LEVAMISOLE AND FLUOROURACIL FOR ADJUVANT THERAPY OF RESECTED COLON CARCINOMA

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Abstract Twelve hundred ninety-six patients with resected colon cancer that either was locally invasive (Stage B_2) or had regional nodal involvement (Stage C) were randomly assigned to observation or to treatment for one year with levamisole combined with fluorouracil. Patients with Stage C disease could also be randomly assigned to treatment with levamisole alone. The median follow-up time at this writing is 3 years (range, 2 to 5½).

Among the patients with Stage C disease, therapy with levamisole plus fluorouracil reduced the risk of cancer recurrence by 41 percent ($P<0.0001$). The overall death rate was reduced by 33 percent ($P \approx 0.006$). Treatment with levamisole alone had no detectable effect. The results in the patients with Stage B_2 disease were equivocal and too preliminary to allow firm conclusions. Toxic effects of levamisole alone were infrequent, usually consisting of mild nausea with occasional dermatitis or leukopenia, and those of levamisole plus fluorouracil were essentially the same as those of fluorouracil alone — i.e., nausea, vomiting, stomatitis, diarrhea, dermatitis, and leukopenia. These reactions were usually not severe and did not greatly impede patients' compliance with their regimen.

We conclude that adjuvant therapy with levamisole and fluorouracil should be standard treatment for Stage C colon carcinoma. Since most patients in our study were treated by community oncologists, this approach should be readily adaptable to conventional medical practice. (N Engl J Med 1990; 322:352-8.)

THIS year, cancer of the colon will afflict over 100,000 persons in the United States.[1] As a cause of death due to cancer, it is second only to lung cancer. There is no established means of preventing colon cancer, and there is no reliable and cost-effective means of screening to ensure early diagnosis. In the main, symptomatic patients must be treated as they present themselves, and in half of them cure has unfortunately not been possible. However, in about 80 percent of patients the diagnosis is made at a stage when all apparent diseased tissue can be surgically removed. In such patients, incurability is likely to be due to residual cancer existing in an occult and probably microscopic stage. Studies of animal models of the disease indicate that at such a stage, effective chemotherapy or immunotherapy is most likely to result in cure. Numerous randomized, controlled trials of adjuvant therapy for resected colon cancer have used various forms of immunotherapy and chemotherapy, often fluorouracil either alone or in combination with other agents. Although these trials have involved several thousand patients, there has been no convincing and reproducible evidence of significant benefit.[2] The consensus has seemed to be that the best standard treatment was surgery alone, and thus that the use of untreated controls was ethically justified in any surgical adjuvant trial.

In 1989 a report from the North Central Cancer Treatment Group (NCCTG) suggested a possible benefit from the use of levamisole, either alone or in combination with fluorouracil, as adjuvant therapy for colorectal cancer in Dukes' Stage B_2 (invasion of serosa or pericolonic fat) or Stage C (metastasis to regional lymph nodes).[3]

Levamisole has had extensive, worldwide use as an anthelmintic drug in both humans and domestic animals.[4] It attracted interest as an agent for cancer therapy because of its presumed immunomodulatory activity.[4,5] Its use in combination with fluorouracil in the NCCTG study was based on the hope that it would add to the marginal activity of fluorouracil.[3] The results of that trial, which had a median follow-up time of seven years, showed that adjuvant therapy with the combination of levamisole and fluorouracil significantly reduced the cancer-recurrence rate as compared with the rate when no adjuvant therapy was given, whereas the use of levamisole alone produced a borderline advantage. Analysis of overall survival showed a suggestive but not definite advantage of the combination therapy. Subset analysis, however, did show that the treatment conferred a significant advantage for survival on patients with Stage C disease. These results were sufficiently promising to justify a larger and more definitive study. The methods of this confirmatory trial were nearly identical to those of the NCCTG study, except that, to avoid possible confusion due to subset analysis, sepa-

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rate studies were conducted for patients in Stage B_2 and those in Stage C.

METHODS

This was a national intergroup trial that was sponsored by the National Cancer Institute and involved the Eastern Cooperative Oncology Group, the NCCTG, the Southwest Oncology Group, and the Mayo Clinic. Enrollment of patients was begun in March 1984, when a preliminary analysis of the NCCTG study[3] indicated the likelihood of a treatment advantage for levamisole plus fluorouracil and for levamisole alone, with regard to time to recurrence. Enrollment was completed in October 1987.

Patient Selection

All patients were required to have undergone a potentially curative en bloc resection of an adenocarcinoma of the colon without gross or microscopic evidence of residual disease. Patients with rectal carcinoma were ineligible. The resected specimen in eligible patients showed one of two indicators of poor prognosis — invasion extending at least to the serosa or pericolonic fat (Stage B_2) or metastasis to regional lymph nodes (Stage C). It was further required that the patient be able to swallow oral medication and have a leukocyte count of at least 4000 per microliter and a platelet count of at least 130,000 per microliter. Patients were ineligible if they had had any other cancer within five years except for superficial skin carcinoma or in situ carcinoma of the cervix. Eligibility was determined by careful review of study forms, operative reports, and pathology reports. Entry into the study was allowed no earlier than one week and no later than five weeks after surgery.

Stratification and Randomization Procedures

Written informed consent was obtained from the patients before they were enrolled in the study. Patients with B_2 lesions were stratified according to the extent of invasion (only into or through the serosa vs. into adjacent organs) and the interval since surgery (7 to 20 days vs. 21 to 35 days). They were then randomly assigned to either observation or therapy with levamisole plus fluorouracil. A dynamic randomization method was employed.[6]

Patients with Stage C lesions were stratified according to the invasion by the primary lesion and the interval since surgery and also according to the number of lymph nodes involved (≤ 4 vs. >4). They were then randomly assigned to observation, therapy with levamisole alone, or therapy with levamisole plus fluorouracil.

Protocol Management

Within 72 hours before randomization a medical history was taken and the following were performed: a physical examination, hematologic testing (hemoglobin measurement, leukocyte count, platelet count, and differential count), a blood-chemistry panel (including at least measurement of bilirubin, alkaline phosphatase, aminotransferases, and creatinine), and chest radiography if it had not been performed preoperatively.

Patients assigned to the control arm were observed after surgery, with no planned treatment. During the first year they were evaluated every 12 weeks, during the second year every four months, and thereafter every six months, for a total of five years. These evaluations consisted of an interim history-taking and physical examination, hematologic testing, a blood-chemistry panel, and chest radiography. In addition, either a proctoscopic examination and radiography of the colon (barium enema) or a colonoscopic examination was performed at 24 weeks, 48 weeks, and annually thereafter. Follow-up was continued beyond five years but without formal protocol requirements.

Patients assigned to levamisole alone had periodic evaluations at the same time as those who received no adjuvant therapy. In addition, they received levamisole by mouth (50 mg every eight hours) for a period of three days; this was repeated every two weeks for one year. Hematologic testing and blood-chemistry panels were repeated every four weeks. If persistent dermatitis or leukopenia developed, levamisole was discontinued.

Patients assigned to levamisole plus fluorouracil were evaluated at the same time as those who received no adjuvant therapy, and were given levamisole in the same dose and on the same schedule as patients assigned to levamisole alone. In addition, no earlier than 21 days after surgery, they received fluorouracil by rapid intravenous injection (450 mg per square meter of body-surface area) daily for five consecutive days. Twenty-eight days after the start of this course, weekly treatment with fluorouracil was begun with an intravenous dose (450 mg per square meter) and continued for 48 weeks. Leukocyte counts were determined before each weekly dose. If stomatitis, diarrhea, or leukopenia developed, weekly fluorouracil treatment was deferred until the side effects subsided. If these side effects were moderate to severe, the dose of fluorouracil was reduced by 20 percent.

Statistical Analysis

Survival was the primary end point of this study; the time to recurrence was also determined. A minimum of 150 eligible patients per treatment arm was planned for the trial involving Stage B_2 disease and 300 patients per arm for Stage C. This ensured that the Stage B_2 trial would have a power of 0.90 to detect a ratio of the control-group hazard to the combination-therapy–group hazard of 2.0. This assumed a pairwise comparison of treatments by a one-sided log-rank test in which a value of 0.05 indicated statistical significance. The Stage C study would have a power of 0.90 to detect a hazard ratio of 1.35.

Statistical analyses were carried out according to the procedures of the Statistical Analysis System.[7] The survival curves were generated by the Kaplan–Meier method.[8] The log-rank statistic[9] was used to compare the distributions of survival times. The Cox proportional-hazards model[10] was used to determine the ratios of relapse and survival rates and to perform all multivariate analyses. Backward regression was used to find the significant prognostic factors; variables were progressively eliminated on the basis of the maximal partial-likelihood estimate (MLE) statistics. To adjust for covariates when evaluating treatments, we kept the variable of treatment in the model and used the backward regression for other covariates, keeping those whose MLE statistics satisfied the criterion of a P value less than 0.01. All P values reported for this study are two-sided.

Results were carefully monitored with periodic formal analyses of survival, recurrence, and other secondary outcomes. Consideration was given at these times to possible early reporting of results. We used the four-stage group sequential boundary of O'Brien and Fleming[11] with an error rate of 0.05, since we thought it clear that early termination should occur only if the results were extreme. Analyses were planned to occur after approximately 125, 250, 375, and 500 deaths were observed among patients with Stage C disease. Any decision about early termination and early reporting was planned to be global in nature, taking into account not only overall survival but also the characteristics of the patients treated, overall recurrence, toxicity, and relevant results reported by other investigators. At the second planned interim analysis in September 1989, the results for survival met the protocol criteria for early reporting. To be specific, because this interim analysis was performed after 301 deaths were observed among patients in Stage C, the O'Brien–Fleming criterion required the two-sided P value to be less than 0.0098 — i.e., $2\,\Phi\,[-4.006\,(125/301)^{1/2}]$, where Φ denotes the cumulative distribution function for the standard normal distribution (see Table 1 of Emerson and Fleming).[12] This criterion, however, was met only in the Stage C part of the study, which is reported in detail. Although the results of the Stage B part of the study will be described in brief, we regard these as inconclusive.

RESULTS

A total of 1296 patients were entered in this trial. Of 325 patients entered in the Stage B_2 study, 7 (2.2 percent) were considered ineligible (2 assigned to observation and 5 to levamisole plus fluorouracil). Of 971 patients entered in the Stage C study, 42 (4.3 percent) were ineligible (12 assigned to observation,

18 to levamisole alone, and 12 to levamisole plus fluorouracil). Ineligibility was most frequently due to the presence of a stage of disease more advanced than that allowed by the protocol. Because ineligibility was not biased by treatment assignment, these patients were excluded from the analysis. Eight patients in the Stage B_2 study and 14 in the Stage C study refused to accept their treatment assignment. Of those who refused in the Stage B study, 5 (63 percent) were assigned to the treatment arm; 13 of 14 (93 percent) of those who refused in the Stage C study were assigned to the observation arm. Because this withdrawal from study could be and undoubtedly was biased by treatment assignment, these patients were included in all analyses. At present, follow-up findings in 98 percent of the patients have been reported in the timely fashion specified by the protocol.

The median follow-up time for this study is now 3 years (range, 2 to 5½). On the basis of the original projections of our protocol, we estimate that among patients with Stage B_2 disease, 80 percent of the anticipated recurrences in the control arm have been recorded but only 27 percent of the deaths expected to occur during the first five years. Therefore, from the standpoint of survival these are rather early results. On the other hand, results in the Stage C study show that 82 percent of the anticipated recurrences have occurred, as well as 60 percent of the anticipated deaths.

Table 1. Clinical and Pathological Characteristics in the Stage C Colon-Cancer Study.

	OBSERVATION (N = 315)	LEVAMISOLE (N = 310)	LEV + 5-FU (N = 304)
Age — median (range)	60 (18–84)	61 (26–83)	61 (25–80)
		percent of patients	
Sex — male	53	57	46
Days since surgery			
7–20	29	26	25
21–35	71	74	75
Location of primary tumor			
Cecum and right colon	31	35	34
Flexures and transverse colon	14	19	17
Left colon	6	5	4
Sigmoid and rectosigmoid	47	38	43
Multiple primaries	3	3	2
Depth of invasion			
Submucosa	3	1	3
Muscular layer	12	12	10
Serosa	85	87	86
Adjacent organ involvement			
Adhesion	15	16	13
Invasion	8	7	3
Obstruction	20	20	18
Perforation	3	4	3
Regional peritoneal implants	5	6	7
No. of nodes involved			
1–4	72	71	74
>4	28	29	26
Histologic differentiation			
Well	9	12	10
Moderately well	73	71	71
Poor	17	14	18
Unknown	2	3	2

Stage B_2 Study

The characteristics of the 318 patients in this study are well balanced between the protocol arms. At the time of this writing, 54 patients have had recurrences (32 on the observation arm and 22 on the levamisole–fluorouracil arm). At 3½ years, 84 percent of the patients who received levamisole plus fluorouracil and 77 percent of the patients who underwent observation are free of recurrence according to Kaplan–Meier estimates. There has been a disproportionate number of deaths due to causes unassociated with recurrence on the levamisole–fluorouracil arm (six, as compared with only one on the observation arm). The preliminary data on survival indicate that 29 patients have died — 18 among those receiving levamisole plus fluorouracil. The survival estimates at 3½ years are 91 percent in the control group and 85 percent in the levamisole–fluorouracil group. However, 22 patients in the control group and 10 in the levamisole–fluorouracil group have had recurrence of cancer but are still living. At least an additional two years of observation will be required before definite conclusions can be drawn.

Stage C Study

The characteristics of the 929 eligible patients with Stage C disease are shown in Table 1. In the main, they were well balanced among the study arms. More men received levamisole alone, and fewer received levamisole plus fluorouracil. There were more patients on the observation arm who had tumors of the sigmoid and rectosigmoid and lesions invading adjacent organs.

At present, 402 patients have had recurrences. Of these, 155 are in the observation group, 144 in the levamisole group, and 103 in the levamisole–fluorouracil group. The estimated overall reduction in the recurrence rate (Cox proportional-hazard model) with levamisole–fluorouracil therapy is 41 percent (95 percent confidence interval, 23 to 54 percent). At 3½ years, 63 percent of the patients receiving levamisole plus fluorouracil and 47 percent of the control patients are free of recurrence according to Kaplan–Meier estimates. Recurrence-free intervals are plotted in Figure 1 and are carried out to 52 months, at which point fewer than 10 percent of the patients can be followed. Therapy with levamisole plus fluorouracil produced an unequivocal advantage over observation ($P<0.0001$). On the other hand, therapy with levamisole alone produced no detectable effect.

Figure 2 shows the effect of therapy with levamisole plus fluorouracil on specific sites of initial recurrence. The rates of recurrence were reduced for all sites. This was most striking (>50 percent) for sites outside the abdominal cavity — i.e., the lungs, retroperitoneal nodes, peripheral nodes, and abdominal wall. It is possible that the more distant sites had a smaller, less well established tumor burden at the time that adjuvant therapy began.

Table 2 shows the relation of the patients' patho-

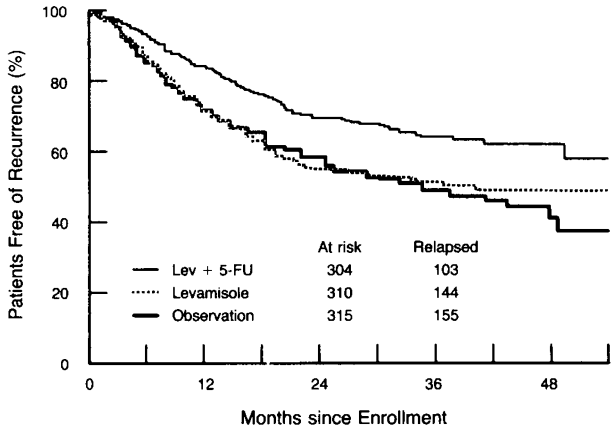

Figure 1. Recurrence-free Interval, According to Study Arm. Lev + 5-FU denotes combination therapy with levamisole and fluorouracil.

logical characteristics to recurrence. By a backward-regression selection procedure, the depth of invasion, the number of metastatic lymph nodes, and histologic differentiation were all found to be independent determinants of recurrence ($P<0.01$). After adjustment for imbalances among prognostic variables, therapy with levamisole plus fluorouracil was again found to have a significant advantage over observation in terms of preventing recurrence ($P = 0.0002$). Levamisole alone had no significant advantage ($P = 0.64$).

In these analyses, data on patients who died without recurrences were censored. Comparable results were observed when death without recurrence was considered an event — i.e., recurrence-free survival.

At present, 301 patients have died: 114 on the observation arm, 109 who received levamisole alone, and 78 who received levamisole plus fluorouracil. The estimated reduction in the death rate by treatment with levamisole plus fluorouracil as compared with observation is 33 percent (95 percent confidence interval, 10 to 50 percent). Eighteen patients died without evidence of recurrence: five on the observation arm, seven who received levamisole alone, and six who received levamisole plus fluorouracil. As would be anticipated in patients with this age distribution, these deaths were largely cardiovascular (11 patients). There are 119 patients who have documented recurrence but are still alive: 46 of these on the observation arm, 42 who received levamisole alone, and 31 who received levamisole plus fluorouracil. These figures make it likely that the survival advantage of treatment with levamisole plus fluorouracil will be sustained. Survival among all eligible patients in the study is plotted in Figure 3. The 3½-year survival estimates were 55 percent for the observation arm and 71 percent for the levamisole–fluorouracil arm. Whereas survival with levamisole therapy alone overlapped that with observation, survival with levamisole plus fluorouracil showed that this treatment had a decided advantage ($P = 0.0064$). This difference exceeded our protocol definition of extreme results that would justify early reporting — i.e., a two-sided P value of less than 0.0098.

The relations between the characteristics of the patients or their cancers and survival (Table 2) were similar to the relations between these characteristics and recurrence. After variables were selected by backward regression, the following characteristics were found to have independent prognostic significance ($P<0.01$): the location of the primary tumor, the depth of invasion, obstruction, the number of metastatic nodes, and histologic differentiation. When the proportional-hazard model was used to correct for the influence of prognostic variables, levamisole plus fluorouracil was again found to have a significant survival advantage over observation ($P = 0.0052$). Levamisole alone showed no effect ($P = 0.92$).

In exploratory subset analyses, levamisole–fluorouracil treatment appeared to have the greatest advantage among male patients (in both survival and recurrence), older patients (recurrence), patients with tumors that were well differentiated to moderately well differentiated (survival and recurrence), patients in whom more than four nodes were involved (survival), and patients treated 21 to 35 days after surgery (recurrence). These results show two striking contradictions to those of subset analyses reported in the NCCTG study, in which levamisole plus fluorouracil was found to be most effective in reducing the risk of recurrence among female patients and younger patients. This underscores the importance of the statement by the authors of that study, that "subset analyses must be interpreted with great caution."[3]

Toxicity

Toxic reactions are presented according to treatment arm in Table 3. For this analysis we have grouped data from the studies of Stages B_2 and C disease. The reactions to levamisole alone were typically mild. One patient, however, had a life-threatening exfoliative dermatitis. A characteristic reaction to levamisole that probably occurred more often than it was recorded was an unusual taste, usually described as metallic and occasionally associated with an altered sense of smell. Uncommon reactions, seemingly co-

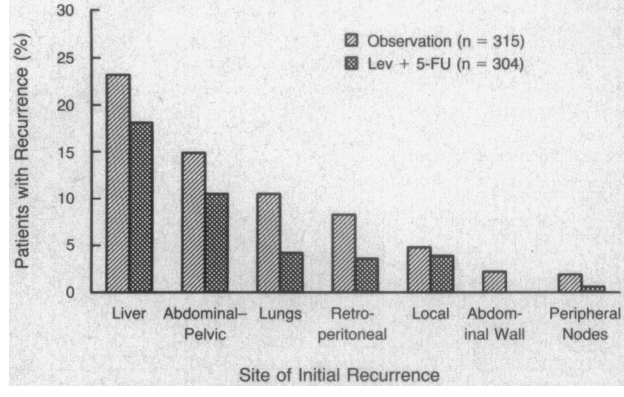

Figure 2. Site of Initial Recurrence, According to Study Arm.

incident with the days of treatment, were arthralgia and myalgia. Mood-altering effects have been attributed to levamisole,[4] and a small proportion of patients experienced anxiety, irritability, depression, somnolence, or insomnia. It was difficult to determine whether these symptoms were in fact treatment-related. Hematologic depression was infrequent and fully reversible. One patient had a minor rise in the bilirubin level at eight months without an accompanying change in liver enzyme levels.

The predominant toxic reactions to levamisole plus fluorouracil were those that might have been anticipated with fluorouracil alone — i.e., nausea, vomiting, diarrhea, stomatitis, dermatitis, and leukopenia. These reactions were rarely severe. Some degree of alopecia was experienced by 22 percent of the patients, but it was judged to be severe in only 2 percent. A variety of neurologic symptoms were experienced by 83 patients (18 percent). These ranged from vague lightheadedness and emotional changes to disabling cerebellar ataxia, and they usually abated when therapy was discontinued.

Eleven patients had evidence of hepatic toxicity (elevated levels of bilirubin, nine patients; alkaline phosphatase, seven patients; and aminotransferases, three patients). In 10 patients these changes occurred during maintenance therapy, reaching a maximum at a median time of 7½ months. In all instances there was improvement after therapy was discontinued, usually to a completely normal state (nine patients).

Leukopenia was the toxic reaction that usually led to dose limitation. It was seldom severe, but the single drug-related death observed in this study was due to profound leukopenia and sepsis.

Second Primary Cancers

Second primary cancers have been documented in 35 of the 1247 patients in this study: 15 of 474 (3.2 percent) in the observation group, 5 of 310 (1.6 percent) in the levamisole group, and 15 of 463 (3.2 percent) in the levamisole-plus-fluorouracil group. As might be expected, the large bowel was the most frequent site (nine cases). A single case of leukemia (acute lymphoblastic) was observed in the group that received levamisole alone, and this first became evident after only three months of therapy. Leukemogenesis has not been a recognized problem in previous trials with levamisole, and among almost 900 patients treated with levamisole or levamisole plus fluorouracil in the present study and the NCCTG study, this is the only documented case.

Table 2. Influence of Prognostic Variables on Recurrence and Survival (Stage C Study).

Variable	No. of Patients	Percent Recurrence-free at 3½ Yr	P Value	Percent Surviving by 3½ Yr	P Value
Sex					
Male	484	55	0.104	59	0.999
Female	445	51		61	
Age					
<61 yr	470	54	0.883	62	0.188
≥61 yr	459	52		58	
Days since surgery					
7–20	247	49	0.082	59	0.098
21–35	682	54		61	
Location of primary tumor*					
Cecum and right colon	308	52	0.111	53	<0.001
Flexures and transverse colon	153	50		51	
Left colon	49	60		64	
Sigmoid and rectosigmoid	397	55		68	
Multiple sites	24	42		67	
Depth of invasion*†					
Submucosa or muscular layer	127	76	<0.001	84	<0.001
Serosa	802	49		56	
Adhesion to adjacent organ					
Yes	58	46	0.068	45	0.020
No	871	54		63	
Invasion of adjacent organ					
Yes	58	43	0.006	29	0.006
No	871	54		61	
Obstruction*					
Yes	180	46	0.019	47	<0.001
No	749	55		63	
Perforation					
Yes	27	36	0.078	54	0.716
No	902	53		60	
Regional peritoneal implants					
Yes	57	38	0.006	42	0.001
No	872	54		61	
No. of nodes involved*†					
1–4	674	61	<0.001	70	<0.001
>4	255	33		34	
Histologic differentiation*†					
Well	93	56	<0.001	57	<0.001
Moderately well	663	55		64	
Poor	150	40		42	

*Independent prognostic variable for survival, P<0.01. †Independent prognostic variable for recurrence, P<0.01.

Treatment Compliance

Compliance with levamisole therapy was monitored both through questioning of the patients by physicians and nurses and through a drug diary kept by the patient. Of the 310 patients assigned to levamisole alone, 286 (92 percent) continued treatment for at least 90 percent of the scheduled year or until death or disease progression. Among the 24 patients in whom therapy was abbreviated, the most common reason was drug toxicity (11 patients) and the most frequent specific side effect was arthralgia (6 patients).

In view of the greater toxicity and practical problems of the levamisole–fluorouracil regimen, it is not surprising that a larger proportion of patients prematurely discontinued treatment (136 of 457, 30 percent), after a median of five months. In 56 of these patients,

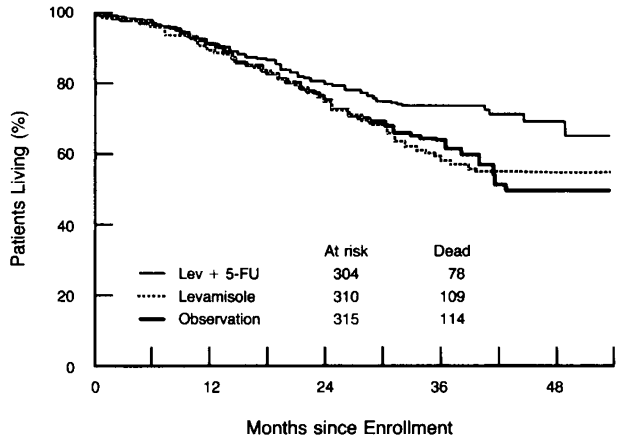

Figure 3. Survival, According to Study Arm.

toxicity was the principal reason, and the usual specific side effect was nausea.

Discussion

The results of this study indicate that therapy with levamisole plus fluorouracil reduces recurrence rates in patients with surgically treated Stage C colon carcinoma. This reduction in recurrence rates should lead to a reduction in deaths due to cancer. There is also evidence that treatment with levamisole plus fluorouracil significantly reduces the overall death rate, at least during the first 3½ years after surgery. These results confirm the significant overall reduction in recurrence rates and the reduction in death rates among patients with Stage C disease (significant only in subset analysis) observed in the NCCTG study.[3]

The improvement produced by levamisole plus fluorouracil was not only statistically significant but also clinically meaningful. The reduction in recurrence and death rates by approximately one third is substantial and justifies the inconvenience of therapy as well as the usually tolerable toxicity.

Approximately 21,000 patients will have surgical treatment for Stage C colon cancer in this country over the next year (Surveillance, Epidemiology and End Results Program, National Cancer Institute: unpublished data). Not all these patients will be suitable for or will desire adjuvant therapy, but there are very few contraindications to this treatment and it seems reasonable for physicians to offer this option. This study was not confined to major cancer centers or university hospitals. The majority of patients were fully treated in community practice. It is therefore reasonable to assume that therapy with levamisole plus fluorouracil could easily be incorporated into standard medical practice, with results comparable to those in this study.

Our results are still too early to allow assessment of the effectiveness of levamisole plus fluorouracil in patients with Stage B_2 cancer. There is a suggestion of reduced recurrence rates, but this has not been accompanied by any improvement in survival. Although advantages in both recurrence and survival were shown in patients with this stage in the NCCTG study, the numbers of patients were small and the differences were not significant. Also, neither the current study nor the NCCTG study offers evidence of the effectiveness of levamisole plus fluorouracil for rectal carcinoma, in which local recurrence is a major problem and emerging evidence indicates that multimodality approaches incorporating radiation therapy may be more rational and more effective.[13,14]

It is difficult to explain why this empirically conceived drug combination is effective. In advanced colorectal cancer, a preliminary report of a small study showed a suggestive but not significant advantage among patients given levamisole plus fluorouracil as compared with those treated with fluorouracil alone.[15] A larger and more mature NCCTG trial of a similar regimen, however, showed no survival advantage.[16] It is possible that the results of the present study could have been obtained with fluorouracil alone, but this seems unlikely in view of past experience. Although tested as a single agent in several trials of therapy adjuvant to colon surgery, fluorouracil has never been

Table 3. Toxic Effects, According to Treatment Arm.

Effect	Levamisole (N = 310)	Levamisole plus Fluorouracil	
		Induction (N = 457)	Maintenance (N = 441)*
		percent of patients	
Gastrointestinal			
Nausea	24	37	56
Severe	1	2	5
Vomiting	7	8	17
Severe	1	2	2
Diarrhea	13	25	47
Severe	1	3	7
Mucocutaneous			
Stomatitis	3	27	28
Severe	0	5	3
Dermatitis	9	8	22
Severe	2	1	1
Alopecia	3	4	22
Severe	0	1	2
Conjunctivitis	1	1	7
Hematologic†			
Leukopenia			
<4000–≥2000	8	38	38
<2000–≥1000	1	4	2
<1000	0	3	0
Thrombocytopenia			
<130,000–≥50,000	2	4	18
<50,000–≥25,000	1	1	3
<25,000	0	1	1
Other			
Fatigue or weakness	8	5	11
Taste change	7	2	7
Arthralgia or myalgia	7	2	4
Headache	3	1	3
Dizziness or vertigo	2	1	4
Ataxia	0	0	3
Anxiety or irritability	3	2	2
Impaired liver function	1	1	2

*Excludes patients who did not receive maintenance therapy.

†Excludes patients in whom counts were not adequately documented.

markedly effective. When all studies of fluorouracil were evaluated in a meta-analysis of 3499 patients, there was only a minor increase in five-year survival (3.4 percent), which barely reached statistical significance ($P = 0.04$).[2] In a small study using a different dosage regimen, patients treated with the combination of levamisole and fluorouracil were reported to have a significant survival advantage not only over untreated controls but also over patients treated with fluorouracil alone.[17] Although a few reports have claimed that the use of levamisole alone as an adjuvant to surgery had a positive effect on other cancers, these claims have been denied in confirmatory trials.[18] Our study and a recently reported trial of the European Organisation for Research on Treatment of Cancer[19] provide convincing evidence that levamisole alone is of little or no value in patients with colon cancer.

Although modulation of immunity has been the presumed mechanism for the antineoplastic activity of levamisole, this agent has a broad spectrum of pharmacologic activities, including the inhibition of fumarate reductase, potent inhibition of mammalian alkaline phosphatases, and inhibition of aerobic tumor glycolysis.[4,20] In animal models levamisole has been shown to improve survival when added to cyclophosphamide, semustine, and carmustine, and to do this in systems in which levamisole alone has no effect.[21] It is entirely possible that the clinical results we have obtained by adding levamisole to fluorouracil represent an example of biochemical modulation completely independent of immune effect. The possibility of biologic modulation is also raised by the finding that levamisole potentiates the activity of human interferon and interleukin-2.[22,23] The arbitrary nature of the dosage regimen for levamisole administration in this protocol probably dates back to the use of the drug as an anthelmintic. The purely empirical nature of the combination regimen we employed makes it likely that a more rational and effective combination regimen could be developed if the nature of the interaction between levamisole and fluorouracil were better known.[24]

We believe that this treatment should be offered to all patients with Stage C colon cancer who meet the eligibility criteria set down in our protocol. Since there appears to be no benefit of starting levamisole therapy soon after surgery, we recommend that therapy be deferred for a minimum of three weeks and begun then only if the patient is ambulatory, in a good state of nutrition, and free of postoperative complications. We have no evidence that starting therapy more than five weeks after surgery will produce any benefit.

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