Cladribine versus fingolimod, natalizumab and interferon β for multiple sclerosis

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Abstract:	<p>Objective</p> <p>This propensity score-matched analysis from MSBase compared the effectiveness of cladribine with interferon β, fingolimod or natalizumab.</p> <p>Methods</p> <p>We identified all patients with relapse-onset multiple sclerosis, exposure to the study therapies, and ≥ 1-year on-treatment follow-up from MSBase. Three pairwise propensity score-matched analyses compared treatment outcomes over one year. The outcomes were hazards of first relapse, disability accumulation and disability improvement events. Sensitivity analyses were completed.</p> <p>Results</p> <p>The cohorts consisted of 37 (cladribine), 1940 (interferon), 1892 (fingolimod) and 1410 patients (natalizumab). The probability of experiencing a relapse on cladribine was higher than on interferon ($p=0.05$), similar to fingolimod ($p=0.31$) and lower than on natalizumab ($p=0.042$). The probability of disability accumulation on cladribine was similar to interferon ($p=0.37$) and fingolimod ($p=0.089$) but greater than natalizumab ($p=0.021$). The probability of disability improvement was higher on cladribine than interferon ($p=0.00017$), fingolimod ($p=0.0025$) or natalizumab ($p=0.00099$). Sensitivity analyses largely confirmed the above results.</p> <p>Conclusions</p> <p>Cladribine is an effective therapy for relapse-onset multiple sclerosis. Its effect on relapses is comparable to fingolimod and its effect on disability accrual is comparable to interferon β and fingolimod. Cladribine may potentially associate with superior recovery from disability relative to interferon, fingolimod and natalizumab.</p>

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Cladribine versus fingolimod, natalizumab and interferon β for multiple sclerosis

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Keywords

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Abstract

Objective

This propensity score-matched analysis from MSBase compared the effectiveness of cladribine with interferon β , fingolimod or natalizumab.

Methods

We identified all patients with relapse-onset multiple sclerosis, exposure to the study therapies, and ≥ 1 -year on-treatment follow-up from MSBase. Three pairwise propensity score-matched analyses compared treatment outcomes over one year. The outcomes were hazards of first relapse, disability accumulation and disability improvement events. Sensitivity analyses were completed.

Results

The cohorts consisted of 37 (cladribine), 1940 (interferon), 1892 (fingolimod) and 1410 patients (natalizumab). The probability of experiencing a relapse on cladribine was higher than on interferon ($p=0.05$), similar to fingolimod ($p=0.31$) and lower than on natalizumab ($p=0.042$). The probability of disability accumulation on cladribine was similar to interferon ($p=0.37$) and fingolimod ($p=0.089$) but greater than natalizumab ($p=0.021$). The probability of disability improvement was higher on cladribine than interferon ($p=0.00017$), fingolimod ($p=0.0025$) or natalizumab ($p=0.00099$). Sensitivity analyses largely confirmed the above results.

Conclusions

Cladribine is an effective therapy for relapse-onset multiple sclerosis. Its effect on relapses is comparable to fingolimod and its effect on disability accrual is comparable to interferon β and fingolimod. Cladribine may potentially associate with superior recovery from disability relative to interferon, fingolimod and natalizumab.

Introduction

Cladribine (2-chlorodeoxyadenosine) is a purinergic antimetabolite with a preferential effect on lymphocytes. Its intracellular metabolite, 2-chlorodeoxyadenosine triphosphate triggers lymphocyte apoptosis through inhibiting DNA synthesis and repair.¹ Cladribine primarily triggers rapid and sustained reductions in numbers of CD4+ and CD20+ cells and transient reduction in numbers of CD8+ and natural killer cells.² The compound has an established role as a treatment for several subtypes of leukaemia and Non-Hodgkin's lymphomas. It has recently been trialled as an immunotherapy for relapsing-remitting multiple sclerosis (MS).³

Superiority of cladribine to placebo in relapsing-remitting MS was demonstrated in the CLARITY phase 3 randomised controlled trial.⁴ Cladribine 3.5 mg/kg was superior to placebo in suppressing relapse activity (annualised relapse rate 0.14 vs. 0.33, respectively) and increasing the probability of remaining relapse-free by 127%. Cladribine was also associated with a relative 55% increase in the probability of remaining free from 3-month confirmed disability progression.

Cladribine was approved for indication relapsing-remitting MS in Australia in 2010, but the application was subsequently rejected in Europe and the US. At the present time, a re-submission to the European Medicines Agency is pending a decision. Evidence for effectiveness of cladribine compared to other agents is critical for determining its position in the current treatment landscape, however, no direct head-to-head comparisons of cladribine to other potent immunotherapies are available.

The Australian Cladribine Product Familiarisation Program in 2011 involved 144 patients with relapse-onset MS who received one course of cladribine in a clinical practice setting.⁵ Data from 90 of these patients, as well as additional 21 patients treated off-label, were captured in MSBase, a global observational cohort study of MS. MSBase has developed a track record of comparing effectiveness of MS immunotherapies using observational data.^{6, 7} Here we conducted a propensity score-matched analysis of observational data from MSBase, including patients from Australian Cladribine Product Familiarisation Program, in order to compare the effectiveness of cladribine to that of three other common immunotherapies, interferon β , fingolimod and natalizumab.

Methods

Ethics Statement

MSBase is an international observational cohort study of MS, registered with the World Health Organization International Clinical Trials Registry Platform, ID ACTRN12605000455662.⁸ The study was approved by the Melbourne Health Human Research Ethics Committee, and by the site institutional review boards (or exemptions were granted, according to local regulations). Written informed consent was obtained from enrolled patients, as required.

Patients

The inclusion criteria were definite relapse-onset MS,^{9, 10} exposure to one of the study therapies, ≥1 year of continuous study monotherapy (in the cladribine group, patients were considered to be treated for 1 year after their exposure to cladribine), no prior exposure to alemtuzumab, mitoxantrone, rituximab or haematopoietic stem cell transplantation, minimum required recorded follow-up (3 months prior to treatment start and 2 disability scores ≥6 months apart with at least one score recorded while on the study therapy) and minimum dataset (consisting of sex, age, date of first MS symptom, dates of clinical relapses, clinical MS course, and disability score at treatment commencement [-1 year to +1 month]).

Procedures

The included patients were treated with one of the study therapies: cladribine (3.5 mg/kg total dose, initial treatment consisting of 2 courses completed in 2 weeks), interferon beta-1a (44 µg s.c. three times weekly), fingolimod (0.5 mg oral daily) and natalizumab (300 µg i.v. every four weeks). Baseline was defined as the first commencement of the study therapy and patients were censored at discontinuing therapy, commencing a post-baseline disease modifying therapy, the last recorded disability score, or at the end of the first year post-baseline, whichever occurred first.

The analysed data were recorded as part of routine clinical practice, mostly at tertiary MS centres, with data entry at the time of clinical visits. The MSBase Observational Plan stipulates minimum annual evaluations of neurological status of the included patients. Data entry portals were iMed or MSBase online data entry system. Rigorous automated quality assurance procedure was applied, assessing erroneous data entries, data density and generalisability as described elsewhere.¹¹

Outcomes

The study endpoints were the proportion of patients free from relapses, disability accumulation events and disability improvement events while on study therapy.

A relapse was defined as new symptoms or exacerbation of existing symptoms persisting for ≥ 24 hours, in the absence of concurrent illness/fever, and occurring ≥ 30 days after a previous relapse. Information about relapses, their date of onset, symptomatology, severity, therapy and recovery was recorded by treated neurologists. Their confirmation with disability score was not required.

Disability was scored using Expanded Disability Status Scale (EDSS) by accredited EDSS scorers (Neurostatus certification was required at the participating centres), excluding the scores recorded within 30 days of a previous relapse. Disability accumulation was defined as an on-treatment increase in EDSS by 1 step (1.5 step if baseline EDSS was 0 and 0.5 steps if baseline EDSS was >5.5) confirmed by subsequent EDSS scores over ≥ 6 months (irrespective of treatment status at confirmation). Disability improvement was defined as a decrease in EDSS by 1 step (1.5 step if baseline EDSS was 1.5 and 0.5 steps if baseline EDSS was >6 ; decrease from EDSS step 1 to step 0 was not be evaluated as confirmed disability improvement) confirmed by subsequent EDSS scores over ≥ 6 months.¹²

Statistical analysis

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Matching and statistical analyses were conducted using R (version 3.0.3),¹³ in three separate matched analyses of cladribine vs. interferon β , cladribine vs. fingolimod, or cladribine vs. natalizumab. Individual patients were matched on their propensity of receiving either of the compared therapies.^{6, 14} Individual propensity scores were calculated using a multivariable logistic improvement model of treatment allocation that used as independent variables the demographic and clinical variables available at the time of treatment assignment: sex, age, time from first MS symptom, EDSS, MS course, number of relapses in the prior 3 months, number of prior MS therapies, and the most effective prior MS therapy.

Patients were matched in a variable 10:1 ratio using nearest neighbour matching within a caliper of 0.5 standard deviations of the propensity score, without replacement and with exact match on EDSS (categorised as 2-EDSS step bins). All subsequent analyses were designed as paired models with weighting to adjust for the variable matching ratio. A maximum cumulative weight for each matched patient was 1. The common on-treatment follow-up was determined in each matched pair as the shorter of the two patient follow-up periods (pairwise censoring) to mitigate attrition bias, informative censoring and the effect of differential treatment persistence.¹⁵

Tests of statistical inference were carried out at $\alpha=0.05$. No correction for false discovery rate was required as the number of tests of statistical inference per pairwise comparison was 9. Cumulative hazards of freedom from relapses, EDSS accumulation and EDSS improvement were evaluated with weighted conditional proportional hazards models (Cox). Where the proportionality of hazards assumption was violated (as per Schoenfeld's global test), interaction term for treatment and time was included or Weibull models were used instead.

Robustness of the statistically significant differences to unidentified confounders was quantified with Rosenbaum sensitivity test for Hodges-Lehmann Γ .¹⁶ Where no statistically significant differences were observed, analytical power was quantified as the minimum effect magnitude detectable within the available cohort at $1-\beta=0.8$ using 200 simulations.

Three sensitivity analyses were completed. The sensitivity analyses evaluated the robustness of the results to potential confounders, such as the matching parameters (using a loose definition of matching consisting of 20:1 variable matching within a large caliper of 5), matching exactly on

MS course but not disability, and follow-up duration (allowing inclusion of patients with <1 year on-treatment follow-up).

Results

Of the 111 patients exposed to cladribine identified in the MSBase cohort, 90 patients took part in the Australian Cladribine Product Familiarisation Program and 21 were treated with cladribine off label (Supplementary Table S1). The numbers of eligible patients available for matching were 37 patients treated with cladribine, 1940 patients treated with interferon β , 1892 patients treated with fingolimod and 1410 patients treated with natalizumab (Figure 1). The 74 patients from the cladribine group who were excluded from the analysis had characteristics similar to the included cohort (mean age 45 years, disease duration 11 years, median EDSS 5, with 74% patients diagnosed with relapsing-remitting MS and 12% with secondary progressive MS). The multivariable logistic regression models that were used to estimate the individual propensity scores indicated that patients treated with cladribine tended to be older than the patients treated with any of the three comparator therapies, with a greater disability than interferon β and fingolimod, and with lower prior relapse activity than natalizumab (Supplementary Table S2).

The numbers of patients retained in the pairwise-matched cohorts are shown in Table 1. The matching procedure decreased the between-group differences in propensity scores from 0.41 to 0.01 (by 97%) for the comparison with interferon β , 0.12 to 0.001 (by 99%) for the comparison with fingolimod, and from 0.30 to 0.01 (by 96%) for the comparison of cladribine with natalizumab. Only mild to moderate differences in baseline characteristics were observed between the matched cohorts. On-treatment follow-up was identical between the matched cohorts as a result of pairwise censoring.

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The patients treated with cladribine (n=22) were less likely to experience a relapse during the first year of treatment (hazard ratio, HR 0.6 [95%CI 0.38-0.95], p=0.050) than the matched patients treated with interferon β (n=167; Figure 2). The proportions of relapse-free patients at the end of year one were 86% and 70%, respectively. The probability of disability accumulation was similar in the two matched cohorts (HR 0.41 [95%CI 0.87-1.47], p=0.37). Patients treated with cladribine were relatively more likely to experience a disability improvement event during the first year on treatment (HR 15, [95%CI 3.6-59], p=0.00017).

For both matched cladribine (n=32) and fingolimod (n=258) cohorts, the proportion of patients who remained relapse-free at the end of year one was 79% (Figure 3). Cumulative hazards of a relapse did not differ between the two groups (HR 1.2 [95%CI 0.83-1.8], p=0.31). The probability of disability accumulation was similar in the cladribine and fingolimod groups (HR 1.8 [95%CI 0.91-3.7], p=0.089). The probability of experiencing a disability improvement event was greater in the cladribine cohort when compared to the fingolimod cohort (HR 3.9 [95%CI 1.6-9.6], P=0.0025).

The patients treated with cladribine (n=26) were more likely to experience a relapse (HR 1.8 [95%CI 1.08-2.97], p=0.042) than the matched patients treated with natalizumab (n=174; Figure 4). However, at the end of year one, the proportions of relapse-free patients were 80% and 81%, respectively. The probability of disability accumulation was greater in the cladribine cohort (HR 2.5 [95%CI 1.2-5.6], p=0.021). The probability of experiencing a disability improvement event was greater among the patients treated with cladribine than those treated with natalizumab (HR 4 [95%CI 1.8-9.2], p=0.00099).

Sensitivity analyses largely confirmed the results of the primary analyses (Supplementary Table S3). The exception was the analysis the probability of a relapse, which did not replicate the difference between cladribine and interferon β from the primary analysis, and the probability of a disability progression event, which showed a difference favouring cladribine over interferon β in two sensitivity analyses. Also, the results of the sensitivity analysis using loose matching criteria showed results that differed from the primary analysis for the cladribine vs. fingolimod comparison.

Analysis of the minimum detectable effect size was completed for each primary analysis that did not show significant difference between the matched groups (Supplementary Table S4). The analyses in which no statistically significant effect was observed were sufficiently powered to detect minimum differences of 62% for the probability of remaining relapse-free, and 2-42% for the probability of experiencing disability accumulation. According to the Rosenbaum sensitivity test for Hodges-Lehmann Γ , the comparisons of annual relapse rates were vulnerable to potential unmeasured confounders.

Discussion

In this observational, propensity score-matched study of patients with relapse-onset MS from MSBase, cladribine was superior to high-dose interferon β , similar to fingolimod and inferior to natalizumab in reducing relapse activity during the first year of treatment. The probability of confirmed disability accumulation during the first year on cladribine was greater than on natalizumab and was comparable to fingolimod and interferon β . Cladribine was more frequently associated with confirmed disability improvement than interferon β , fingolimod or natalizumab.

In a phase 3 placebo-controlled trial in relapsing-remitting MS (CLARITY), the two tested doses of cladribine (3.5 mg/kg and 5.25 mg/kg) were superior to placebo in reducing relapse activity and accumulation of disability.⁴ At 96 weeks, 80% of the cladribine 3.5 mg group and 60% of the placebo group were free from relapses, and 91% of the cladribine 3.5 mg group and 85% of the placebo group were free from 6-month confirmed increase in EDSS.¹⁷ Even though the follow-up in our study was shorter than in CLARITY, the proportions of patients free from relapses (80-90%) and 6-month confirmed disability progression (90%) were in keeping with the data from the randomised trial. Two-year extension of the CLARITY trial (CLARITY-EXT) showed that in a majority of patients, the clinical benefits of cladribine used for 2 years were maintained for at least 4 years.¹⁸

A phase 2b placebo-controlled trial of cladribine as an add-on to interferon β (ONWARD) included 15% of patients with secondary progressive MS, a proportion that is similar to the representation of SPMS in our study.¹⁹ The study showed 63% reduction in the risk of a relapse

in add-on cladribine when compared to placebo. A phase 3 placebo-controlled randomised trial (ORACLE MS) in clinically isolated syndrome showed that cladribine was associated with a 67% reduction in the risk of second relapse indicative of conversion to clinically definite MS.²⁰ In our study, the magnitude of the effect of cladribine on relapses was relatively lower, which could be explained by the **comparison to** active therapies.

Currently, there are no randomised or observational studies comparing the efficacy of cladribine to other disease modifying therapies. Therefore, the information that cladribine is superior to interferon β , similar to fingolimod and inferior to natalizumab in reducing relapse activity could help define the place of cladribine in the context of the other available MS therapies. With the exception of natalizumab, which was marginally superior in preventing disability accumulation during the first year on treatment, we were unable to demonstrate differences in disability accrual between cladribine and the comparator therapies. Finally, 6-month confirmed improvement of disability was observed in 10-20% of the cladribine cohort during the first year, which was superior to all three comparator therapies. **This is of interest in the context of the comparison to natalizumab, which** is known to be associated with a marked improvement in disability early after its commencement.^{6, 7, 21} It is also of note that, on average, the matched patients included in this study had relatively long-standing MS (median MS duration 10-15 years), with a median age of 44-50 years, were usually previously treated with other immunotherapies, and suffered from moderately severe disability (median EDSS scores of 3.5-4). Improvement in disability in a cohort with this profile is unexpected. **Further validation of these results in patients with advanced MS is needed.**

We conducted multiple steps to mitigate potential biases, including matching, pairwise censoring, and adjusting the statistical models,¹⁵ an approach mirroring our previous studies.⁶ A large number of patients treated with natalizumab, fingolimod, or interferon β are available from the MSBase cohort; we were therefore able to achieve a satisfactory match on patients' demographic and clinical characteristics while maximising the power by one-to-multiple matching. Because the patients were exposed to only one dose of oral cladribine (**i.e. two courses over two weeks**) before the drug was withdrawn in Australia, we only analysed treatment outcomes during the initial year post-treatment. **This study therefore did not evaluate**

treatment outcomes after a full dose of oral cladribine (i.e. after the second dose at 12 months).

The primary analysis was conservative and limited to patients with a minimum 1-year follow-up.

The main limitation of this study consists in the limited size of the cladribine cohort. Only a small number of patients with MS were exposed to cladribine world-wide, and the Australian Cladribine Product Familiarisation Program is, to our knowledge, the only available real-world MS cohort treated with oral cladribine. We maximised the use of this cohort by capturing, in MSBase, two thirds of the Australian treated population, of whom one quarter had sufficient longitudinal data to enable comparison of treatment outcomes, including relapse incidence, disability accumulation or disability improvement. While the power of this study is thus limited, it demonstrated a number of statistically significant differences between cladribine and the three comparator therapies. Where no difference was observed, the minimum detectable effect size was of moderate magnitude. In these instances, small or moderate differences in relapse frequency and the probability of disability accumulation or improvement would not have been detected. Another limitation is the restricted duration of the cladribine follow-up. We are therefore only able to comment on short-term outcomes on cladribine relative to the comparator therapies. While a good overall balance between the compared cohorts was achieved using propensity score matching, a potential residual imbalance was observed in some of the variables. In particular, patients treated with cladribine tended to be older and with longer time from MS onset than those treated with natalizumab (mean differences of 6 years and 4 years, respectively) and the cladribine cohort was enriched for patients with secondary progressive MS relative to the matched fingolimod cohort. These residual imbalances could potentially influence the outcomes of the comparative analyses, deflating relapse frequency and inflating disability accrual recorded in the cladribine cohorts when compared to the fingolimod and natalizumab cohorts. The absence of systematically captured safety data represents another limitation. Therefore, risk–benefit ratios of cladribine and the other therapies should be carefully considered by clinicians for each patient individually. Finally, the lack of MRI data prevented us from using MRI activity as a matching or an outcome variable. The results presented were sensitive to hypothetical unmeasured confounders. To mitigate this risk, we demonstrated that the results were robust to variation in inclusion criteria and matching

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parameters. When using a loose match, thus simulating an unmatched scenario while preserving the paired structure of the models, the results differed from the primary analysis (namely for the cladribine vs. fingolimod comparison). This demonstrates the impact of stringent matching on the results of treatment comparisons, especially where the compared samples are heterogeneous.

In conclusion, cladribine is a proven effective therapy in relapse-onset multiple sclerosis. In our study, its effect on reducing relapse activity was comparable to fingolimod and its effect on disability accrual was comparable to interferon β and fingolimod, while its association with sustained recovery from previously accrued disability was superior to interferon β , fingolimod and natalizumab. Currently, the submission of cladribine to the European Medicines Agency for relapsing-remitting MS is pending. Therefore, our comparative effectiveness results, while limited by the small cohort size, will help inform the role of cladribine in the management of MS.

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The MSBase Study contributors are listed in the Online Supplement.

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Claudio Solaro did not declare any competing interests.

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Table 1
Baseline characteristics of matched patient groups

	Cladribine (n=22)	Interferon β (n=167)	d	Cladribine (n=32)	Fingolimod (n=258)	d	Cladribine (n=26)	Natalizumab (n=174)	d
female, nr (%)	17 (77%)	125 (75%)		26 (81%)	201 (78%)		20 (77%)	125 (72%)	
age, yr, mean $\pm$ SD	49 $\pm$ 10	45 $\pm$ 8	0.43	50 $\pm$ 10	48 $\pm$ 8	0.24	50 $\pm$ 9	44 $\pm$ 10	0.57
disease duration, yr, median (quartiles)	13.5 (5.6-18.3)	11.2 (6.5-17.1)	0.21	14.1 (7.6-23.8)	13.8 (8-19.5)	0.20	14 (6.2-17.9)	10 (4.5-17.2)	0.34
disability, EDSS, median (quartiles)	3.75 (2.25-5.9)	3.5 (2-4.5)	0.21	4.5 (3-6)	3.5 (2-5.5)	0.20	4 (2-6)	3.5 (2-5.5)	0.34
disease course, patients									
relapsing-remitting	19 (86%)	151 (90%)		24 (75%)	216 (84%)		22 (85%)	154 (89%)	
secondary progressive	3 (14%)	16 (10%)		8 (25%)	42 (16%)		4 (15%)	20 (11%)	
relapses 3 months pre-baseline, mean $\pm$ SD	0.1 $\pm$ 0.4	0.2 $\pm$ 0.4	0.23	0.2 $\pm$ 0.4	0.1 $\pm$ 0.4	0.07	0.1 $\pm$ 0.3	0.2 $\pm$ 0.4	0.27
visit interval, months, median (quartiles)	6 (4-12)	6 (3-6)	0.68	6 (4-12)	6 (3-6)	0.24	6 (4-12)	4 (3-6)	0.47
previous therapies, nr, median (quartiles)	1 (1-2)	1 (1-2)	0.29	2 (1-3)	2 (1-3)	0.01	1 (1-3)	1 (1-2)	0.05
most active previous therapy, patients									
IFN/GA	20 (91%)	150 (90%)		23 (72%)	187 (72%)		24 (92%)	155 (89%)	
Dimethyl fumarate	0 (0%)	0 (0%)		1 (3%)	5 (2%)		1 (4%)	3 (2%)	
Fingolimod	0 (0%)	1 (1%)		0 (0%)	0 (0%)		1 (4%)	10 (6%)	
Natalizumab	1 (5%)	4 (2%)		7 (22%)	60 (23%)		0 (0%)	0 (0%)	
none	1 (5%)	12 (7%)		1 (3%)	6 (2%)		0 (0%)	6 (3%)	

d, Cohen's d (standardised difference); EDSS, Expanded Disability Status Scale; SD, standard deviation

Figure legends

Figure 1

Patient disposition

CIS, clinically isolated syndrome; EDSS, Expanded Disability Status Scale; MS, multiple sclerosis; PPMS, primary progressive multiple sclerosis

Figure 2

Probability of first relapse (A), disability accumulation (B), disability improvement (C) events and disability in EDSS [median $\pm$ 95% confidence interval] (D) in matched patients treated with cladribine or interferon β .

95%CI, 95% confidence interval; EDSS, Expanded Disability Status Scale

Figure 3

Probability of first relapse (A), disability accumulation (B), disability improvement (C) events and disability in EDSS [median $\pm$ 95% confidence interval] (D) in matched patients treated with cladribine or fingolimod.

95%CI, 95% confidence interval; EDSS, Expanded Disability Status Scale

Figure 4

Probability of first relapse (A), disability accumulation (B), disability improvement (C) events and disability in EDSS [median $\pm$ 95% confidence interval] (D) in matched patients treated with cladribine or natalizumab.

95%CI, 95% confidence interval; EDSS, Expanded Disability Status Scale

Patient disposition
CIS, clinically isolated syndrome; EDSS, Expanded Disability Status Scale; MS, multiple sclerosis; PPMS, primary progressive multiple sclerosis

199x54mm (300 x 300 DPI)

Peer Review

Probability of first relapse (A), disability accumulation (B), disability improvement (C) events and disability in EDSS [median $\pm$ 95% confidence interval] (D) in matched patients treated with cladribine or interferon β . 95%CI, 95% confidence interval; EDSS, Expanded Disability Status Scale

149x124mm (300 x 300 DPI)

Probability of first relapse (A), disability accumulation (B), disability improvement (C) events and disability in EDSS [median $\pm$ 95% confidence interval] (D) in matched patients treated with cladribine or fingolimod. 95%CI, 95% confidence interval; EDSS, Expanded Disability Status Scale

149x124mm (300 x 300 DPI)

Probability of first relapse (A), disability accumulation (B), disability improvement (C) events and disability in EDSS [median $\pm$ 95% confidence interval] (D) in matched patients treated with cladribine or natalizumab. 95%CI, 95% confidence interval; EDSS, Expanded Disability Status Scale

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Online-Only Supplement

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Supplementary Table S1**Patient disposition per centre and therapy**

Centre	Patients - total	Cladribine	Interferon β	Fingolimod	Natalizumab
Hospital Fernandez, Capital Federal, Argentina	1	0	0	0	1
Hospital Italiano, Buenos Aires, Argentina	2	0	1	1	0
Sir Charles Gairdner Hospital, Nedlands, Australia	3	3	0	0	0
Brain and Mind Centre, Sydney, Australia	2	1	0	1	0
University of Melbourne, Melbourne, Australia	39	2	3	20	14
University Newcastle, Newcastle, Australia	25	6	0	15	4
Geelong Hospital, Geelong, Australia	1	1	0	0	0
St Vincents Hospital, Fitzroy, Melbourne, Australia	1	0	0	0	1
Monash Medical Centre, Melbourne, Australia	8	7	0	1	0
Liverpool Hospital, Sydney, Australia	7	1	0	3	3
Box Hill Hospital, Melbourne, Australia	47	11	1	17	18
Westmead Hospital, Sydney, Australia	6	0	1	5	0
Flinders University, Adelaide, Australia	10	2	2	5	1
University of Queensland, Brisbane, Australia	5	1	0	4	0
Royal Hobart Hospital, Hobart, Australia	1	0	0	0	1
The Alfred, Melbourne, Australia	1	0	0	1	0
Austin Health, Melbourne, Australia	3	0	0	2	1
Cliniques Universitaires Saint-Luc, Brussels, Belgium	4	0	1	2	1
Universidade Metropolitana de Santos, Santos, Brazil	1	0	0	0	1
CSSS Saint-Jérôme, Saint-Jerome, Canada	7	0	0	5	2
Jewish General Hospital, Montreal, Canada	3	0	0	2	1
Hopital Notre Dame, Montreal, Canada	18	0	4	9	5
CISSS Chaudière-Appalache, Levis, Canada	31	0	9	15	7
Neuro Rive-Sud, Quebec, Canada	14	0	3	6	5
General University Hospital and Charles University in Prague, Prague, Czech Republic	100	0	40	35	25
Kommunehospitalet, Arhus C, Denmark	4	0	0	0	4
Hospital Universitario Virgen de Valme, Seville, Spain	6	0	4	1	1
Hospital Universitario Donostia, San Sebastián, Spain	6	0	0	4	2
Hospital Clinico San Carlos, Madrid, Spain	8	0	5	0	3
Hospital Universitario Virgen Macarena, Sevilla, Spain	38	0	4	23	11
Hospital de Galdakao-Usansolo, Galdakao, Spain	1	0	0	0	1
Hospital Germans Trias i Pujol, Badalona, Spain	4	0	1	1	2

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Centre	Patients - total	Cladribine	Interferon β	Fingolimod	Natalizumab
Hospital Universitario de la Ribera, Alzira, Spain	1	0	0	1	0
South East Trust, Belfast, United Kingdom	1	0	1	0	0
Veszprém Megyei Csolnoky Ferenc Kórház zrt., Veszprem, Hungary	1	0	0	0	1
Jahn Ferenc Teaching Hospital, Budapest, Hungary	2	0	0	0	2
University of Debrecen, Debrecen, Hungary	2	0	0	0	2
Szent Imre Hospital, Budapest, Hungary	1	0	0	0	1
Assaf Harofeh Medical Center, Beer-Yaakov, Israel	2	0	2	0	0
University "G. d'Annunzio ", Chieti, Italy	43	0	11	25	7
Azienda Sanitaria Unica Regionale Marche - AV3, Macerata, Italy	9	0	5	3	1
University of Bari, Bari, Italy	43	0	24	10	9
University of Florence, Florence, Italy	2	0	2	0	0
C. Mondino National Neurological Institute, Pavia, Italy	2	0	0	0	2
Ospedali Riuniti di Salerno, Salerno, Italy	5	0	5	0	0
University of Parma, Parma, Italy	6	0	1	2	3
Azienda Ospedaliera di Rilievo Nazionale San Giuseppe Moscati Avellino, Avellino, Italy	9	0	2	4	3
Nuovo Ospedale Civile Sant'Agostino/Estense, Modena, Italy	9	0	4	2	3
Ospedale P. A. Micone, Genova, Italy	17	0	3	2	12
Amiri Hospital, Kuwait City, Kuwait	5	0	0	3	2
University Hospital Nijmegen, Nijmegen, Netherlands	1	0	0	1	0
Zuyderland Ziekenhuis, Sittard, Netherlands	11	0	1	1	9
Jeroen Bosch Ziekenhuis, Den Bosch, Netherlands	1	0	0	0	1
Groene Hart Ziekenhuis, Gouda, Netherlands	7	0	3	1	3
Hospital São João, Porto, Portugal	2	0	0	0	2
KTU Medical Faculty Farabi Hospital, Trabzon, Turkey	9	0	3	4	2
19 Mayis University, Samsun, Turkey	15	0	2	13	0

Supplementary Table S2

Multivariable logistic regression models used to calculate propensity scores

	interferon β		fingolimod		natalizumab	
	coefficient	p value	coefficient	p value	coefficient	p value
sex		n.s.		n.s.		n.s.
age, year	-0.13	10^{-6}	-0.10	10^{-5}	-0.13	10^{-7}
disease duration		n.s.		n.s.		n.s.
disability, EDSS step	-0.32	0.045	-0.30	0.014		n.s.
relapses 3 months pre-baseline		n.s.		n.s.	1.61	0.28
disease course		n.s.		n.s.		n.s.
prior therapies, nr	-1.01	0.0003		n.s.		n.s.
most active prior therapy				n.s.		
fingolimod	-4.35	0.011		n.s.		n.s.
interferon beta / glatiramer acetate		n.s.		n.s.		n.s.
natalizumab	-4.52	0.002		n.s.		n.s.
dimethyl fumarate		n.s.		n.s.		n.s.
teriflunomide		-		n.s.		n.s.

reference: cladribine; n.s., no evidence for the association of independent variable with treatment allocation ($p > 0.05$)**Supplementary Table S3**

Results of the primary and sensitivity analyses

analysis	patients (matched)		outcomes		
	cladribine	comparator	probability of a relapse	disability accumulation hazard	disability improvement probability
cladribine vs. interferon β-1a SC					
primary analysis	22	167	HR=0.6, $p=0.05$ *	HR=0.4, $p=0.37$	HR=15, $p=0.00017$ *
no minimum follow-up	24	177	HR=0.8, $p=0.52$	HR=0.2, $p=0.008$ *	HR=11, $p=10^{-5}$ *
exact match on MS course	24	175	HR=0.95, $p=0.6$	HR=0.2, $p=0.068$	HR=4, $p=0.015$ *
loose matching criteria	37	404	HR=0.95, $p=0.7$	HR=0.3, $p=0.012$ *	HR=3.8, $p=0.00024$ *
cladribine vs. fingolimod					
primary analysis	32	258	HR=1.2, $p=0.31$	HR=1.8, $p=0.089$	HR=3.9, $p=0.0025$ *
no minimum follow-up	33	255	HR=0.62, $p=0.15$	HR=0.93, $p=0.80$	HR=2.1, $p=0.059$
exact match on MS course	35	265	HR=1.3, $p=0.15$	HR=1.7, $p=0.087$	HR=5.1, $p=0.00021$ *
loose matching criteria	37	727	HR=1.3, $p=0.03$ *	HR=3.8, $p=0.001$ *	HR=3.3, $p=10^{-6}$ *
cladribine vs. natalizumab					
primary analysis	26	174	HR=1.8, $p=0.042$ *	HR=2.5, $p=0.021$ *	HR=4, $p=0.00099$ *
no minimum follow-up	26	188	HR=1.2, $p=0.80$	HR=3.5, $p=0.0025$ *	HR=3.2, $p=0.0031$ *
exact match on MS course	29	209	HR=5, $p=0.00022$ *	HR=4.1, $p=0.00011$ *	HR=3, $p=0.0032$ *
loose matching criteria	37	705	HR=1.3, $p=0.030$ *	HR=3.2, $p=0.0095$ *	HR=2.2, $p=0.00028$ *

HR, hazard ratio; *evidence of statistically significant difference / association ($p > 0.05$)

Supplementary Table S4
Results of the power analyses

analysis	outcomes		
	probability of a relapse	disability accumulation hazard	disability improvement probability
cladribine vs. interferon β	-	2%	-
cladribine vs. fingolimod	62%	42%	-
cladribine vs. natalizumab	-	-	-

The table shows minimum detectable differences for the disease outcomes whose analyses did not reach the level of statistical significance in the primary analyses. The differences are shown as proportion of the cumulative hazards.

For Peer Review

STROBE Statement—checklist of items that should be included in reports of observational studies

Title: Cladribine versus fingolimod, natalizumab and interferon β for multiple sclerosis

	Item No.	Recommendation	Page No.
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	2
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	3-4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	3
Participants	6	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	3
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case	4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	3-4
Data sources/measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	3-4
Bias	9	Describe any efforts to address potential sources of bias	4-5
Study size	10	Explain how the study size was arrived at	3-4

Continued on next page

Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	4-5
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	4-5
		(b) Describe any methods used to examine subgroups and interactions	4-5
		(c) Explain how missing data were addressed	3-5
		(d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed	4
		<i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed	
		<i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy	
		(e) Describe any sensitivity analyses	
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	5
		(b) Give reasons for non-participation at each stage	Fig 1
		(c) Consider use of a flow diagram	Fig 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Tab 1
		(b) Indicate number of participants with missing data for each variable of interest	Fig 1
		(c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	Tab 1
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	5-6
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	
		<i>Cross-sectional study</i> —Report numbers of outcome events or summary measures	
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	5-6, Fig 2-4
		(b) Report category boundaries when continuous variables were categorized	n/a
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a

Continued on next page

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	6
Discussion			
Key results	18	Summarise key results with reference to study objectives	6
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	7
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	8
Generalisability	21	Discuss the generalisability (external validity) of the study results	6-7
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	9

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.