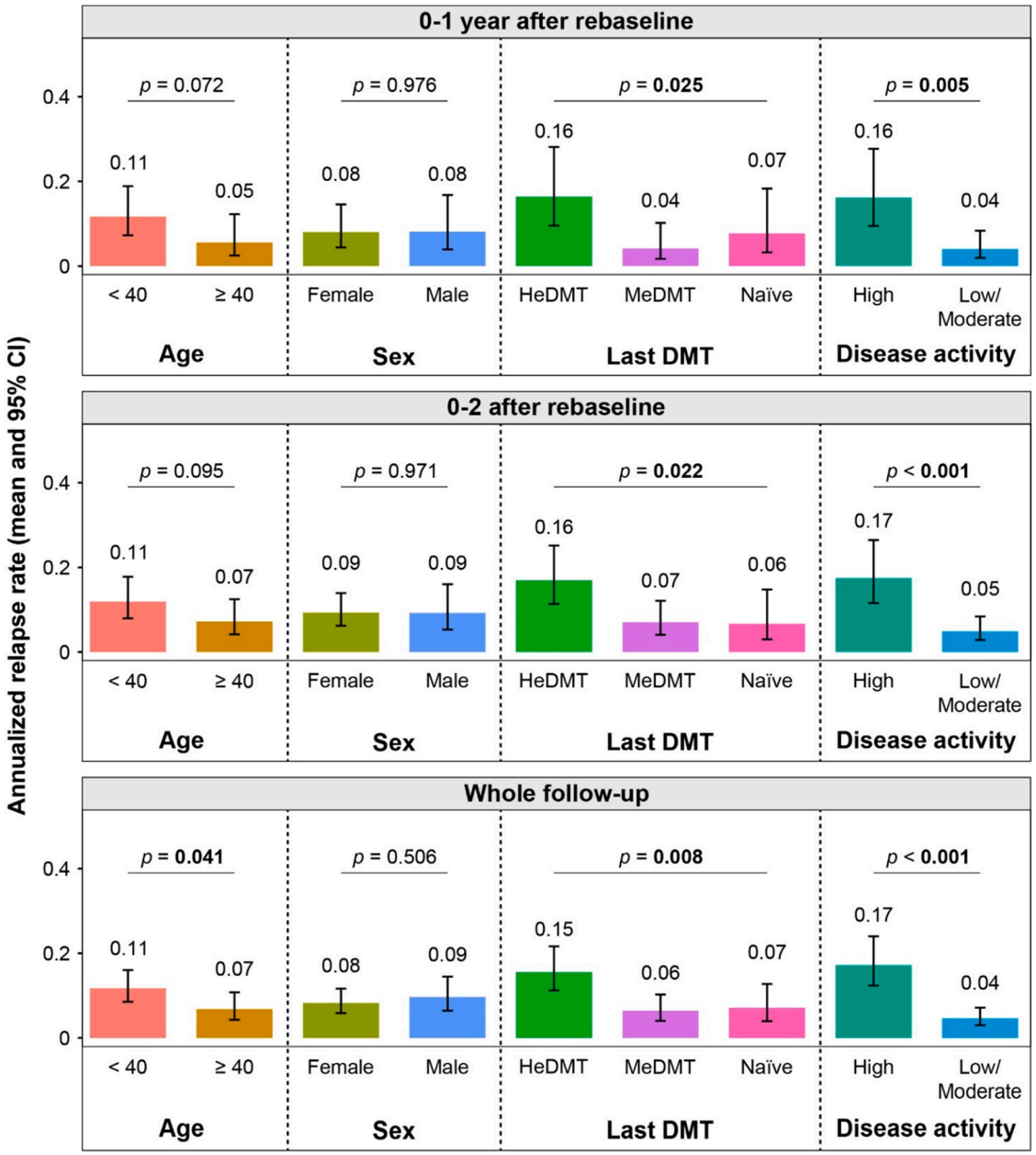

Fig. 2. Adjusted annualized relapse rate for different groups of patients in the three follow-up periods considered. Significant p-values are showed in bold. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
MeDMT: moderately effective disease-modifying treatment; HeDMT: highly effective disease-modifying treatment; Naïve: treatment naïve.

1.408 – 4.512, $p = .002$), whereas none of the other variables included had a significant effect.

3.2.4. NEDA-3

By the end of the follow-up, 47 out of 113 patients (41.6%) included in the analysis had still NEDA-3 status. This was again comparable to what was estimated in the KM survival analysis at the median follow-up time of 37 months (40.6% [95% CI 31.9 – 51.6] of the patients had NEDA-3 status preserved; Fig. 5). None of the baseline variables included in the Cox regression analysis had a significant effect on the hazard of losing NEDA-3 status.

3.3. Safety

A total of 44 patients reported at least one AE (Table 4). Adverse events were mild or moderate. The most reported event was lymphopenia (8 patients). Herpes zoster was only reported in 2 patients and was dermatomal in both. No malignancies were reported. In total, 30 (11.2%) patients discontinued cladribine treatment, of whom 7 (2.6%) discontinued because of adverse effects and 12 (4.5%) discontinued because of disease activity. After discontinuation of cladribine therapy 18 patients switched to another DMT: 9 to ocrelizumab, 6 to other HeDMTs and 3 to MeDMTs.

4. Discussion

In this real-world nationwide population-based longitudinal cohort study with prospectively enrolled patients we found that cladribine tablets reduced ARR with 84.8% compared with the year prior to start of cladribine. Patients switching from a HeDMT had a higher risk of experiencing relapses in all the follow-up time periods considered, suggesting that these patients belonged to the group highly active

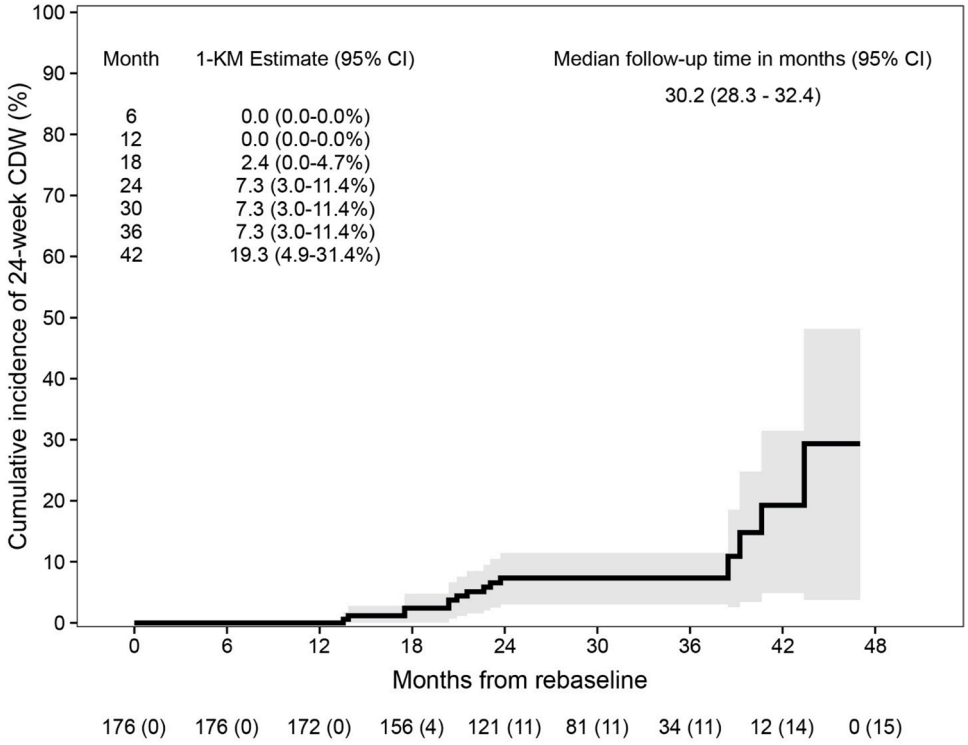

Fig. 3. Kaplan-Maier (KM) curve for 24-week confirmed disability worsening (CDW). Number of patients at risk and cumulative number of events (in parenthesis) are showed below the x-axis.

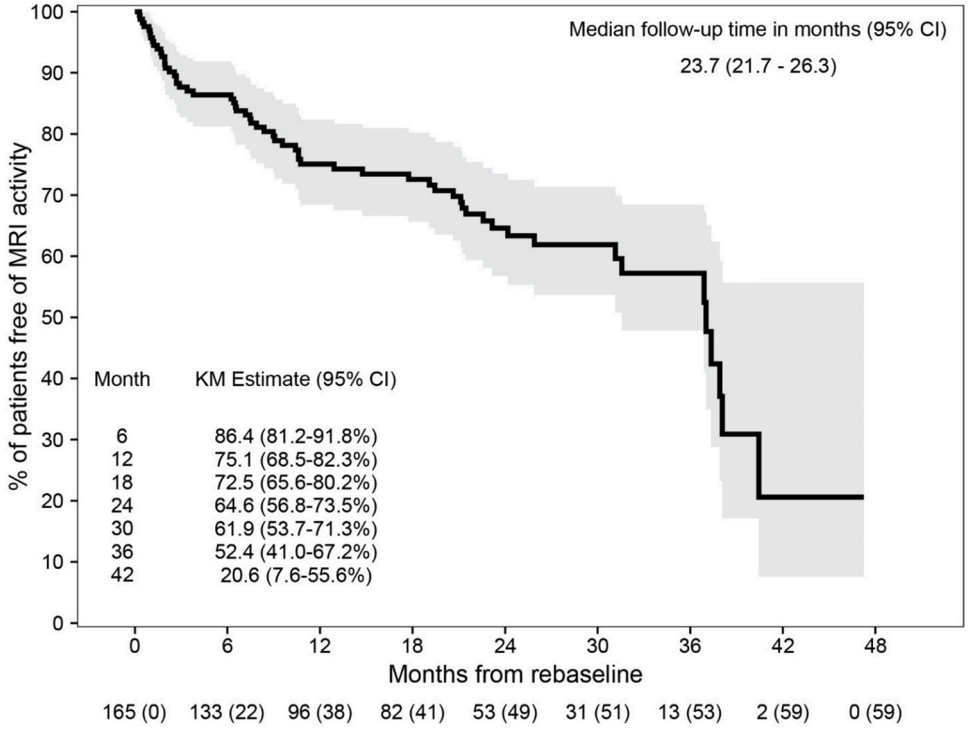

Fig. 4. Kaplan-Maier (KM) curve for MRI activity. Number of patients at risk and cumulative number of events (in parenthesis) are showed below the x-axis.

patients and many of these patients had been effectively controlled on HeDMTS like alemtuzumab and natalizumab. In the present study, the ARR during cladribine treatment (0.10) was comparable to that seen in the CLARITY study (3.5 mg/kg group) 0.14) (Giovannoni et al., 2010). Disease activity-free (NEDA-3) status after 1 year was achieved by 71.7% and after 2 years by 49.0% compared to 54% and 44%, respectively, in the CLARITY trial (Giovannoni et al., 2011a).

In a 2-year CLARITY EXTENSION study AAR remained low in patients, who in the CLARITY trial were treated with cladribine 3.5 mg/kg (ARR 0.14; 95% CI 0.12–0.17), independently whether patients were

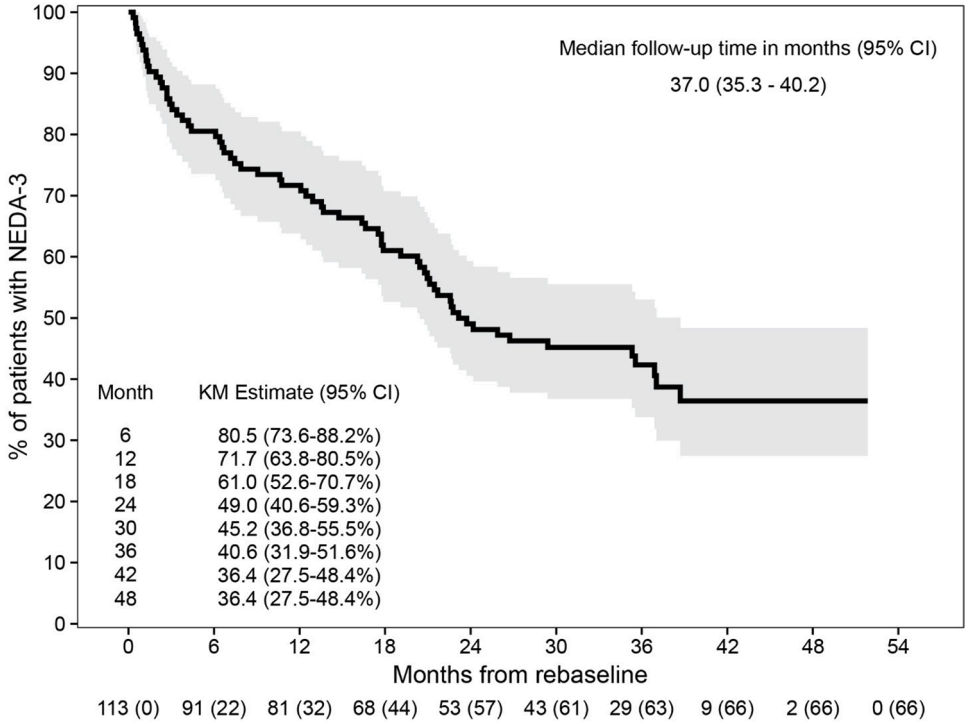

Fig. 5. Kaplan-Maier (KM) curve for NEDA-3. Number of patients at risk and cumulative number of events (in parenthesis) are showed below the x-axis.

Table 4
Adverse events (AEs) recorded in relation to cladribine treatment.

Adverse event	N patients (%)*
Lymphopenia	8 (3.0)
Influenza-like symptoms	5 (1.9)
Toxic liver disease	5 (1.9)
Herpes simplex infections	3 (1.1)
Upper respiratory infection/Pneumonia	3 (1.1)
Urinary infection	< 3 **
Alopecia	< 3 **
Amenorrhea	< 3 **
Condyloma	< 3 **
Post-herpetic neuralgia	< 3 **
allergy	< 3 **
diarrhea	< 3 **
Thyrotoxicosis	< 3 **
Other	7 (2.6)
Total	44 (16.4)

* Percentages expressed out of the total cohort ($N = 268$).
** Less than 3 incidences are anonymized (< 3) to comply with GDPR.

randomized to placebo ($N = 98$) (ARR 0.15; 95% CI 0.09–0.21) or cladribine 3.5 mg/kg ($N = 186$) (ARR 0.10; 95% CI 0.06–0–13) in the CLARITY EXTENSION (NS) (Giovannoni et al., 2017). In the CLARITY EXTENSION study no evidence of disease activity-3 (NEDA-3) was seen in 29.6% of patients treated with 3·5 mg/kg in the CLARITY trial and placebo in the CLARITY EXTENSION, which owing to the variable bridging interval of 0.1–29 months between CLARITY and CLARITY EXTENSION is equivalent with NEDA-3 in 29.6% at 48 to 77 months after treatment with 3·5 mg/kg (Giovannoni et al., 2021).

Our subgroup analyses did not suggest a reduced effect in patients ≥ 40 years, which is in line with a recent study of 62 patients <50 years and 35 patients ≥50 years showing that there was no difference in time to evidence of disease activity and occurrence of adverse events between the age groups (Disanto et al., 2022).

For all analyses of clinical endpoints, we used a re-baseline 90 days after the baseline date, to account for the fact that cladribine might not be working in the first months after initiation of therapy, although a recent study has shown that the effect on MRI activity was present already from month 1 and increasing over time (de Stefano et al., 2022).

In the present study, adverse events were relatively infrequent and usually mild, which is in line with the observations in clinical trials. In clinical trials cladribine tablets were well tolerated with no symptoms in relation to drug administration. Across all studies cladribine showed a favorable safety profile. As a reflection of the mechanism of action of cladribine (Leist and Weissert, 2011), lymphopenia was frequent in the cladribine groups in the CLARITY trial (Giovannoni et al., 2010). However, 86–89% of patients recovered to Grade 0 or 1 lymphopenia by week 48 in each treatment year. One patient had severe pancytopenia, and the patient turned out to have a reactivation of latent tuberculosis and subsequently died (Cook et al., 2011). Infections or infestations were reported as serious adverse events in 2.3% of cladribine-treated patients and 1.6% of placebo-treated patients, and the incidence of infections in the cladribine groups showed an inverse correlation with the lowest lymphocyte count. Herpes zoster infections, all dermatomal restricted, developed in 2.2% patients who received cladribine, most frequently seen in patients suffering lymphopenia grade 3 or 4 (Giovannoni et al., 2010). A summary of side effects from cladribine tablets treatment in the first 12 weeks of two clinical studies called CLARITY and ORACLE-MS showed that most side effects were mild and only 1.6% of patients on cladribine tablets and 1.4% placebo treated patients discontinued treatment due to side effects (Oh et al., 2022).

PML has not been reported in MS patients treated with cladribine but has been observed in patients treated with cladribine for malignant diseases (Alstadhaug et al., 2017).

Observations from all clinical trials and observational studies of cladribine in MS, including the present study, do not support any suspicion of an increase in rate of malignancies.

The results of the present study were in line with a recently published real-world data report from two centers, in which 270 RRMS patients treated with cladribine were followed for a median of 25 months. Of these 234 had received a full treatment and 142 had been followed for ≥ 24 months. 97 (36%) of the patients were treatment naïve and 74 (27%) had been treated with one DMD previously. The ARR rate dropped from 1 to approximately 0.2 after 6 months and remained at the same level. In

total, 69 patients suffered a relapse, of whom 40 patients had a relapse within the first year of treatment. Sixty-five patients experienced confirmed worsening of disability during the observation period. Previously natalizumab treatment was reported as a risk factor for new clinical and MRI activity (Pfeuffer et al., 2021).

A Finnish nationwide register study of 179 patients found a mean annualized relapse rate of 0.1 after a median follow-up time of 19 months compared with 1.0 before initiation of cladribine therapy (Rauma et al., 2022).

A single center study retrospectively reviewed 46 patients treated with alemtuzumab and 65 patients treated with cladribine (51 i.v. cladribine and 14 cladribine tablets) with a follow-up time of 3.3 years. Alemtuzumab had a higher rate of 2-year NEDA than cladribine (OR 4.78, 95%CI: 1.57–14.50, p=.006), but beyond 2 years the difference was not statistically significant (HR 0.50, 95%CI: 0.25–1. 30, p=.061). It is difficult, however, to interpret the results as the cladribine group was older (p=.0002), with higher baseline EDSS (p=.0015), and more likely secondary progressive ($p<.0001$) (Bose et al., 2021).

A propensity score-matched analysis from MSBase compared the effectiveness of cladribine with interferon β, fingolimod or natalizumab, comprising 37 patients with cladribine treatment out of 5279 patients. After 1 year the probability of experiencing a relapse on cladribine was lower than on interferon, comparable to fingolimod and higher than on natalizumab, while the probability of disability accumulation on cladribine was similar to interferon and fingolimod but greater than natalizumab, whereas the probability of disability improvement was higher on cladribine than on the other drugs (Kalincik et al., 2018). The very low number of cladribine treated patients and the short observation time make it difficult to assess the efficacy of cladribine compared to other DMDs.

An indirect network meta-analysis comparing cladribine with fingolimod, dimethyl fumarate and teriflunomide was performed including six randomized trials using NEDA-3 as outcome. The rate of NEDA-3 was significantly higher in cladribine tablets vs dimethyl fumarate: OR (odds ratio) =1.76 (95% CI: 1.02–3.03) and teriflunomide: OR=2.78 (95% CI: 1.60–4.83), but not vs fingolimod: OR=1.04 (95% CI: 0.65–1.64) (Bartosik-Psujek et al., 2021).

A study of 73 patients who had been treated with fingolimod and were switched to either cladribine ($N = 33$) or rituximab ($N = 40$) found that relapses and MRI disease activity was observed more frequently in patients receiving cladribine than in patients receiving rituximab, which could suggest that rituximab had a faster or higher effect than cladribine. The study, however, was retrospective and not randomized (Nygaard et al., 2022).

In a long-term follow-up study, a cohort of 90 cladribine-treated relapsing forms of MS (RRMS 70, SPMS 18) patients were retrospectively followed for median 3.5 (2.0–4.3) years. Mean age on starting cladribine was 47 years; mean age at MS onset was 34 years, and median baseline EDSS score was 5.25. Of these 66 had follow-up with EDSS. Approximately 80% of patients were EDSS progression-free, 65% remained relapse-free after 2 years, and median time to next DMT in 62 (69%) was 1.7 years with fingolimod (26 patients), dimethyl fumarate (13 patients), natalizumab (10 patients) and teriflunomide (6 patients) being the most frequently used DMTs after cladribine (Lizak et al., 2021).

The present study is one of the largest series of cladribin-treated patients yet published and the data represent a nationwide cohort, capturing every cladribine-treated patient in Denmark. Reporting of data to the MS Registry is mandatory at every clinical visit which ensures uniform prospectively collected real-time data. The study is a population-based review that provides important information regarding prescription of cladribine in Denmark and the therapeutic results in a large, unselected cohort of Danish patients starting therapy with cladribine tablets in a real-world setting.

Our study has some limitations. More than one third of the patients did not have a baseline MRI (date of treatment initiation ± 3 months) which had several explanations: A part of the study covered the COVID-19 pandemia period and during this period many patients had planned MRIs canceled or postponed more than 3 months and some of missing values are probably due to a lack of data-entry by the treating neurologist. The COVID-19 pandemia also caused cancelation or postponement of planned regular clinical follow-up visits and follow-up scans and postponed the 1-year treatment cause. These irregularities also made it difficult to measure 1- and 2-year NEDA.

5. Conclusion

This nation-wide review provides important information regarding prescription of cladribine and the therapeutic results in a large, unselected cohort of Danish patients starting therapy with cladribine tablets in a real-world setting.

The study showed that cladribine treatment was safe, and the therapeutic response was as expected from previous clinical trials. A prolonged observation period is necessary to assess the long-term benefit and risk of cladribine.

CRediT authorship contribution statement

Per Soelberg Sorensen: Conceptualization, Methodology, Formal analysis, Writing – original draft. **Luigi Pontieri:** Conceptualization, Methodology, Formal analysis, Writing – review & editing. **Hanna Joensen:** Formal analysis, Writing – review & editing. **Alex Heick:** Resources, Writing – review & editing. **Peter Vestergaard Rasmussen:** Resources, Writing – review & editing. **Jakob Schäfer:** Resources, Writing – review & editing. **Rikke Ratzer:** Resources, Writing – review & editing. **Caroline Ellinore Pihl:** Resources, Writing – review & editing. **Finn Sellebjerg:** Resources, Writing – review & editing. **Melinda Magyari:** Conceptualization, Methodology, Formal analysis, Resources, Writing – review & editing.

Declaration of Competing Interest

Per Soelberg Sørensen has received personal compensation for serving on scientific advisory boards, steering committees, independent data monitoring committees or have received honoraria as speaker from Biogen, Merck, Novartis, TEVA, GlaxoSmithKline, Sanofi/Genzyme, and BMS/Celgene.

Alex Heick has served on scientific advisory boards for Biogen, Sanofi-Genzyme, Novartis, Janssen and Merck. Honoraria for lecturing has been received from Biogen, Merck, Novartis and Sanofi. Support for congress participation has been received from Biogen, Sanofi-Genzyme, Teva, Roche, Merck, Novartis, Bayer, Schering, Pfizer and Janssen.

Peter Vestergaard Rasmussen has served on scientific advisory boards for, served as consultant for, received support for congress participation or received speaker honoraria from Alexion, Biogen, Bristol Myers Squibb, Merck, Novartis, Roche and Sanofi Genzyme.

Jakob Schäfer has served on a scientific advisory board with Sanofi, received speaker honoraria from Novartis and received travel compensation from Merck, Roche, Sanofi.

Rikke Ratzer has served on scientific advisory boards, received speaker honoraria and received support for congress participation from Roche, Merck, Sanofi, Medtronic and Ipsen.

Finn Sellebjerg holds a professorship at the Faculty of Health Sciences and Medicine, University of Copenhagen, sponsored by the Danish Multiple Sclerosis Society. He has served on scientific advisory boards for, served as consultant for, received support for congress participation or received speaker honoraria from Alexion, Biogen, Bristol Myers Squibb, Merck, Novartis, Roche and Sanofi Genzyme. His-laboratory has received research support from Biogen, Merck, Novartis, Roche and Sanofi Genzyme.

Melinda Magyari has served in scientific advisory board for Sanofi, Novartis, Merck, and has received honoraria for lecturing from Biogen,

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