

A Double-Blind, Placebo-Controlled, Randomized Trial of Cladribine in Relapsing-Relapsing Multiple Sclerosis

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We conducted an 18-month, placebo-controlled, double-blind study to evaluate cladribine in the treatment of 52 patients with relapsing-relapsing multiple sclerosis. Patients received either placebo or cladribine 0.07 mg/kg/day by subcutaneous injection for 5 consecutive days as six monthly courses for a total cumulative dose of 2.1 mg/kg. Analysis of results revealed a statistically significant favorable effect of cladribine on the joint frequency and severity of relapses and magnetic resonance imaging (MRI) findings. MRI-enhancing lesions were completely suppressed in the cladribine patients by the sixth month of treatment. Mild segmental herpes zoster occurred in two cladribine-treated patients and one patient receiving placebo. Otherwise, there were no side effects or adverse events. We conclude that cladribine shows promise as a treatment for relapsing-relapsing multiple sclerosis.

Although the cause and cure of multiple sclerosis (MS) remains unknown, it is generally accepted that immunological mechanisms play an important role in the inflammatory demyelination of the central nervous system that is the pathological hallmark of the disease. For this reason, current approaches to the treatment of MS patients are focused on modulation or suppression of the immune system.

The drug cladribine [2-chlorodeoxyadenosine (2-CdA), Mylinax, Ortho McNeil, Raritan, NJ] is a selective immunosuppressive molecule synthesized by Carson to mimic the immunodeficiency state seen in hereditary adenosine deaminase deficiency (1). It is a purine nucleoside with chlorine substituted for hydrogen at the 2 position of the purine ring. This makes the molecule resistant to adenosine deaminase, leading to accumulation of deoxynucleotides and selective killing of lymphocytes with relatively little toxicity in other tissues (2). The drug is used widely as a treatment for lymphoid malignancies and is especially effective in hairy cell leukemia (3,4). Because of positive results from our therapeutic trial of cladribine in progressive MS (5,6), we undertook this study to evaluate cladribine in patients with the relapsing-relapsing form of MS.

Key words: immunosuppression; 2-chlorodeoxyadenosine.

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METHODS

Patient Selection

The study subjects were 52 patients with clinically definite relapsing-relapsing MS for at least 1 year. All patients had a history of two or more relapses in the previous 2 years and Extended Disability Status Score (EDSS) scores of 6.5 or less at time of study entry. The majority of patients had been followed at Scripps Clinic. Exclusion criteria included: 1) prior treatment with an immunosuppressive drug within 3 months; 2) a serum creatinine of >1.5 mg/dl; 3) serum glutamic-oxaloacetic transaminase/serum glutamic-pyruvic transaminase or alkaline phosphatase elevated to twice the upper limit of normal; 4) baseline neutrophil counts of $<1600/\mu\text{l}$ or platelet counts of $<130,000/\mu\text{l}$; and 5) previous total lymphoid irradiation or prior extensive myelosuppressive chemotherapy.

The study plan, risks, and potential benefits were explained to each patient in detail. All patients gave informed consent to participate in the study.

Study Design

An 18-month, randomized, placebo-controlled, double-blind study was conducted in the facilities of the General Clinical Research Center (GCRC) of Scripps Clinic. After completion of screening evaluations, 52 patients were stratified according to gender, age (in 10-year intervals), and degree of disability as mea-

sured by Scripps Neurological Rating Scale (SNRS; 7) (in 10-point intervals). The stratified groups were then randomized in blocks of four to either the placebo arm or the cladribine arm. In all, 27 patients were randomized onto the cladribine arm and 25 onto the placebo arm. Throughout the study, patients, neurologists, nurses, and the neuroradiologist remained blinded to treatment assignment. A pharmacist was informed of patient assignment by code in order to dispense placebo or the appropriate dose of cladribine to each patient.

In all patients, clinical neurological exams plus SNRS and EDSS rating scales were performed at baseline and repeated by the same neurologist every month for the first year, every 3 months for the second year, and within 48 hr or less of report by a patient of a relapse. A clinical relapse was defined as the appearance of new symptoms or worsening of an existing symptom, attributable to MS and accompanied by objective worsening of neurological findings. To be scored as a relapse the alterations must have been preceded by disease stability or improvement lasting for at least 30 days, and the worsening must have lasted at least 24 hr and occur in the absence of fever. Relapse severity was rated as follows: 1) mild relapse—decrease in SNRS of 1–7; 2) moderate relapse—decrease in SNRS of 8–14; or 3) severe relapse—decrease in SNRS of 15 or greater.

Magnetic resonance imaging (MRI) of the brain was performed on a 1.5 T Signa scanner (General Electric, Milwaukee, WI) for each patient at baseline, and then monthly for the first year and every 6 months the second year. T1-weighted scans were obtained in the sagittal and axial planes. Axial scans of 3 mm thickness and zero interslice gaps were done about 10 min after the intravenous injection of gadopentetate dimeglumine (Magnevist, Berlex Laboratories). Special attention was given to careful repositioning of patients to guarantee reproducible slice positions. The regions of contrast enhancement on T1-weighted scans were outlined by hand on filmed images. All scans were interpreted and marked by the same neuroradiologist (J.Z.), who had no knowledge of patient treatment assignment. These were then duplicated by a technologist using the taped raw data and a computer workstation [ANALYZE (8), Rochester, MN]. Quantitation of MRI findings involved the determination of lesion areas on the consecutive sections of the T1-weighted scans as interpreted by one of two skilled technologists, then calculation of volumes by assuming homogeneity of lesions across the sections. Initially, the taped raw data from the individual scans were read into a volume-rendering software program, ANALYZE, running on a Hewlett-Packard 712/60 workstation. Our methodology for lesion area deter-

mination is a semiautomated quantitative technique adapted from Wicks et al. (9) and Filippi et al. (10).

Drug Administration

In contrast to our earlier study of intravenous cladribine in progressive MS (5), the drug was administered subcutaneously because of greater ease of administration and because it has now been established that the pharmacological properties and response rates of cladribine in lymphoproliferative diseases are the same if the drug is given either intravenously or subcutaneously (11). Each patient received a course of five consecutive daily subcutaneous injections of cladribine, 0.07 mg/kg/day or an equivalent volume of saline placebo, fractionated into two or three injection sites, and given monthly for 6 months for a total cumulative dose of 2.1 mg/kg of cladribine. A complete blood count was obtained before each monthly course of treatment and reviewed by the pharmacist, and the next dose of cladribine was given only if blood count safety criteria were met according to an algorithm designed for this purpose by one of us (E.B.; Table 1). If these criteria were not met, a placebo dose was substituted. The study design included eight monthly courses. The last two courses ordinarily consisted of placebo, but if a drug dose had been omitted because of blood count inadequacy, then active drug could be given at month 7 or 8 instead of placebo.

Statistical Considerations

Two primary outcome measures were identified: 1) the joint frequency and severity of clinical relapses as judged by neurological examination; and 2) the numbers of enhancing lesions on T1-weighted MRI brain

Table 1. Pretreatment safety criteria for monthly courses of cladribine in multiple sclerosis

- | | |
|----|---|
| 1. | Platelet count must be: |
| a. | 200,000 or higher, or |
| b. | Between 150,000 and 200,000 and represent more than 50% of previous pretreatment platelet count, or |
| c. | Between 125,000 and 150,000 and represent at least 80% of previous pretreatment platelet count |
| 2. | Absolute granulocyte count must be greater than 1000 |
| 3. | Hemoglobin level must not have declined: |
| a. | More than 1.5 g/dl from previous monthly pretreatment level, or |
| b. | 3 g/dl or more from baseline |

scans. Outcomes were to be assessed at 1 year. A sample size of 25 patients per group would be sufficient to detect a decline in the annual rate of exacerbations, from 1 in the placebo group to 0.5 in the cladribine group, with a two-sided Poisson test at alpha level 0.05 (12). Similarly, on the basis of findings from our chronic progressive MS trial, we postulated that the frequency of enhancing lesions in the placebo group would remain at 50% throughout the course of this study, whereas the frequency of enhancing lesions in the cladribine-treated group would decline from 50% to <10% at 1 year. A sample size of 25 patients per treatment group would be sufficient to detect a difference of 50% versus 10% with a power of 0.90, using a two-sided binomial test at alpha level 0.05.

Our analyses were intent-to-treat, in that all data from every patient initially randomized either to placebo or to cladribine are included and reported for that initial treatment group. Blinded observations were undertaken up to 18 months from baseline (trial entry); hence, information is reported out to this period. We present primary analyses—that is, analyses of the primary outcomes at 1 year—and identify other analyses as secondary. We did not impute data values for any patient not observed out to 18 months.

Comparison of the joint frequency and severity of relapses between the two treatment groups was undertaken using Mantel's (13) extension of the Mantel-Haenszel procedure, here denoted Q_M . (Mantel's procedure can incorporate arbitrary scores for the degree of relapse, but we chose to score objectively by means of ranks based on drop in SNRS score, separately in each monthly summary table of relapses, as cross-classified by treatment group.) A stratified version of Mantel's test and a general linear model with Poisson link (that is, a Poisson regression model; 14) were also used to evaluate the significance of covariate information as predictors of clinical relapse. Confidence intervals for relapse rates were calculated under the assumption that the numbers of events followed a Poisson distribution in each treatment group. Comparison of the frequency of enhancing lesions on T1-weighted MRI scans over time was done with McNemar's test for paired data (within treatment groups) and Fisher exact test (between treatment groups); logistic regression was also used to assess the significance of covariate information. A nonparametric repeated measures analysis of variance procedure (15) was used to compare neurological performance scores (EDSS and SNRS) between the two treatment groups over the course of the study. The EDSS and SNRS scores were considered to be secondary outcome measures since little change might be expected between the two groups over the relatively short time frame of

the study. Two-sided p values relative to the null distributions of the observed test statistics are reported.

Intrarater reliability of the determination of presence of enhancing lesions on T1-weighted MRI scans was assessed by means of a test-retest of 20 scans by the examining neuroradiologist (J.Z.). Discrepancies between the two independent evaluations were 15% (3/20). In a similar spirit, both examining neurologists (J.R. and J.S.) participated in a study of inter-rater and intrarater reliability, with regard to the neurological rating scales. Twenty patients (J.R., 10; J.S., 10) were assessed by the same examiner twice on the same day, the period between examinations ranging from 135 min to 240 min. Intrarater agreement for one examiner (J.S.) on the EDSS was perfect; the weighted κ coefficient of agreement (16) for the other examiner was 0.997. The weighted κ coefficients of agreement between the paired SNRS scores were 0.999 for both examiners. Separately, 20 patients were independently assessed by each examiner on the same day. Inter-rater agreement was high: the weighted κ coefficient of association was 0.990 for the EDSS and 0.957 for the SNRS. Inter-rater agreement on the EDSS was 100% for all sets of examinations when agreement was defined as a difference of less than or equal to 1.0, and 95% when agreement was defined as a difference of less than or equal to 0.5. Inter-rater agreement on the SNRS was 95% when agreement was defined as a difference of no more than 10 points, and 90% when agreement was defined as a difference of no more than 5 points.

RESULTS

Trial Considerations

There was one withdrawal on the placebo arm at 3 months (conversion disorder complicating assessment of underlying MS), and one withdrawal on the cladribine arm at 4 months (patient moved out of state); all of the remaining patients received standard intervention without deviations, as specified in the protocol. Thus, 26 cladribine patients and 24 placebo patients were available for evaluation at 12 months. During the period from 12 to 18 months, five patients on placebo withdrew: two patients moved out of state, two withdrew for unspecified reasons, and one withdrew because of worsening MS. One patient receiving cladribine also withdrew because of worsening MS. Thus, 25 cladribine patients and 19 placebo patients were available for evaluation the entire 18-month period. Figure 1 depicts the trial profile. During the period from 12 to 18 months, the blinding was removed from two cladribine patients and two placebo patients.

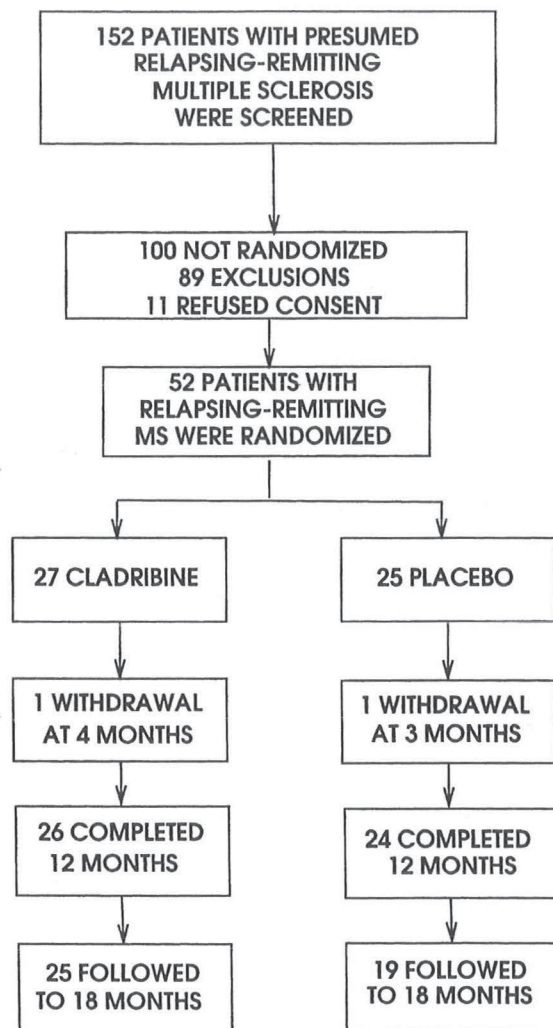


Figure 1. Trial profile of the cladribine relapsing-remitting MS clinical trial.

Because of potential bias, information from these patients concerning their frequency and severity of exacerbations subsequent to the point of unblinding is not used in the calculation and comparison of exacerbation rates between the two treatment groups.

Demographic and Baseline Characteristics in the Two Treatment Groups after Randomization

The two groups were similar in terms of baseline clinical characteristics (Table 2). Each group had an approximate 2:1 female-to-male preponderance and comparable mean age, disease duration, and baseline EDSS. Patients randomized to cladribine therapy averaged a slightly greater number of exacerbations in the 12 months prior to study entry than patients randomized to placebo.

Table 2. Baseline demographic and clinical characteristics

	Placebo (n = 25)	Cladribine (n = 27)
Sex		
Male	7	9
Female	18	18
Race		
White	25	24
Other	0	3
Age (years)		
Mean	39.8	43.4
25th percentile	36.5	38.5
50th percentile	41	44.5
75th percentile	44	49.5
Range	31–52	30–52
Years with symptoms		
Mean	9.1	10.2
25th percentile	3.5	4.5
50th percentile	9	8
75th percentile	12.5	12.5
Range	1–25	1–29
Number of exacerbations in previous year		
1	13	5
2	5	16
3 or 4	7	6
Baseline EDSS		
Mean	3.8	3.9
25th percentile	2.5	2.3
50th percentile	3.5	5.5
75th percentile	5.3	5.5
Range	2–6.5	2–6.5
Baseline SNRS		
Mean	75.8	76.1
25th percentile	67	66
50th percentile	75.5	78.5
75th percentile	86	86.5
Range	54–98	41–93

EDSS, Extended Disability Status Score. SNRS, Scripps Neurological Rating Scale.

Effect of Cladribine on Outcome Measures

Figure 2 depicts the frequency and severity of exacerbations for all patients enrolled in the study. We examined the joint distribution of frequency and severity over months 7 through 12 for treatment comparisons: on the basis of our prior experience (17), we expected the maximum immunosuppression on cladribine therapy would not be achieved prior to month 7. Using the extended Mantel-Haenszel procedure, we found that there is a statistically significant reduction in the frequency and severity of exacerbations in the cladribine group compared to the placebo group over months 7 through 12 ($Q_M = 2.30$, $2p = .021$). Over this period, the relapse rate in the cladribine group

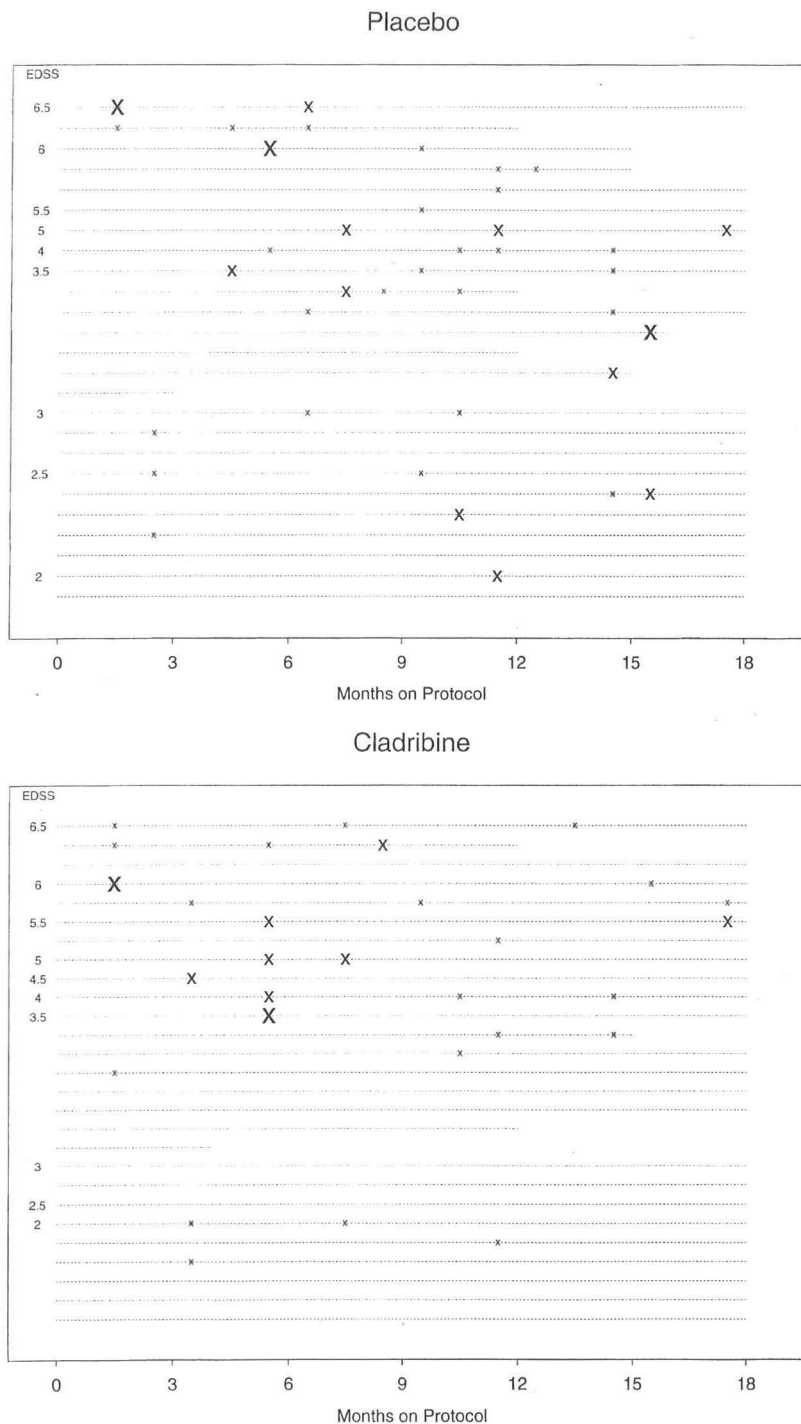


Figure 2. Event charts for frequency and severity of exacerbations over the course of the study in placebo-treated and cladribine-treated patients.

Within treatment groups, patients are ordered in terms of decreasing EDSS scores at baseline; patients with identical EDSS scores at baseline are ordered by numbers of new exacerbations. Mild, moderate, or severe exacerbations are denoted by progressively heavier X's.

was 0.77 per year [95% confidence interval (CI), 0.37–1.41], compared to 1.67 per year in the placebo group (95% CI, 1.02–2.57). With Poisson regression, we identified treatment along with two other covariates, baseline EDSS and number of exacerbations in the year prior to start of treatment, as significant predictors of relapse over months 7 through 12 (with fewer

relapses being associated with cladribine therapy, lower EDSS scores at baseline, and fewer exacerbations in the year prior to start of treatment). In secondary analyses, we found that the reduction in the distribution of frequency and severity of exacerbations in the cladribine group relative to the placebo group is sustained at 18 months: $Q_M = 2.59$, $2p = .010$ over

the 1-year period from month 7 through month 18. Over this extended period, the exacerbation rate in the cladribine group was 0.66 per year (95% CI, 0.37–1.05) compared to 1.34 per year in the placebo group (95% CI, 0.90–1.93).

Figure 3 compares the frequency and severity of relapses at 6-month intervals during the study with the frequency of relapses in the 12 months preceding treatment with drug or placebo. It is apparent that there was striking improvement in the first 6 months of the study, when injections of placebo or cladribine were being given 5 days of each month, regardless of whether the patients received placebo or active drug. When injections were stopped, the frequency of relapses in the placebo group returned to its baseline, pretreatment frequency, but the frequency and severity of relapses continued to decline in the patients who received cladribine.

MRI results (Fig. 4, Table 3) revealed complete suppression of enhancing lesions after study month 6 in the cladribine group, whereas lesion enhancement persisted in the placebo group. Formally, with regard to the primary outcome measure at 12 months, there is a highly significant decrease in the occurrence of enhancing lesions at 12 months relative to baseline in the cladribine group ($2p < .0003$ by McNemar's test). In contrast, there is a slight increase in the occurrence

of enhancing lesions at 12 months relative to baseline in the placebo group ($2p = .109$ by McNemar's test); and, the frequency of enhancing lesions is significantly greater at 12 months in the placebo group than in the cladribine group ($2p = .0001$ by Fisher exact test). In secondary analyses, we find a significant reduction in the frequency of enhancing lesions experienced by the cladribine group relative to baseline already at month 7 and persisting at month 18 ($2p < .0005$ by McNemar's test at each time point). Moreover, the frequency of enhancing lesions in the placebo group is already significantly greater than that in the cladribine group by 7 months ($2p = .0001$ by Fisher) and remains so at 18 months ($2p = .002$ by Fisher). No significant predictors of enhancing lesion presence other than treatment were found by logistic regression.

We found no significant differences between the treatment groups in either EDSS or SNRS scores, the secondary outcome parameters, over 18 months ($p > .5$ for each; Fig. 5).

Adverse Events and Side Effects

Infections were limited to an episode of mild segmental herpes zoster that occurred in two cladribine-

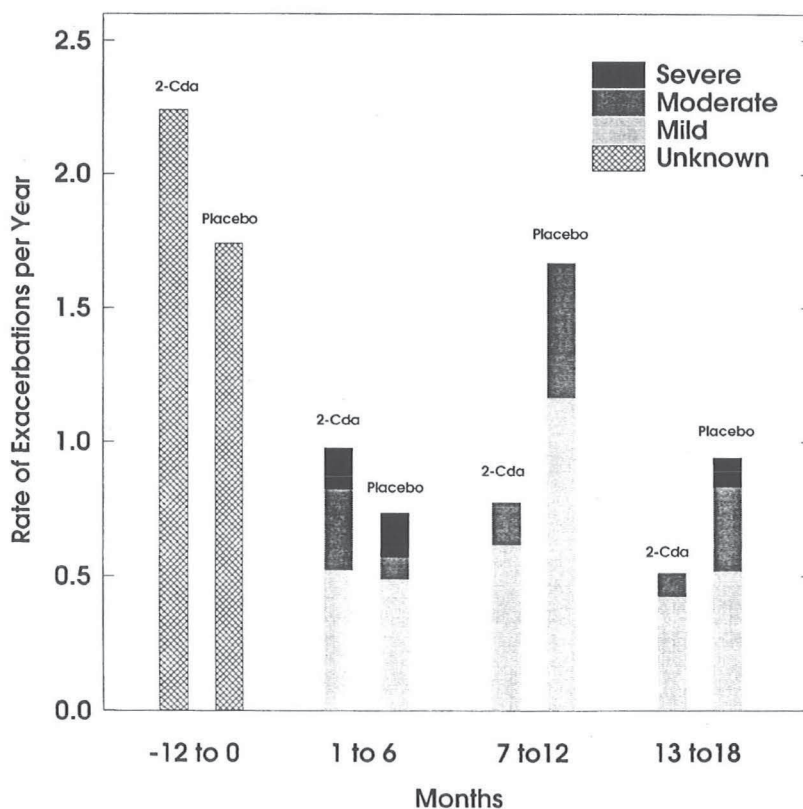


Figure 3. Rates of exacerbations for the two treatment groups.

The number of exacerbations for the year prior to initiation of treatment was obtained from the patient's history. Over the course of the trial itself, exacerbations were documented by the neurologist and classified as mild, moderate, or severe as explained in the text. For purposes of comparison, rates are presented as relapses per year.

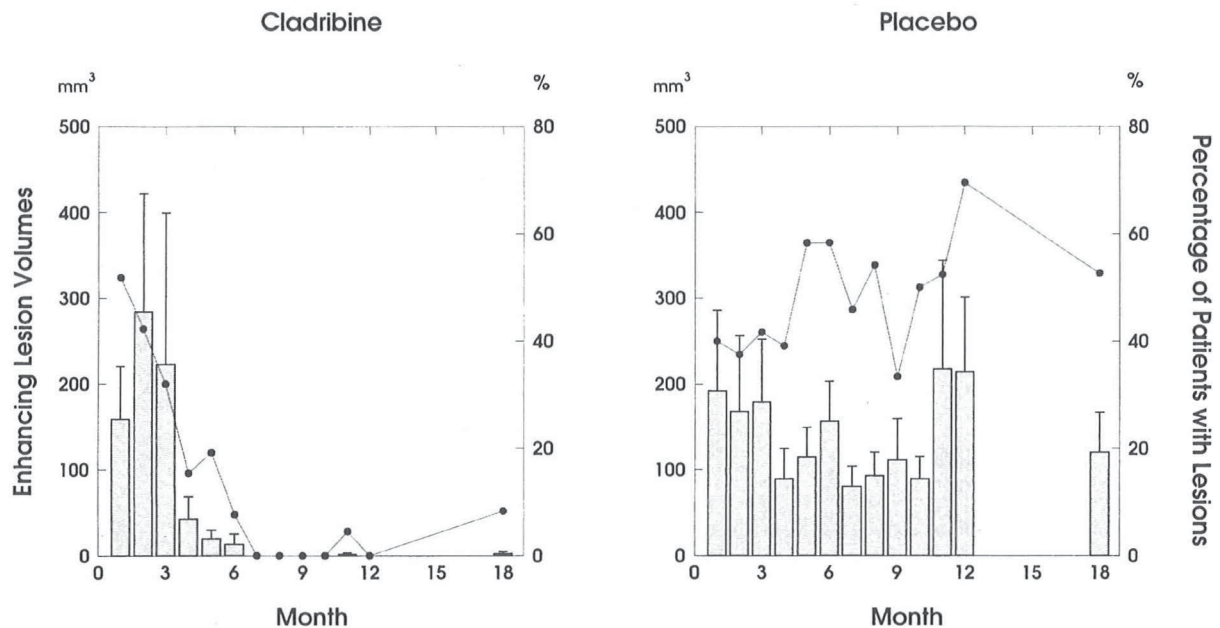


Figure 4. Average volumes of enhancing lesions on T1-weighted scans (bars, left vertical axis), and proportions of patients with enhancing lesions (dots, right vertical axis), for the two treatment groups over the course of the trial.

Averages and proportions were computed on the basis of all available MRI data at each time point. Standard error bars are also given for the MRI volumes.

treated patients and in one patient receiving placebo. Acyclovir was administered orally in each case. Cladribine-treated patients experienced no side effects that might have led to unblinding of patients or examining neurologists. Average lymphocyte counts in the cladribine group declined as expected to a nadir at 7 months of $0.4 \times 10^3/\mu\text{l}$. The average platelet count in the cladribine group declined modestly to a low of $>200 \times 10^3/\mu\text{l}$ at 6 months, and the average hemoglobin to a low of >13.5 g/dl at 11 months, but there were no individual cases of significant thrombo-

cytopenia, anemia, granulocytopenia, or generalized marrow suppression (Fig. 6).

DISCUSSION

In view of the long-lasting lymphopenia and relatively low toxicity from cladribine, we began studies in 1990 with progressive MS patients in the hope that depletion of immunocytes might be beneficial. Because initial observations were encouraging, we undertook a 2-year, placebo-controlled, double-blind study in 51 patients in which cladribine (total dose = 2.8 mg/kg) or placebo was administered during the first year via a central venous access device using a portable infusion pump. A favorable effect on neurological performance scores and on MRI findings was documented in patients treated with cladribine (5,6).

A multicenter trial has subsequently been conducted in 159 patients with progressive MS using cladribine subcutaneously at a total dose of 2.1 mg/kg. Unexpectedly, there was no worsening of neurological performance scores in the placebo arm and therefore no significant differences were seen between cladribine and placebo-treated groups. This study was probably underpowered due to inclusion of more patients with advanced disability (18). However, marked suppression of enhancing MRI lesions with cladribine treatment was confirmed in the pro-

Table 3. Presence of enhancing lesions by MRI over the course of the trial

Time	Placebo		Cladribine	
	Lesions absent	Lesions present	Lesions absent	Lesions present
Baseline	15	10	13	14
7 months	13	11	25	0
12 months	7	16	25	0
18 months	9	10	22	2

Note: One cladribine patient did not have an MRI during month 7, one placebo patient and one cladribine patient did not have an MRI during month 12, and one cladribine patient did not have an MRI at month 18.

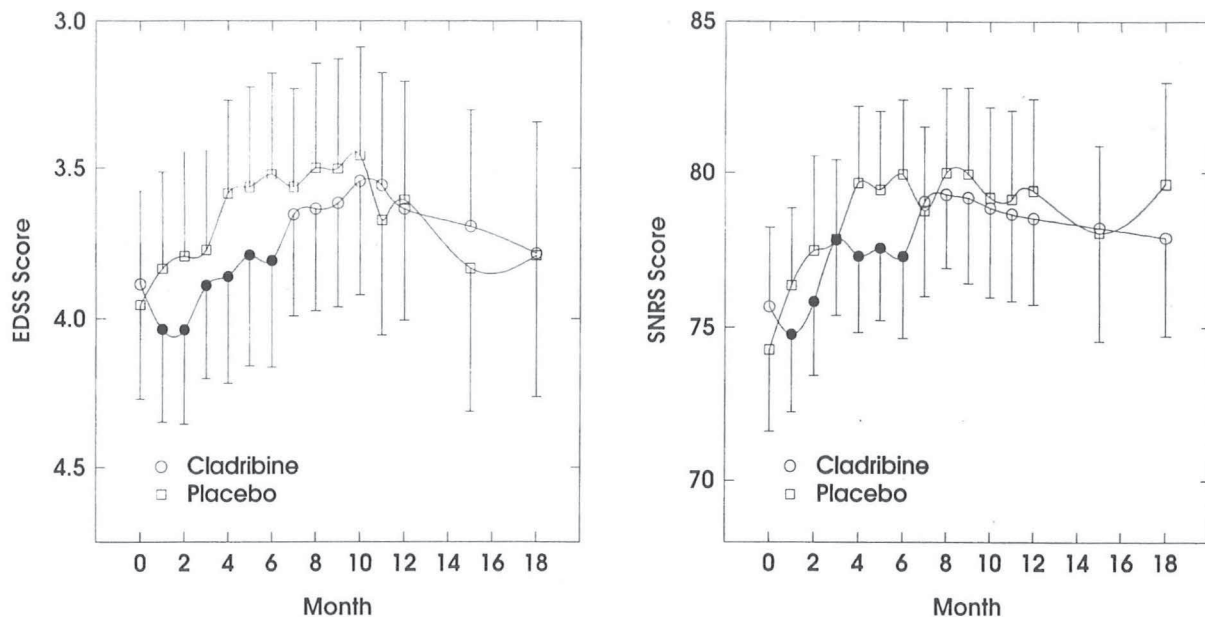


Figure 5. Changes in the Kurtzke (EDSS) and Scripps (SNRS) rating scores.

Solid symbols indicate when cladribine was administered. Means and pointwise standard error bars are shown.

gressive MS multicenter study and no significant toxicity was observed.

The results in relapsing-remitting MS, as reported in the current study, indicate that cladribine given subcutaneously at a total dosage of 2.1 mg/kg appears to be safe and effective in reducing the rate and severity of clinical exacerbations for at least the relatively short duration of the study. Particularly striking was the placebo effect noted in the first 6 months of our study, when patients were receiving injections; the relapse frequency dropped to about one half in both groups of patients. In the second 6 months, however, when no injections were given but after immunosuppression had been achieved in the drug-treated group, the relapse frequency returned to baseline in patients who had received placebo, but patients who had received drug continued to enjoy relative freedom from exacerbations, an effect that continued into the second year of the study.

Our primary analyses are predicated on outcomes at 1 year following randomization. During this period, all patients received the treatment to which they had been randomized; that is, treatment allocation and treatment actually received were identical for everyone. Two patients, one randomized to placebo and the other to cladribine, withdrew from the study during the first year. Hence, the attrition rate was <5% on each arm over the formal length of the trial. The results of our primary analyses at 1 year, comparing the joint frequency and severity of exacerbations between

the treatment groups, and comparing the frequencies of enhancing lesions, are insensitive to the loss of information from these patients. With regard to the second primary end point, the presence of enhancing lesions of T1-weighted scans, it is clear from Table 3 that even under the least favorable scenario for cladribine—no enhancing lesions in the placebo withdrawal, but enhancing lesions in the cladribine withdrawal—the favorable outcome of cladribine therapy relative to placebo at 1 year would remain overwhelmingly significant. The lack of any demonstrable difference in neurological disability (EDSS and SNRS scores) between the cladribine and placebo groups is of uncertain significance since neither treatment group, including the placebo group, showed significant worsening over the duration of the study.

The almost complete suppression of MRI-enhancing lesions with cladribine is a very robust treatment effect similar to that previously reported with cladribine in progressive MS (5,6,18) and exceeding the effect of interferon beta-1a (19). Since enhancement of MRI lesions is thought to reflect active disease (20), the question arises as to how some cladribine-treated patients continued to have clinical relapses, but with no apparent lesion enhancement on serial MRI scans.

The mechanism of action of the beneficial effect of cladribine on MS is presumably related to the selective and sustained depletion of lymphocytes. In a previous study of cladribine in chronic progressive

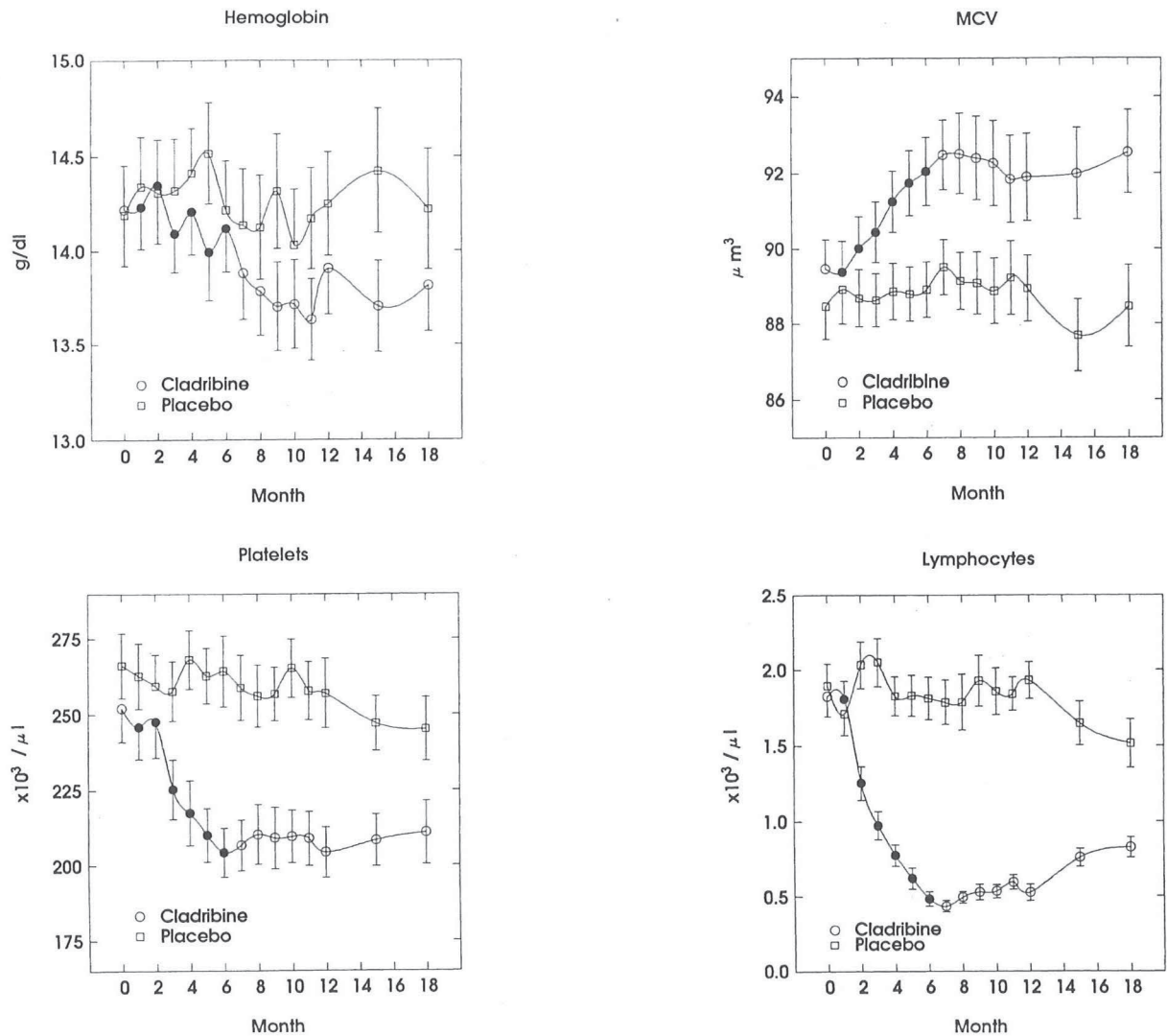


Figure 6. Serial blood cell counts over the course of the trial. Solid symbols indicate when drug was administered. Means and standard error bars are shown.

MS (6), there was an approximately four-fold decrease in the CD4/CD8 “helper/suppressor” ratio and an approximately five-fold reduction of CD25-activated T and B lymphocytes, which lasted for many months after a 4-month course of the drug. By comparison, treatment of MS with the general immunosuppressive drugs cyclophosphamide (21) or chlorambucil (22) produced a relatively transient two-fold decline in the CD4/CD8 ratio. This difference suggests that cladribine may have a more potent immunosuppressive effect in MS than other drugs.

The duration of the beneficial effect on relapsing MS after a course of cladribine could not be determined from this study since the patients were unblinded after 18 months. However, an estimate based on observations of cladribine in chronic progressive

MS (6) would indicate that beneficial effect should eventually decline and would not be expected to last much beyond 2 years. The lengthy but impermanent duration of effect of cladribine means that retreatment will be necessary if cladribine is to become a practical long-term therapy for MS. At present, there are few data on the safety of long-term retreatment with cladribine in a nonmalignant disease such as MS. Twenty-four patients from our initial study of cladribine in chronic progressive MS have been retreated ($0.07 \text{ mg/kg} \times 5 \text{ days} \times 4 \text{ courses}$) because of a recurrence of disease worsening. There were no instances of marrow suppression or thrombocytopenia in these patients, but 6 (25%) developed herpes zoster with a second course of cladribine as compared to 2 of 51 (4%) with the first treatment. Repeated treatment

raises long-term toxicity issues including: 1) susceptibility to opportunistic infection; 2) marrow stem cell depletion; and 3) an increase in the incidence of malignancy, known to occur in association with other long-term immunosuppressive treatments (23).

Since currently available treatments for relapsing MS are only partially effective and because, in the case of interferons, there is concern that the beneficial effect may be lost due to induction of neutralizing antibodies (24), new treatments need to be developed. In this regard, cladribine shows promise as a relatively safe and at least temporarily effective treatment in reducing the frequency and severity of MS exacerbations and in suppressing new contrast-enhancing MRI lesions.

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