



# The treatment of chronic progressive multiple sclerosis with cladribine

(immunosuppression/double-blind study/crossover/2-chlorodeoxyadenosine/deoxypurine)

E. BEUTLER\*<sup>†</sup>, J. C. SIPE<sup>‡</sup>, J. S. ROMINE<sup>‡</sup>, J. A. KOZIOL\*, R. McMILLAN\*, AND J. ZYROFF<sup>§</sup>

\*Department of Molecular and Experimental Medicine, The Scripps Research Institute, and <sup>‡</sup>Division of Neurology and <sup>§</sup>Department of Radiology, Scripps Clinic and Research Foundation, La Jolla, CA 92037

Contributed by E. Beutler, November 6, 1995

**ABSTRACT** A 2-year, placebo-controlled, double-blind, crossover study was started in 1992 to evaluate cladribine, an immunosuppressive drug, in the treatment of chronic progressive multiple sclerosis. In the first year patients were given cladribine 0.10 mg/kg per day for 7 days as four monthly courses for a total of 2.8 mg/kg or placebo. During the second year patients treated with placebo during the first year were given i.v. infusions of 0.10 mg, 0.05 mg, and 0.05 mg of cladribine per kg of body weight per day for 7 consecutive days in three successive monthly courses, for a total dose of 1.4 mg/kg. Patients who had been treated previously with cladribine were crossed over to placebo. Analysis of the results revealed a favorable influence on the neurological performance scores, both in the Kurtze extended disability status and the Scripps neurological rating scale, and on MRI findings in patients treated with cladribine. In the first year the most striking finding was that while clinical deterioration continued in the placebo-treated patients, the condition of patients who received cladribine stabilized or even improved slightly. Toxicity and therapeutic response were dose-related.

Although the underlying cause of multiple sclerosis (MS) remains a mystery, considerable evidence exists that damage to the central nervous system is mediated by immunopathologic mechanisms (1, 2). For this reason, immunosuppression is a rational approach to treatment of this disorder.

2-Chlorodeoxyadenosine (2-CdA; cladribine; Leustatin) is an adenosine deaminase-resistant purine nucleoside, designed by Carson *et al.* (3–5) to simulate the immunodeficiency state of hereditary adenosine deaminase deficiency by causing the accumulation of deoxynucleotides in lymphocytes. This simple compound has been widely used for the treatment of lymphoid malignancies (6) and has a very favorable toxicity profile relative to other lymphocytolytic drugs.

Since there is no satisfactory treatment for chronic progressive MS, the use of cladribine was considered because of its relatively low toxicity and the long-lasting lymphopenia that it produces. Open-label feasibility studies were begun in 1990 with a small number of patients. The results in terms of both apparent benefit on neurological performance and lack of toxicity were favorable and encouraged us to proceed with a larger 2-yr placebo-controlled crossover study to further explore issues of safety and therapeutic effect. The first-year results of this study showed a positive effect (7). We now report observations from the entire 2 yr of this double-blind study and an additional 6-mo unblinded follow-up.

## METHODS

**Patient Selection.** The study subjects were 51 patients with clinically definite or laboratory-supported definite chronic

progressive MS (8) for >2 yr. The patients had been followed at Scripps Clinic by the neurology group for periods of from 6 mo to 20 yr. The study plan and risks and potential benefits were explained to each patient in detail, and all patients gave informed consent to participate in the investigations, which were performed under investigator-initiated INDs no. 29,111 and no. 93,777 from the Food and Drug Administration.

Patient characteristics are summarized in Table 1.

**Study Design. Drug dosage.** A phase III double-blind crossover study was started in January of 1992. In the first year of this study patients on the cladribine arm were given four monthly 7-day courses of 0.10 mg of cladribine per kg per day (0.7 mg/kg per course) for a total of four courses (total dose = 2.8 mg of cladribine per kg), except as noted below. During the second year blinding was maintained, and the patients who had received placebo were given active drug, but at one-half the total dose that had been administered during the first year. The four infusions given to these patients were divided so that the first consisted of 0.7 mg of cladribine per kg, the second and third each consisted of 0.35 mg of cladribine per kg of body weight, and the fourth consisted of saline placebo (total dose = 1.4 mg of cladribine per kg). The patients who had originally received cladribine were given four monthly saline placebo infusions from the beginning of the second year.

Cladribine or placebo was administered monthly on an outpatient basis by 7-day i.v. infusions through a central venous catheter using a portable infusion pump. Blood counts and chemistry panels were done before each infusion, and the counts, reviewed by an unblinded hematologist/internist, had direct contact with only four of the patients when a medical problem required internal medicine consultation.

Throughout the study, patients, neurologists, nurses, and the neuroradiologist were unaware of the treatment assigned to each patient. Cladribine causes no symptoms on infusion and cannot be distinguished from placebo by patients or professional staff. Drug was withheld on occasions when blood counts did not meet the safety standards that had been established; this occurred on four occasions in patients receiving active drug and in two patients receiving placebo. Corticosteroid therapy was permitted when the examining neurologist considered them necessary for treatment of the patient. Two patients received corticosteroids during the study; one patient required one course during placebo administration, and another patient required two courses during cladribine therapy.

**Sample size and patient compliance.** On the basis of the results of the open label study, we had estimated that a sample size of 22 pairs of patients would be sufficient to detect a 15% improvement in the Scripps neurological rating scale (SNRS)

Abbreviations: MS, multiple sclerosis; SNRS, Scripps neurological rating scale; EDSS, Kurtze extended disability status.

<sup>†</sup>To whom reprint requests should be addressed at: Department of Molecular and Experimental Medicine, The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, CA 92037.

Table 1. Demographic characteristics of patients at study entry

| Characteristic                                       | Initial placebo | Initial cladribine |
|------------------------------------------------------|-----------------|--------------------|
| Sex (F/M)                                            | 16/8            | 18/9               |
| Race (White/other)                                   | 22/2            | 27/0               |
| Mean age, yr (range)                                 | 42.5 (21–54)    | 43.4 (28–54)       |
| Mean duration of clinical symptoms of MS, yr (range) | 10.5 (2–31)     | 12.7 (2–24)        |

while on cladribine, compared with no improvement while receiving placebo, with a statistical power of 0.90, using a one-sided test at an  $\alpha$  level of 0.05. Initially 24 pairs of patients were matched by age, sex, and disease severity. Each matched pair was randomized by the statistician (J.A.K.) using random number tables so that one patient was assigned to the group initially receiving cladribine and the other to the group receiving placebo. One additional individual, for whom no suitable match was identified, was started on the cladribine arm; this individual left the study after 8 mo on the protocol. Two patients, both initially assigned to cladribine, were lost to follow-up at 2 and 3 mo on the study, respectively. Because the loss of these two patients on the initial cladribine arm was not attributed to treatment, we recruited two additional patients, appropriately matched by the “blinded” neurologists (J.C.S. and J.S.R.) and assigned by the statistician to cladribine, as replacements. One patient receiving placebo withdrew from the study after 4 mo on the protocol for reasons related to treatment (i.e., lack of stabilization of disease); this patient was not replaced. The analyses reported are based on the experience of the 24 matched pairs of patients and exclude the three patients initially on cladribine who did not complete a full year of the study, as described above. Four additional patients were lost from the study in the second year for various reasons unrelated to the study. Accordingly, 24-mo or longer follow-up of 21 of the 24 patients who had received cladribine in the first year and 22 of the 24 patients who had received placebo in the first year was possible.

**Deviations from the original protocol.** There were a few deviations from the stated drug dosages. In the original protocol a total of six monthly doses had been planned, but this was modified to four courses after the study had been initiated because of a greater than expected decline in the platelet count in some patients. One patient received five courses before the decision to reduce dosage had been made. One patient received only two courses, and two patients received only three courses because of thrombocytopenia. At the beginning of the second phase of the study, five patients who were to have received placebo were given a single 0.7 mg/kg of infusion cladribine by error. Separate analysis showed that the response of these patients was not greater than those of the other patients, and the data of these patients have been retained.

**Observations and Data Analysis.** *Neurologic evaluation.* Patients were evaluated monthly by means of the Kurtzke extended disability status scale (EDSS) (9) and with the SNRS (10). MRI brain scans with contrast enhancement were performed in these patients before treatment and at 6-mo intervals.

To assess inter-rater variability 20 patients were independently assessed by each examiner (J.S.R., J.C.S.) on the same day. Interrater agreement (11) on each scoring instrument was quite high: the weighted  $\kappa$  coefficient of agreement was 0.976 for the EDSS scores and 0.828 for the SNRS scores. Interrater agreement, defined as a difference of  $\leq 1.0$  EDSS points, reached 100% for all sets of examinations. This result compared favorably with results reported in other clinical trials of investigative therapeutic agents in MS (12). In comparison, interrater agreement on the SNRS was 85%, with agreement defined as a difference of  $\leq 10$  SNRS points. In a separate evaluation 18 patients were assessed by the same examiner

twice on the same day, the period between examinations ranging from 135 min to 240 min. Intra-rater agreement on the EDSS was perfect with both examiners, and weighed  $\kappa$  coefficients of agreement between the two SNRS scores were 0.978 (J.S.R.) and 0.998 (J.C.S.).

*Hematologic evaluation.* Monthly blood counts were obtained during drug administration. Less frequent counts were obtained thereafter. Lymphocyte subset evaluation was done monthly during the course of drug administration and less frequently thereafter.

*MRI.* MRI was performed on a 1.5-T General Electric Signa scanner. T<sub>2</sub> and proton density-weighted images were obtained by using a conventional spin-echo sequence with repetition times of 2500 msec and echo-delay times of 30 and 90 msec. Axial scans of 3-mm thickness and 0 interslice gap were obtained  $\approx 10$  min after gadopentetate dimeglumine (Magnevist, Berlex Laboratories) injection to assure optimal time for transmigration of the contrast agent across the blood–brain barrier.

*Statistical Methods.* The SNRS was designated as the primary outcome parameter. Summary statistics are reported as mean and range or SEM. Analyses of the neurological scores and MRI findings were undertaken with parametric methods appropriate for two-period repeated-measurements crossover designs (13) as well as a nonparametric repeated-measures ANOVA technique (14). Last available observations were carried forward for patients who had completed at least 18 mo of the study. Similar analyses were done in which these data remained missing and in which they were modeled under the representation that they were missing at random; these analyses yielded similar results to those reported here. Kaplan–Meier curves and log–rank statistics were also used to compare neurological rating-scale outcomes between the two treatment groups during the first year of the study. Two-sided *P* values are reported throughout.

## RESULTS

**Neurologic Findings.** *Clinical performance scores.* Fig. 1 depicts the EDSS and SNRS scores of the patients. The average EDSS and SNRS scores of patients receiving cladribine improved modestly during the first year of the study, whereas the scores of patients with placebo continued to deteriorate. The improvement in SNRS scores appeared to peak at  $\approx 18$  mo and be well maintained for the 24 mo of follow-up in the patients treated with 2.8 mg of cladribine per kg, even though they received no active drug after the first 4 mo of the study. After 24 mo, in unblinded observations, fairly rapid deterioration was documented. While the scores of the patients who received placebo in the first year of the study deteriorated during that year, the lower dose of cladribine they received (1.4 mg/kg) also seemed to be effective in stabilizing their disease, albeit for a shorter time period, with peak improvement at  $\approx 8$  mo after treatment initiation. Inspection of the curves thus suggests that the stabilization of disease produced by the lower dose of cladribine may be of shorter duration than that seen with the larger dose and that a rebound worsening of disease may occur between 24 and 30 mo after initiation of therapy with the higher dose. ANOVA based on the two-period crossover design with absolute changes in EDSS and SNRS as end points revealed no significant carryover effects between subjects or period effects within subjects, but highly significant treatment effects: the *F*-statistics for assessing treatment effects with subjects were  $F_{1,44} = 10.19$ ,  $P = .0026$  for EDSS and  $F_{1,44} = 23.46$ ,  $P < 0.0001$  for SNRS.

Kaplan–Meier time-to-failure plots show that whether failure is defined as a gain of 0.5, 1, or 1.5 points on the EDSS scale or loss of 5, 10, or 15 points on the SNRS scale, patients receiving cladribine fared better than those who received placebo in the first study year. Plots showing time-to-gain of 1.0

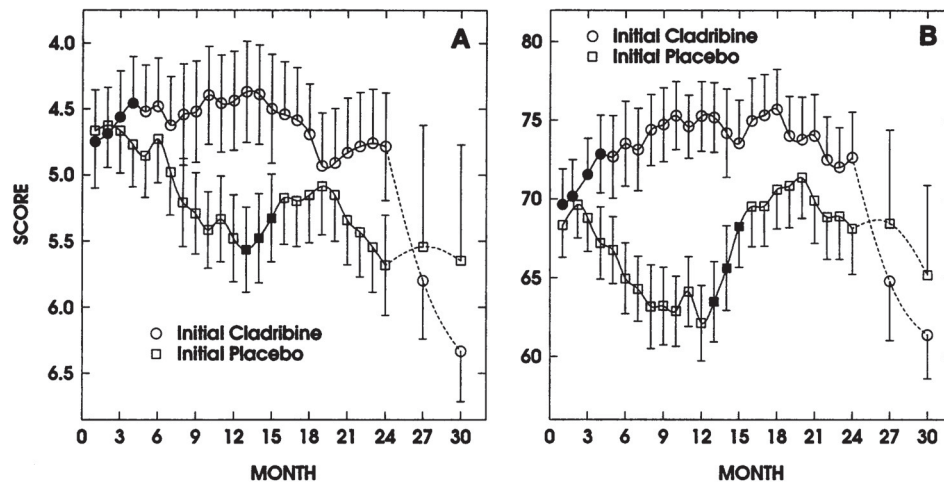


FIG. 1. The EDSS (A) and SNRS (B) of 48 patients treated with cladribine in a double-blind crossover study. The solid symbols indicate months during which drug was administered. The group of patients denoted by circles was given 0.7 mg of cladribine per kg of body weight in a 7-day infusion during 4 mo. The group that received placebo first ( $\square$ ) was given 0.7 mg of cladribine per kg of body weight as a first infusion (first solid square) and two subsequent 7-day infusions of 0.35 mg per kg of body weight (second and third solid square). The fourth infusion in these patients was placebo, as denoted by the open square. Bars represent 1 SEM. Dashed lines represent changes occurring after the study had been unblinded.

EDSS or loss of 10 SNRS points are shown in Fig. 2. Surprisingly, time-to-improvement plots (data not shown) also showed a statistically significant advantage for patients receiving cladribine.

Log-rank statistics were used to compare times-to-failure between the two treatment groups of the first year of the study, where failure was defined as a gain of 1 or 1.5 points on the EDSS or a loss of 10 or 15 points on the SNRS relative to baseline value. Each of these statistics was highly significant ( $\Delta$ EDSS = 1.0:  $L = 6.313$ ,  $P = 0.012$ ;  $\Delta$ EDSS = 1.5:  $L = 5.254$ ,  $P = 0.024$ ;  $\Delta$ SNRS = -10:  $L = 8.299$ ,  $P = 0.004$ ;  $\Delta$ SNRS = -15:  $L = 6.800$ ,  $P = 0.009$ ), indicating that patients receiving cladribine fared better than those who received placebo.

**MRI findings.** Neither nonparametric ANOVA nor parametric ANOVA based on the two-period crossover design revealed a significant treatment effect on demyelinated volumes on MRI (data not shown). In contrast, a highly significant difference was seen in the enhancing volumes, a measurement of current disease activity (15). For analysis, enhancing volume findings were dichotomized as being either present or absent; the results of this analysis are summarized in Table 2. At the end of 1 yr, 12 patients in the group given placebo were scored as having no enhancing lesions: 9 because they had no enhancing lesions throughout the period, and 3 because

they had lesions that disappeared. However, the other 12 patients were scored as having lesions: 10 because enhancing lesions persisted throughout the 12-mo period, and 2 because they developed new enhancing lesions. In contrast, at the end of the first year, 22 of the patients who had received cladribine were classified as having no lesions: 11 patients had had no enhancing volumes at baseline, and 11 had enhancing lesions that disappeared. Only two individuals in this group had lesions: one had continuing enhancing lesions, and one developed new enhancing lesions where there had been none ( $P < 0.001$ , McNemar's test).

Further analysis was made of patients after the crossover of treatments at 12 mo. Twenty-two of the patients in the group that received placebo first and who were then treated with 1.4 mg of cladribine per kg of body weight were evaluated at 24 mo. Eleven had no lesions at both 12 and 24 mo, one had lesions at both 12 and 24 mo, and 10 had lesions at 12 mo but none at 24 mo ( $P < 0.001$ , McNemar test). From this crossover analysis, there is clear evidence that the reduced dose of cladribine received by the initial placebo group during the second year on the study significantly reduced the occurrence of enhancing lesions on MRI scans. Among the 20 patients in the group that received cladribine first who were evaluated at

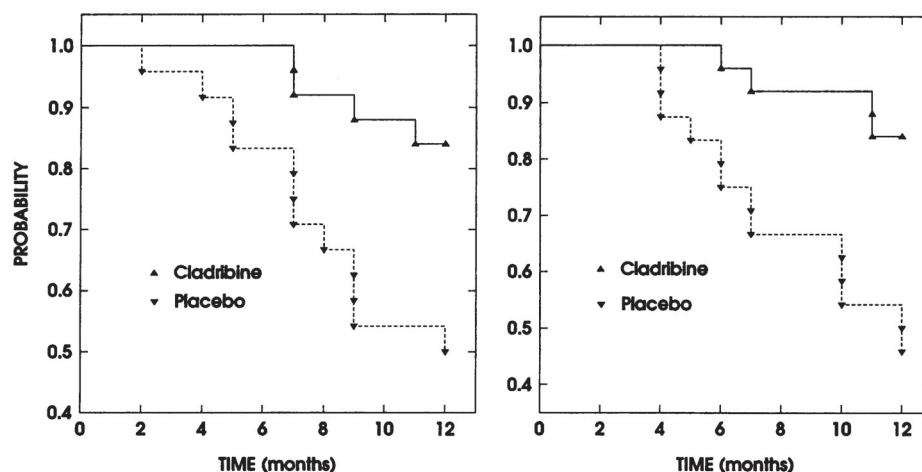


FIG. 2. Kaplan-Meier time-to-failure plots of patients in the double-blind crossover study. (Left) Time-to-increase of EDSS score by 1.0 point. (Right) Time-to-failure as defined by a decrease of SNRS score by 10 points. The difference in time-to-failure estimated by logarithmic-rank statistics was highly significant (EDSS,  $P = 0.012$ ; SNRS,  $P = 0.004$ ).

Table 2. Presence of enhancing lesions by MRI over the study

| Time     | Outcome         |                 |                    |                 |
|----------|-----------------|-----------------|--------------------|-----------------|
|          | Initial placebo |                 | Initial cladribine |                 |
|          | Lesions absent  | Lesions present | Lesions absent     | Lesions present |
| Baseline | 11              | 13              | 12                 | 12              |
| 6 mo     | 13              | 11              | 18                 | 6               |
| 12 mo    | 12              | 12              | 22                 | 2               |
| 18 mo    | 22              | 1               | 23                 | 0               |
| 24 mo    | 21              | 1               | 20                 | 0               |

24 mo, 19 had no lesions both at 12 and at 24 mo, and one had lesions at 12 but none at 24 mo. Thus, the treatment effect found in the group that received cladribine in the first year seemed to carry over for an additional 12 mo.

**Toxicity and adverse events.** The average platelet counts remained normal throughout the study, but the platelet counts of seven patients receiving the larger cladribine dose in the first year of the study fell to  $<100,000/\mu\text{l}$ , and one of these, a patient who had taken carbamazepine (Tegretol) and who was ingesting large amounts of phenytoin (Dilantin), developed severe thrombocytopenia with platelet counts  $<10,000/\mu\text{l}$ . The effect of cladribine on the blood counts of these patients has been reported in detail elsewhere (16). In the second year of the study, when patients received only one-half of the dose of cladribine administered in the first year, platelet counts of  $<100,000/\mu\text{l}$  were encountered in only one patient, the lowest count observed being  $83,000/\mu\text{l}$ . Changes in lymphocyte subsets are depicted in Fig. 3. There was marked depletion of CD4 cells with both the high- and low-dose schedule.

Six patients developed herpes zoster, one of them only after retreatment with cladribine under another protocol. All of these infections were segmental, mild, and responded rapidly to oral acyclovir. One patient developed fatal, fulminating, newly acquired hepatitis B immediately after her second dose of cladribine. As discussed elsewhere (7), it seems unlikely that the administration of cladribine played a role in this patient's illness. One patient developed a severe *Salmonella* infection with near-perforation of the bowel while receiving placebo during the first study year. She responded well to antibiotic therapy and continued the study.

## DISCUSSION

Although numerous immunosuppressive and immunomodulatory drugs have been given to patients with MS, conventional immunosuppression has not demonstrated sufficient promise to be considered as a routine treatment for the chronic progressive form of MS (17–19). Why then, might another immunosuppressive agent produce a greater effect? The suppression of CD4 cells and sparing of CD8 cells that was observed with cladribine administration is much greater than that observed with other immunosuppressive agents. Treatment of MS patients for a year with chlorambucil (18) and cyclophosphamide (20) each produced a relatively transient 2-fold decline in the CD4/CD8 ratio. In contrast, treatment with cladribine for only a few months produced an  $\approx 4$ -fold decrease in this ratio, and the effect was sustained for many months after a 4-mo course of the drug.

Because of its selective and prolonged effect on T cells, cladribine appeared to be a reasonable candidate drug for MS treatment. Our studies clearly show that cladribine retards the progression of neurologic impairment of patients with chronic

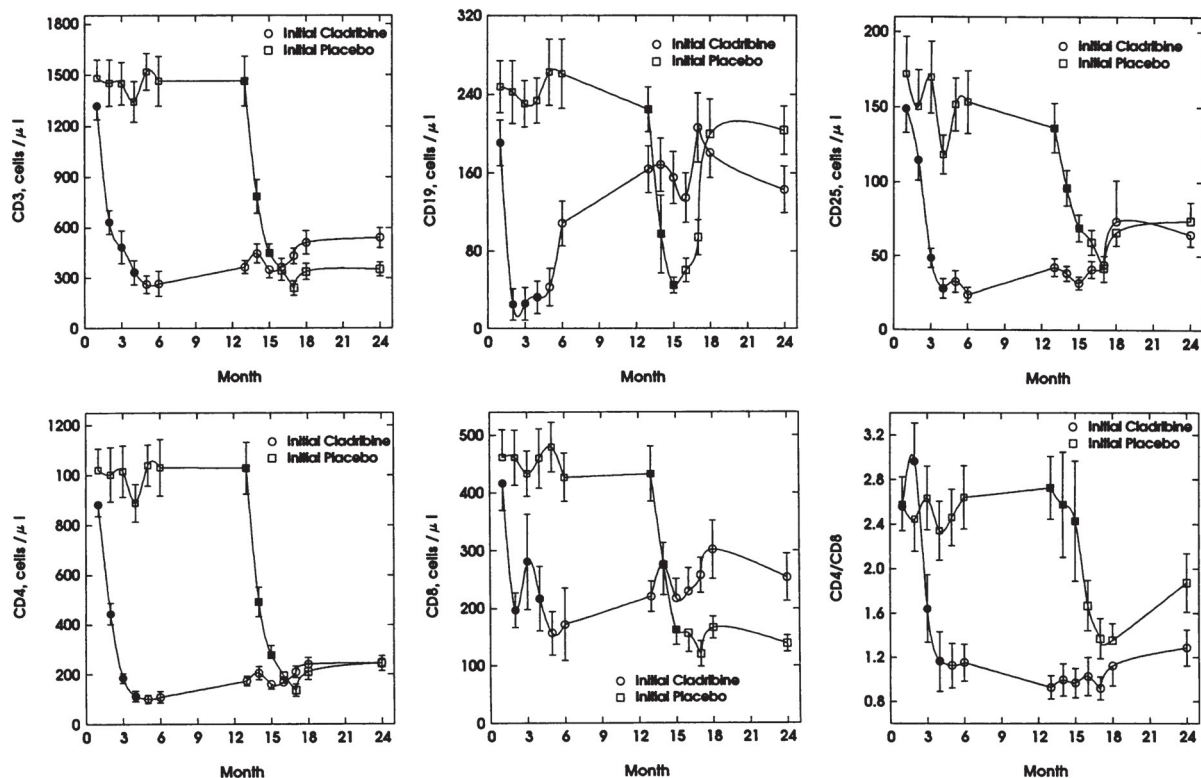


FIG. 3. Changes in lymphocyte subsets in patients. Symbols and drug dosages are as in Fig. 1. The decline of T lymphocytes (CD3) was more marked than that of B lymphocytes. A rapid and profound fall in the inducer/helper (CD4) T lymphocytes was accompanied by a more modest and less sustained decline in the cytotoxic/suppressor (CD8) T lymphocytes. Accordingly, there was an  $\approx 4$ -fold decline in the CD4/CD8 ratio. The number of activated T and B lymphocytes (CD25) fell  $\approx 5$ -fold and remained at subnormal levels for the 14 mo after cessation of drug administration. The slight dip in some of the lymphocytes in the group that received cladribine first is due to the erroneous administration of a single dose of drug to a few of the patients in that group on month 13.

progressive MS. The effect is of sufficient magnitude that highly statistically significant results could be obtained by the study of only 24 pairs of patients in a double-blind study. The smaller effects produced by other drugs have required that much larger groups of patients be investigated. At a total dose of 2.8 mg/kg given over a period of 4 mo, the stabilization of disease appears quite durable.

As for other cytotoxic agents such as methotrexate and cyclophosphamide, marrow suppression is a known side-effect of cladribine (6, 21). In the present study occasional patients developed significant thrombocytopenia at higher drug dosages; one patient developed severe but reversible marrow suppression. The lower dose of cladribine used in the second arm of the present study appears to provide a larger margin of safety. From this point of view, we were encouraged by the therapeutic effect of 1.4 mg/kg of drug given over a 3-mo period, a dose that appeared to be very well tolerated. The therapeutic effect of the lower dose appeared to be less durable than that observed at higher doses, this dosage-response relationship lending further weight to the validity of our results.

The usefulness of MRI as a measure of clinical activity and potentially as an objective means for assessing response to therapy has recently been documented (19). In our studies we observed marked improvement in the appearance and disappearance of lesions visualized on MRI after gadolinium enhancement. These highly statistically significant changes persisted for the full 2 yr of observation in the patients who had received 4 mo of therapy in the first arm of the study and were seen in patients receiving the low cladribine dose as well as in those receiving the high dose.

Treatment with immunosuppressive drug is not without risk. The occurrence of herpes zoster in six of the patients is surely a manifestation of their immunosuppressed state, but no other opportunistic infections attributed to cladribine therapy were encountered, in spite of the fact that the CD4 cell count remained low for long time periods. Many immunosuppressive drugs are also myelosuppressive, and cladribine does exhibit a dose-dependent effect on hematopoietic stem cells. In the current study thrombocytopenia was noted in some patients receiving higher doses of the drug, and severe, transient marrow suppression occurred in one patient who was also ingesting other potentially myelosuppressive drugs (16). Although the short-term risks of cladribine seem acceptable for patients with severely progressive disease, little can be written about the long-term risk. We began using this drug, primarily in patients with malignant disorders, in 1981. Most patients treated before 1987 had end-stage malignant disease, and only a few are alive today. In the past 8 yr, however, many patients with hairy cell leukemia and with relatively early-stage lymphomas have been treated with the drug. No long-term toxic results have been documented, but since the drug is incorporated into DNA (3), the possibility of malignancies occurring long after administration of this purine analogue cannot be dismissed.

Cladribine was given by continuous i.v. infusion on an outpatient basis to all of the patients reported here. This route of infusion was used because the safety and efficacy of the drug given by other routes had not been established at the time this study was initiated. We now recognize that the drug may be

given by the much more convenient subcutaneous route without causing local irritation and with pharmacokinetic (22) and therapeutic (23) results that appear entirely equivalent to the i.v. route. We have designed a study of the effect in both chronic progressive and relapsing/remitting MS of subcutaneously administered cladribine, 0.07 mg/kg per day given as six monthly courses each consisting of five daily injections. It is notable, that this method of administration has recently been used in MS patients by Grieb *et al.* (24) who report preliminary data suggesting beneficial results in the treatment of relapsing/remitting disease with cladribine.

This is manuscript 9176-MEM from The Scripps Research Institute. This work was supported by National Institutes of Health Grants NS30218 and RR00833, Food and Drug Administration Grant FD-R-000280, and the Stein Endowment Fund.

1. Ford, H. C. (1987) *Med. Hypotheses* **24**, 201–216.
2. Weiner, H. L. & Hafler, D. A. (1988) *Ann. Neurol.* **23**, 211–222.
3. Carson, D. A., Wasson, D. B., Taetle, R. & Yu, A. (1983) *Blood* **62**, 737–743.
4. Carson, D. A., Wasson, D. B. & Beutler, E. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 2232–2236.
5. Carson, D. A., Wasson, D. B., Kaye, J., Ullman, B., Martin, D. W., Jr., Robins, R. K. & Montgomery, J. A. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 6865–6869.
6. Beutler, E. (1994) *Semin. Hematol.* **31**, 40–45.
7. Sipe, J. C., Romine, J. S., Koziol, J. A., McMillan, R., Zyroff, J. & Beutler, E. (1994) *Lancet* **344**, 9–13.
8. Poser, C. M., Paty, D. W., Scheinberg, L., McDonald, W. I., Davis, F. A., Ebers, G. C., Johnson, K. P., Sibley, W. A., Silberberg, D. H. & Tourtellotte, W. W. (1983) *Ann. Neurol.* **13**, 227–231.
9. Kurtzke, J. F. (1983) *Neurology* **33**, 1444–1452.
10. Sipe, J. C., Knobler, R. L., Braheny, S. L., Rice, G. P., Panitch, H. S. & Oldstone, M. B. (1984) *Neurology* **34**, 1368–1372.
11. Cohen, J. (1968) *Psychol. Bull.* **70**, 213–220.
12. Goodkin, D. E., Cookfair, D., Wende, K., Bourdette, D., Pulicino, P., Scherokman, B. & Whitham, R. (1992) *Neurology* **42**, 859–863.
13. Jones, B. & Kenward, M. G. (1989) *Design and Analysis of Cross-Over Trials* (Chapman & Hall, London).
14. Koziol, J. A. & Maxwell, D. A. (1983) *Stat. Med.* **1**, 83–89.
15. Miller, D. H., Barkhof, F., Berry, I., Kappos, L., Scotti, G. & Thompson, A. J. (1991) *J. Neurol. Neurosurg. Psychiatr.* **54**, 683–688.
16. Beutler, E., Koziol, J., McMillan, R., Sipe, J. C., Romine, J. S. & Carrera, C. J. (1994) *Acta Haematol. (Basel)* **91**, 10–15.
17. Goodkin, D. E., Bailly, R. C., Teetzen, M. L., Hertsgaard, D. & Beatty, W. W. (1991) *Neurology* **41**, 20–25.
18. Chiappelli, F., Myers, L. W., Ellison, G. W., Liao, D. & Fahey, J. L. (1991) *Int. J. Immunopharmacol.* **5**, 455–461.
19. Khoury, S. J., Guttmann, C. R., Orav, E. J., Hohol, M. J., Ahn, S. S., Hsu, L., Kikinis, R., Mackin, G. A., Jolesz, F. A. & Weiner, H. L. (1994) *Neurology* **44**, 2120–2124.
20. Moody, D. J., Fahey, J. L., Grable, E., Ellison, G. W. & Myers, L. W. (1987) *J. Neuroimmunol.* **14**, 175–182.
21. Beutler, E. (1992) *Lancet* **340**, 952–956.
22. Liliemark, J., Albertioni, F., Hassan, M. & Juliusson, G. (1992) *J. Clin. Oncol.* **10**, 1514–1518.
23. Juliusson, G., Liliemark, J., Hippe, E., Blichfeldt, P., Wallman, K., Hagberg, H., Stolt, C.-M., Bergheim, J., Winqvist, I., Hedenus, M., Carlsson, M., Velin, L., Hellquist, L., Kristofferson, R., Ly, B. E. & Väärt, J. (1992) *Blood* **80**, Suppl. 1, 359 (abstr.).
24. Grieb, P., Ryba, M., Stelmasiak, Z., Nowicki, J., Solski, J. & Jakubowska, B. (1994) *Lancet* **344**, 538.