Evaluation of Levamisole as an Adjuvant to Chemotherapy for Treatment of ANLL

KERRY VAN SLOTEN, RN, PETER H. WIERNIK, MD, CHARLES A. SCHIFFER, MD, AND STEPHEN C. SCHIMPFF, MD

Levamisole is a synthetic, orally administered, relatively nontoxic compound with immunorestorative ability. Levamisole was tested in this study of 60 adults with acute nonlymphocytic leukemia (ANLL) to determine if augmentation of response rate or duration, or survival over that obtained with a standard chemotherapy regimen alone would result. The chemotherapy regimens for all patients consisted of daunorubicin and cytosine arabinoside for induction and consolidation, methotrexate with citrovorum factor reversal in a cytoreductive phase, and late intensification with thioguanine and cytosine arabinoside. The first 30 patients received chemotherapy alone; a second group of 30 patients were scheduled to receive levamisole in addition to chemotherapy. Levamisole, 45 mg/m^2, was administered orally twice daily for three consecutive days each week beginning one week after the initiation of induction chemotherapy and continuing until relapse. No significant difference emerged between the two groups with respect to remission rate, time to achieve remission, postrelapse survival, or total survival. However, a trend towards improved postcomplete remission survival ($P = 0.072$) was noted in the levamisole group, and patients who received levamisole had a significantly greater reinduction rate after relapse ($P = 0.019$).

Cancer 51:1576–1580, 1983.

INDUCTION of complete remission is now achieved in 60–70% of adults with acute nonlymphocytic leukemia (ANLL) with regimens that include at least an anthracycline and cytosine arabinoside.[1,2] Unfortunately, the median duration of remission in complete responders is usually less than one year despite aggressive maintenance chemotherapy.[3,4] Based on the concept that a natural defense mechanism exists against neoplasia involving primarily cell-mediated immunity, various nonspecific immunotherapeutic agents have been tested in patients with ANLL in an effort to enhance response rate, remission duration, and survival. Among the agents available, levamisole is one of the more appealing for a variety of reasons. The drug is of synthetic origin and, therefore, easier to obtain and standardize. It is relatively nontoxic with only mild nausea and fatigue reported with regularity.[5,6] Hematologic toxicity, manifested as either leukopenia or agranulocytosis, occurs in about 2% of cancer patients who receive the drug and is readily reversible.[7] Other infrequent side effects include dysosmia and metallic taste, nervousness and sleep disturbances, and skin rashes.[8] The drug is administered orally and is therefore more readily accepted by the patient than other potentially immunotherapeutic agents. Levamisole acts as an immunorestorer rather than immunostimulant and has its most pronounced effect on lymphoid cells from an immunodepressed host.[8–10]

Levamisole has been reported to enhance disease-free and/or overall survival in patients with a variety of neoplasms, including breast,[11,12] lung,[13] and gastrointestinal cancer.[14] In addition, similar results have recently been reported in children with acute lymphocytic leukemia.[15] Therefore, levamisole was tested in this study of patients with ANLL to determine if it could augment the results obtained with chemotherapy alone in that disease.

Materials and Methods

Sixty patients with ANLL, newly diagnosed and previously untreated, were entered into the study. Characteristics of the patients studied are listed in Table 1. Treatment was begun when serial marrow and peripheral blood studies indicated progressive disease. All patients had at least 50% leukemic cells in the marrow when treatment began.

All patients received the same chemotherapy regimen. Patients received daunorubicin (45 mg/m^2/day) on the

From the Baltimore Cancer Research Center, University of Maryland at Baltimore, Baltimore, Maryland.

Address for reprints: Peter H. Wiernik, MD, Albert Einstein College of Medicine, 111 East 210th Street, New York, NY 10467.

Accepted for publication February 17, 1982.

0008-543X/83/0501/1576 $1.05

first 3 days of treatment and cytosine arabinoside (100 mg/m^2/day) by continuous intravenous infusion on the first 7 days of treatment as induction therapy. Upon achievement of complete remission, 2 courses of consolidation therapy with these agents (2 days and 5 days, respectively, at the same doses) were given.

Immediately following recovery from the last consolidation treatment, a cytoreductive treatment phase was begun. Patients received methotrexate monthly in 3 escalated doses of 50, 100 and 150 mg/kg in a 6 hour infusion followed 2 hours later by citrovorum factor 12–15 mg IV every 6 hours for 24 hours and then orally for an additional 48 hours.

The third course of methotrexate was followed by a three-month rest period without chemotherapy. Then, intensification therapy using cytosine arabinoside 100 mg/m^2 IV every 12 hours and thioguanine 100 mg/m^2 orally, every 12 hours, was administered until profound marrow hypoplasia was achieved. Such courses of cytosine arabinoside and thioguanine were given every three months until relapse. During periods of granulocytopenia following this treatment, patients received oral nonabsorbable antibiotics, consisting of 200 mg of gentamycin liquid, 500 mg of vancomycin liquid, 4 $\times$ 10^6 units of nystatin tablets, and 1 $\times$ 10^6 units of nystatin liquid.[16] In addition, patients were placed in laminar air flow protective isolation whenever rooms were available.[17]

The first 30 patients were treated with chemotherapy alone, prior to levamisole availability. The second 30 patients began treatment after the drug became available for study and were designated to receive levamisole in addition to chemotherapy. Patients designated to receive levamisole began treatment with that drug one week after initiation of induction chemotherapy at a dose of 45 mg/m^2 twice daily for 3 consecutive days each week. Levamisole was continued until relapse. The study was closed to patient accrual July 1, 1977 and data available as of March 1, 1981 are evaluated in this report.

TABLE 1. Characteristics of Evaluable Patients: Levamisole *Versus* Control

	Levamisole	Control	Total
Male/female	15/12	16/13	31/25
Age (yrs)			
Median	53	52	52.5
Range	20–67	16–72	16–72
Pretreatment leukocyte count ($\times 10^3$)			
Median	3.4	6.7	4.7
Range	0.7–700	0.8–90	
Pretreatment hematocrit (%)			
Median	30	29	29
Range	22–45	8–44	
Pretreatment platelet count ($\times 10^3$)			
Median	51	49	50
Range	12–200	9–726	
Infected on admission	11/27	5/29	16/56

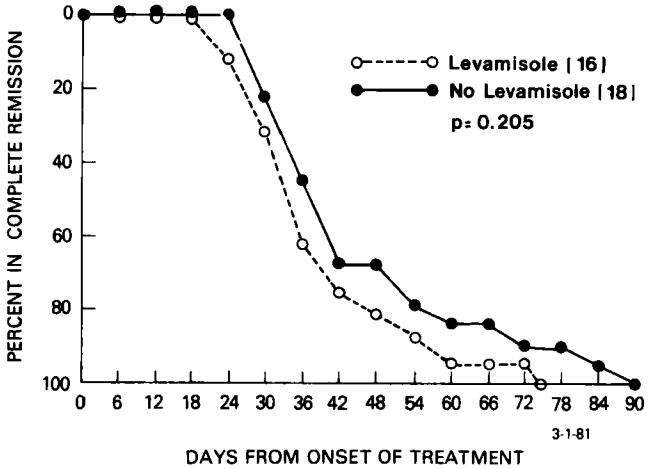

FIG. 1. Time to complete remission after chemotherapy for ANLL with and without levamisole.

Results

Of the 60 patients entered into this study, 56 proved to be evaluable. One patient in the no levamisole group was disqualified from evaluation because of a history of previous malignancy treated with chemotherapy; three patients designated to receive levamisole died prior to receiving the drug. Complete remission was achieved by 62% (18/29) of patients who did not receive levamisole and 59% (16/27) of those who did. Time to remission (Fig. 1) was similar (P = 0.205) for both groups (no levamisole: median, 36.5 days; range, 24–91; levamisole: median, 32 days; range, 22–72). The duration of first complete remission was also similar (P = 0.387) for both

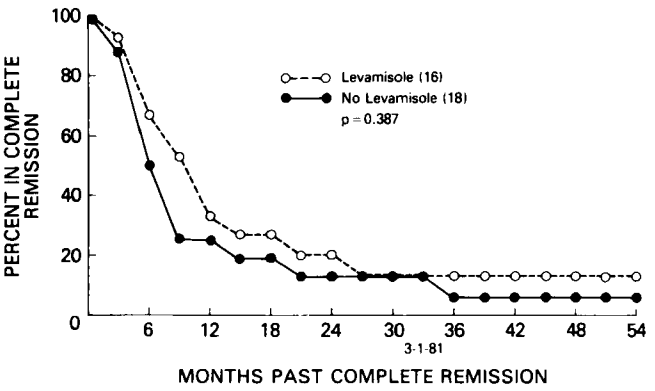

FIG. 2. Duration of complete remission.

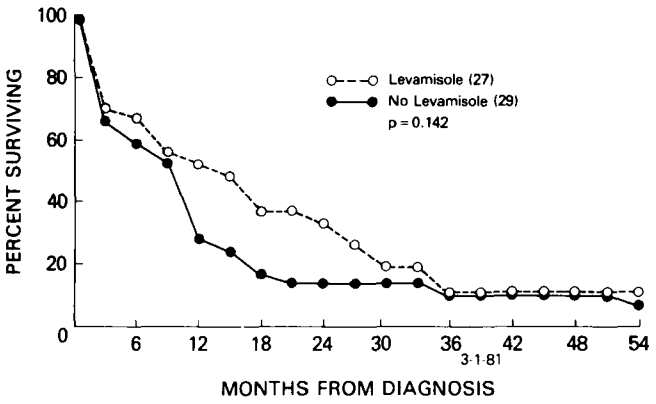

FIG. 3. Patient survival after diagnosis of ANLL.

groups (no levamisole: median, 178 days; range, 74–1984+; levamisole, median 304 days, range 40–1946+) although life table analysis (Fig. 2) reveals the levamisole curve to be consistently superior to that of the control group.

An earlier evaluation of this study suggested a trend toward better overall survival for levamisole-treated patients.[18] However, analysis at five years (Fig. 3) shows no significant difference in survival from the date of diagnosis ($P = 0.142$). Curiously, there is a trend toward superior survival of the levamisole patients from the date complete remission was achieved ($P = 0.072$) (Fig. 4).

Three patients remain in first complete remission as of March 1, 1981 at 54+, 65+ and 68+ months. Two received levamisole and one did not. The levamisole patient at 54+ months continues on both levamisole and late intensification chemotherapy. The levamisole patients at 65+ months discontinued the drug at 56 months and chemotherapy at 60 months. The no levamisole patient still in first complete remission at 68 months discontinued chemotherapy at 61 months. Five patients, including the three still in first complete remission, are alive at 54+, 55+, 65+, 66+, and 68+ months. Three received levamisole and 2 did not.

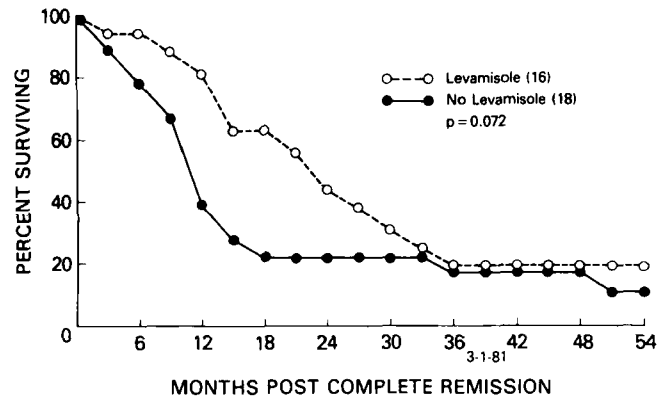

FIG. 4. Patient survival after achieving complete remission of ANLL.

TABLE 2. Second Remission by Chemotherapy Agents Used

	Levamisole patients	Control patients
Cytosine arabinoside and daunorubicin	9/9*	3/7
VP 16 (etoposide)	—	0/3
VP 16 (etoposide) and thioguanine	0/2	—
VP 16 (etoposide) and azacytidine	—	0/4
Azacytidine and pyrazofuran	1/1	—
Vincristine and prednisone	1/1	—
Cytosine arabinoside and thymidine	0/1	—
Overall	11/14	3/14

* Remissions achieved/patients treated.

A variety of agents was used to attempt reinduction of remission in 14 levamisole-treated patients who relapsed and 14 relapsed patients who did not receive levamisole. These agents are listed in Table 2. Overall, there were 11 second complete remissions in the levamisole group and 3 second complete remissions in the group that had not received levamisole. Despite this difference, postrelapse survival (Fig. 5) is not significantly different between the groups ($P = 0.103$).

In 16 of the 28 patients in whom reinduction was attempted, daunorubicin and cytosine arabinoside were employed in the same dose and schedule successful initially. Within this group of 16 patients a significant advantage is seen for the levamisole patients with respect to reinduction therapy success. All nine patients who had received levamisole achieved a second complete remission compared to three of seven patients who had not received it ($P_2 = 0.038$). When characteristics of the patients in these two groups are examined, they are found to be comparable in terms of age (levamisole: median age, 35; range, 20–64 years; no levamisole: median age, 24; range, 16–62 years), but not entirely equivalent in sex distribution, with six of nine levamisole patients and three of seven patients who did not receive levamisole being female. When adjusted for sex, the difference in successful second remission achievement is less significant ($P_2 = 0.085$).

Another potentially prognostic factor in complete remission rate is presence or absence of infection at time of treatment.[4] Although several patients developed infections during the granulocytopenic period following reinduction therapy, none of the 9 levamisole patients treated with daunorubicin and cytosine arabinoside had a clinically apparent infection at the time of treatment whereas one of the seven no levamisole patients did have an infection at the time of treatment which resulted in early death.

Levamisole was generally well-tolerated with three of 27 patients discontinuing the drug prior to relapse for possible drug-related side effects: one patient after six weeks for rash, one patient after two months for continued fever of unknown etiology, and one patient for prolonged agranulocytosis after consolidation therapy, more likely related to the patient's alcohol abuse, hepatitis, and malnutrition.

Discussion

This study was designed to test the efficacy of levamisole used as an adjuvant to chemotherapy for the treatment of acute nonlymphocytic leukemia. The complete remission rate for all patients in the study, 61%, was comparable to that achieved in other studies also using daunorubicin and cytosine arabinoside as induction chemotherapy. The median first remission duration, six months, was also comparable to other studies of similar design.

Both clinical trials and animal studies demonstrate the dose dependency of levamisole response. In a multicenter trial of levamisole adjuvant to surgery in bronchial cancer,[13] levamisole was given at a fixed dose of 150 mg/day. Statistically significant prolongation of the disease-free interval and total survival was obtained, but only in those patients weighing <70 kg, demonstrating the importance of adjusting dosage to either body weight or surface area. On the other hand, in a study of rats with breast cancer,[20] tumor inhibition was demonstrated at one dose while no inhibition occurred at higher doses. This correlated with the author's finding that human lymphocyte response was suppressed rather than augmented at high concentrations of levamisole.[20] Drug dosage in this study was optimal, based on all available data, being adjusted for body surface area and falling within the range of dosage used in successful clinical trials in other tumor systems.

Levamisole was well tolerated with only three of 27 patients discontinuing the drug for possible drug-related side effects. No serious levamisole toxicity occurred. One case of agranulocytosis possibly related to levamisole eventually resolved, coinciding with both discontinuation of the drug and resolution of the patient's hepatitis. Unfortunately, no augmentation of complete remission rate, time necessary to achieve remission, duration of first remission or total survival, occurred in the patient group that received levamisole.

Some advantage was seen for levamisole patients with respect to reinduction success. However, an unequal distribution of certain prognostic factors between the two groups of relapsed patients, such as sex and significant infection makes this observation difficult to interpret. If the observed difference in reinduction rate with the same chemotherapy that was initially successful is valid, it is of minor biologic significance since postrelapse and overall survival are not different between the group treated with levamisole and the group that was not.

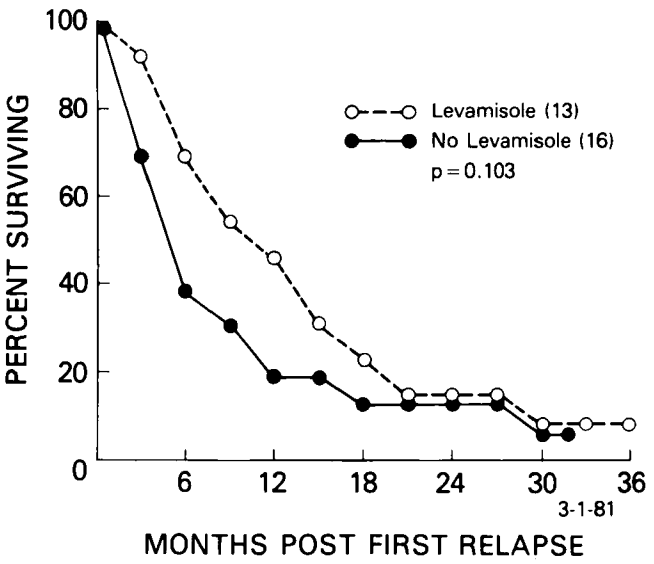

FIG. 5. Patient survival after first relapse of ANLL.

It seems unlikely that further study of levamisole in ANLL, as used in this study, will result in clinically useful data.

ADDENDUM

The five surviving patients on this study continue with no change in remission status as of March 1, 1982 from that described above. Three remain in complete remission at 66+, 77+ and 79+ months. Two received levamisole and one did not. Including these three, five patients are alive at 66+, 67+, 76+ and 79+ months. Three received levamisole and two did not.

REFERENCES

1. Chang P, Wiernik PH, Reich SD *et al.* Prediction of response to cytosine arabinoside and daunorubicin in acute nonlymphocytic leukemia. In: Mandell F, ed. Therapy of Acute Leukemias: Proceedings of the Second International Symposium. Rome: Lombardo Editore, 1979; 148–156.

2. Yates JW, Wallace HJ, Ellison RR *et al.* Cytosine arabinoside and daunorubicin therapy in acute nonlymphocytic leukemia. *Cancer Chemother Rep* 1973; 57:845.

3. Bodey GP, Rodriguez V, McCredie KB *et al.* Early consolidation chemotherapy for adults with acute leukemia in remission. *Med Pediatr Oncol* 1975; 2:299–307.

4. Wiernik PH, Glidewell OJ, Hoagland HC. A comparative trial of daunorubicin, cytosine arabinoside, and thioguanine, and a combination of the three agents for the treatment of acute myelocytic leukemia. *Med Pediatr Oncol* 1979; 6:261–277.

5. Lichtenfeld JL, Wiernik PH. Levamisole in a phase I trial. *Cancer Treat Rep* 1976; 60:963–4.

6. Renoux G. The general immunopharmacology of levamisole. *Drugs* 1980; 19:89–99.

7. Symoens J, Veys E, Mielants M *et al.* Adverse reactions to levamisole. *Cancer Treat Rep* 1978; 62:1721–1730.

8. Amery WK, Spreafico F, Rojas AF *et al.* Adjuvant treatment with levamisole in cancer. A review of experimental and clinical data. *Cancer Treat Rev* 1977; 4:167–194.

9. Symoens J, Rosenthal M. Levamisole in the modulation of the immune response: The current experimental and clinical state. A review. *J Reticuloendothel Soc* 1977; 21:175–221.

10. Spreafico F. Use of levamisole in cancer patients. *Drugs* 1980; 19:105–116.

11. Rojas AF, Feverstein JN, Glait HM *et al.* Clinical Action of Levamisole and Effects of Radiotherapy on Immune Response. In: Chirigos MA, ed. Control of Neoplasia by Modulation of the Immune System. Progress in Cancer Research and Therapy, vol. 2. New York: Raven Press, 1977.

12. Hortobagyi GN, Gutterman JU, Blumenschein GR *et al.* Combined chemoimmunotherapy for advanced breast cancer: Comparison of BCG and levamisole. *Cancer* 1979; 43:1112–1122.

13. Amery WK. Final result of a multi-center placebo-controlled levamisole study of resectable lung cancer. *Cancer Treat Rep* 1978; 62:1677–1683.

14. Verhaigen H. Post-operative levamisole in colorectal cancer. In: Rainer H, ed. Immunotherapy of Malignant Diseases. Stuttgart: Schattaner Verlay, 1978; 94–101.

15. Pavlovsky S, Muriel FS, Garay G *et al.* Chemoimmunotherapy with levamisole in acute lymphoblastic leukemia. *Cancer* 1981; 48:1500–1507.

16. Hahn DM, Schimpff SC, Fortner CL *et al.* Infection in acute leukemia patients receiving oral nonabsorbable antibiotics. *Antimicrob Agents Chemother* 1978; 13:958–964.

17. Schimpff SC, Greene WH, Young VM *et al.* Infection prevention in acute nonlymphocytic leukemia: Laminar air flow room reserve isolation with oral nonabsorbable antibiotic prophylaxis. *Ann Int Med* 1975; 82:351–358.

18. Janssen Pharmaceutica Clinical Research Report #R12, 564/ June 1980.

19. Amery WK, Verhagen H. Effects of levamisole treatment in cancer patients. *Rheumatology* 1978; 5 (Suppl 4):123–135.

20. Sampson D. Immunopotentiation and tumor inhibition with levamisole. *Cancer Treat Rep* 1978; 62:1623–25.