COMBINED CHEMOIMMUNOTHERAPY FOR ADVANCED BREAST CANCER

A Comparison of BCG and Levamisole

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One hundred and fourteen evaluable patients with metastatic breast cancer were treated with a program consisting of 5-FU, Adriamycin, cyclophosphamide (FAC) and nonspecific immunotherapy with Levamisole. The results of this treatment program were compared to those observed with FAC and Bacillus Calmette Guerin (BCG) and FAC chemotherapy alone, both groups treated prior to the study reported in this paper. The overall response rates and complete response rates for all three treatment regimens were identical. The duration of remission, survival of all patients and survival of responders was similar for both chemoimmunotherapy regimens, being superior to the FAC chemotherapy alone group. Immunotherapy with Levamisole was well tolerated and side-effects were experienced by less than one-fourth of the patients. Overall, Levamisole was better tolerated than BCG and was easier to administer than the latter drug. These results suggest that nonspecific immunotherapy with Levamisole might prolong remission and survival of patients with metastatic breast cancer. Since the results achieved with BCG and Levamisole appear similar, the therapeutic ratio favors the use of Levamisole.
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INTENSIVE COMBINATION CHEMOTHERAPY with 5-fluorouracil (5-FU), Adriamycin (ADM) and cyclophosphamide (CTX) (FAC) produces objective regressions in over 70% of patients with metastatic breast cancer.[1,2,15,26] While this combination has been reported to be the most effective in the treatment of breast cancer,[1,15] complete remissions are observed in less than 20% of patients[3] and the median durations of remission obtained are approximately 9 months. Only 50% of the patients survive more than 15 months.[2] Advanced metastatic breast cancer as well as the modalities used in its treatment (surgery, radiotherapy and chemotherapy) depress the immune response of a large percentage of patients.[8,21,25] A number of investigators have correlated immunocompetence with a good prognosis, and improvement in previously depressed immune responsiveness with better responses to therapy and survival.[9] We recently reported that nonspecific immunotherapy with BCG, when added to combination chemotherapy prolongs remission and survival of patients responding to chemotherapy.[6,11] Other investigators have reported similar results with the use of *Corynebacterium parvum (C. parvum),*[23] and the methanol extracted residue of BCG (MER).[31]

Levamisole (LMS) is a synthetic substance originally utilized as an anti-helminthic drug, which has shown encouraging results in cancer treatment.[12,24,28,30,32,34] LMS is considered an immunodulator and is known to restore cell-mediated immune responses in com-

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promised hosts. It does not stimulate the immune response above normal levels.[30] LMS improves T cell or macrophage dependent functions such as delayed type hypersensitivity, graft-versus-host reaction and blood clearance of colloidal particles. It does not seem to influence B lymphocytes directly. It has several potential advantages over BCG or other bacterial immunoadjuvants: oral administration, known pharmacology, good tolerance by patients. We explored the question whether Levamisole added to combination chemotherapy would improve the partial and/or complete response rate, or prolong remission and survival in patients with metastatic breast cancer. This report presents our final results and a comparison with our earlier experience with FAC-BCG.

PATIENTS AND METHODS

All patients with metastatic breast carcinoma were eligible for this treatment protocol provided they satisfied the following criteria: clearly measurable metastatic lesions, histological proof of breast cancer, no demonstrable refractoriness to previous therapy with CTX and/or 5-FU and/or ADM and/or Methotrexate, and no prior exposure to LMS. There were no exclusions based on age, extent of metastatic disease or metabolic abnormalities. Patients were excluded if uncompensated congestive heart failure was present. All patients were off prior radiotherapy or chemotherapy for at least 3 weeks prior to the initiation of this treatment.

Treatment Program

All patients were treated with a program consisting of 5-FU 500 mg/m^2 iv on days 1 and 8 of each course, ADM 50 mg/m^2 iv on day 1, CTX 500 mg/m^2 iv on day 1 (FAC) and LMS 100 mg/m^2 p.o. in three divided doses, on days 9, 10, 13, 14, 17 and 18 of each 21 day course of chemotherapy. Courses were repeated every 21 days if hematological or other toxicity permitted. A white blood count of 3000 with an absolute granulocyte count of 2000 and a platelet count of greater than 100,000 were required to start a new course of chemotherapy. The total cumulative dose of Adriamycin was limited to 450 mg/m^2 in an attempt to reduce the risk of cardiotoxicity secondary to this drug. When Adriamycin was discontinued, maintenance treatment consisting of methotrexate 30 mg/m^2 im on days 1 and 8, 5-FU 500 mg/m^2 by mouth on days 1 and 8 and cyclophosphamide 500 mg/m^2 by mouth on day 2 of each course was started. Levamisole was continued at the same dose and schedule. Maintenance courses of chemotherapy were also repeated every 21 days. In the absence of infectious or hemorrhagic complications, dose escalation or de-escalation was performed in order to maintain the lowest granulocyte count between 1000 and 2000 per cubic mm and the lowest platelet count above 50,000. A major infection (pneumonia, sepsis) and/or hemorrhage required a dose reduction of 25% regardless of the degree of myelosuppression. Chemoimmunotherapy was continued until progressive disease was detected for patients with stable disease or in partial remission; patients who achieved a complete remission continued chemoimmunotherapy for 2 years after the moment they achieved such remission. Unless poor performance status required hospitalization, treatment was administered on an ambulatory basis.

Initial evaluation included a complete history and physical examination, a complete blood count, urinalysis, SMA-100 (liver and renal function tests), chest x-ray, metastatic bone survey, liver and bone scanning and bone marrow aspiration and biopsy. Skin testing with a battery of four recall antigens (dermatophytin, Varidase, Candida and mumps), Keyhole Limpet Hemocyanin (KLH), and PPD was performed prior to starting treatment. A complete staging work-up was repeated at 3–4 month intervals.

Evaluation of Response

In this study, a complete remission was defined as a complete disappearance of all objective and subjective evidence of disease including complete recalcification of bone lesions on x-rays; a partial remission was interpreted as a 50% or greater reduction in the product of the greatest diameters of measurable lesions and/or partial recalcification of bone metastases. Patients with less than 50% reduction or less than 25% increase in tumor size for a period of at least 2 months were considered to have stable disease provided no new lesions appeared during that time; progression or relapse was defined as more than a 25% increase in existing tumor masses or the appearance of any new lesions.

Remission duration was determined from the date remission was achieved until the date

TABLE 1. Pretreatment Population Characteristics

	FAC	FAC-BCG	FAC-Levamisole
No evaluable patients	44	105	114
Age (years)			
Median	51	53	53
(range)	(29–67)	(25–72)	(23–73)
Race (%)			
White	88	90	88
Black	5	7	5
Latin	7	3	7
Disease-free interval (months)			
Median	15	15	15
(range)	(0–104)	(0–140)	(0–186)
Menopausal status			
Premenopausal (%)	25	15	11
Postoophorectomy (%)	9	21	27
Postmenopausal (%)	66	68	62
Prior therapy (%)			
Hormonal	79	65	64
Chemotherapy	7	14	10
Dominant site (%)			
Soft tissue	4.5	12.3	4
Osseous	18	25.7	30
Visceral	77	62	66
Number of disease sites			
1–2	59	66	54
>3	41	34	46

No statistically significant difference was detected in any of the subgroups.

of progression or relapse. Survival was measured from the start of treatment to the date of death or last follow-up examination. Survival was also measured from the date of first recurrence after surgery and from the date of first diagnosis of breast cancer. For patients with bilateral breast cancers, the date of diagnosis of the second breast cancer was used.

The results of this series were compared to the two previously reported groups of patients treated with FAC chemotherapy alone[2] or FAC with nonspecific immunotherapy with BCG.[11] Patients in the FAC chemotherapy alone group were entered in the study between August 1973 and February 1974; patients treated with FAC and BCG were entered on the study between March 1974 and March 1975, while the current study was completed between September 1975 and January 1976.

TABLE 2. Response Rates

	FAC No. pts. (%)	FAC BCG No. pts. (%)	FAC LEV No. pts. (%)
No. evaluable pts.	44	105	114
Complete remission	6 (14)	20 (19)	15 (13)
Partial remission	26 (59)	60 (57)	67 (59)
Stable	12 (27)	19 (18)	26 (23)
Progressive	—	6 (6)	6 (5)
Overall remission	32 (73)	80 (76)	82 (72)

No statistically significant differences were noted.

Sixty-four patients were entered on the FAC chemotherapy study. Two patients died before completing the first course, two were lost to follow-up and two had major protocol violations. Fifty-eight patients were evaluable for response rate which was 79% (46 of 58). However, only 44 were evaluable for remission duration and survival, since 14 responding patients were started on BCG after completing the FAC induction regimen, when starting CMF maintenance. These patients were not included in the groups reported here for analysis. Thus, 44 patients treated with FAC chemotherapy alone represent our chemotherapy alone control group. The overall response rate for these 44 patients was 73% (Table 2).

One hundred and twenty-eight patients were entered on the FAC-BCG program. Twenty-three patients were found to be inevaluable for the following reasons: three patients died before completing their first course of chemoimmunotherapy with pro-

gressive disease, thus, having an inadequate trial; five were lost to follow-up; six major protocol deviations were observed and in 9 patients no BCG or BCG at inadequate dose or schedule was administered.

One hundred and thirty-eight patients were entered on the FAC-Levamisole treatment program. Twelve patients were later found to be ineligible for the protocol, 6 patients having only stage III disease (either regionally advanced primary or inflammatory carcinoma) and six for having adenocarcinoma of unknown primary site. Some of these patients have been reported elsewhere (10,18). Twelve additional patients were considered inevaluable for the following reasons: 3 patients were lost to follow-up before information about response could be obtained; 5 patients never received Levamisole; and 4 patients had treatment with major protocol deviations, unacceptable for evaluation in this study.

One hundred fourteen patients received one or more courses of FAC and Levamisole and represent the material reported in this paper.

The Chi-Square test was used to assess the comparability of the two groups for prognostic factors, or to compare response rates in different subgroups. The Kaplan and Meier method was used to calculate and plot remission and survival curves[16] and a generalized Wilcoxon test with a two-tailed analysis was used to test differences between remission and survival curves.[4]

Results

To assure the comparability of the three groups of patients reported in this paper, we analyzed them according to factors of known prognostic significance, as well as factors considered of prognostic significance in the past. Table 1 shows the distribution by age, race, menopausal status, disease-free interval and prior hormonal and chemical treatment. The minor differences observed in the distribution of oophorectomized patients, or patients with predominant visceral disease were not statistically significant.

Our own evaluation of clinically significant prognostic factors showed the number of disease sites involved to be the single best predictor for response and survival.[29] The three groups showed virtually identical distribution in this respect.

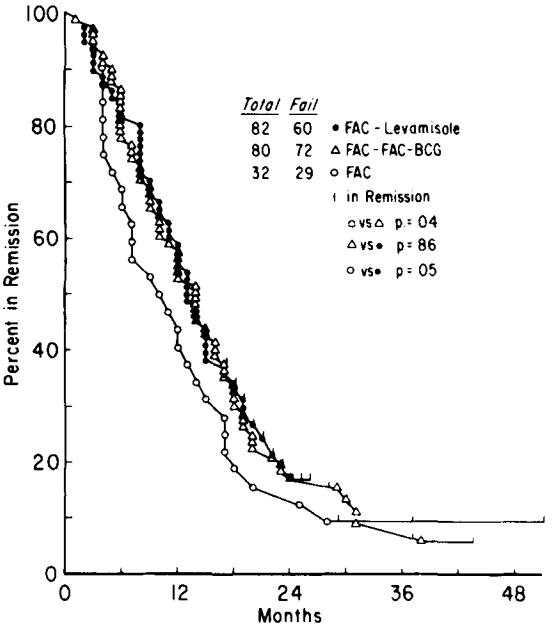

FIG. 1. Duration of remission from onset of response to progression or relapse for all three treatment programs in Stage IV breast cancer.

Table 2 shows the response rate to all three treatment programs. As in our previous report, the response rate was updated for the first 24 months of treatment to identify a small percentage of patients whose response (particularly in bony lesions) may take 6 to 18 months to be manifest.[11] There was no significant difference in the overall or complete remission rate between the three treatment programs, being 74% and 14%, 76% and 19%, and 72% and 13% for FAC, FAC-BCG and FAC-Levamisole respectively. The proportion of patients with stable and progressive disease from the start was also similar between the three groups. Those clinical factors traditionally thought to have prognostic significance, such as age, menopausal status, disease-free interval, dominant site of disease or prior therapy had no influence on the ability of patients to achieve an objective response.

The duration of remission from onset of response till progression for all three treatment programs is shown in Fig. 1. The FAC-BCG and FAC-Levamisole responders had virtually identical remission durations as shown on the graph ($p = 0.86$). Both were superior to that observed with FAC treatment alone ($p = 0.04$ and 0.05, respectively). The median duration of remission for FAC-

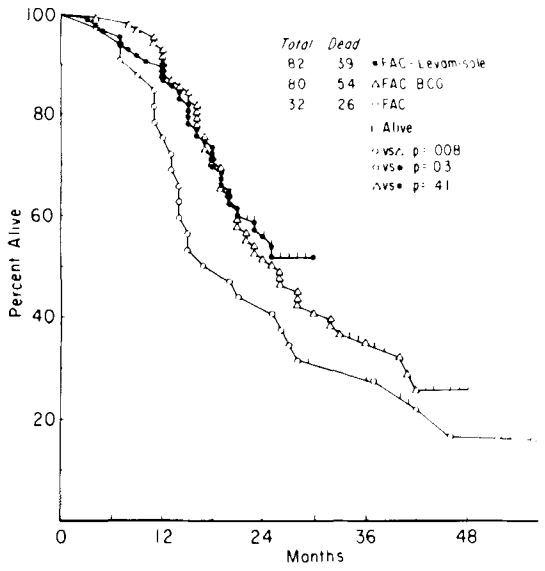

FIG. 2. Length of survival of patients achieving an objective response on all three treatment programs.

Levamisole was 13 months, for FAC-BCG 14 months and for FAC alone 9 months.

While the median duration of complete remission was slightly better than that observed for partial remission, the difference between these two curves was certainly not as dramatic as that observed in the case of acute leukemias or lymphomas. Of the 15 patients who achieved a complete remission on FAC-Levamisole, 8 remain in a complete remission. Of these, 5 have discontinued chemotherapy and two remain in a complete remission on immunotherapy with Levamisole. With a much longer follow-up, five of twenty patients treated with the FAC-BCG protocol remain in remission after discontinuing therapy; one of six patients on FAC chemotherapy alone had a similar clinical course.

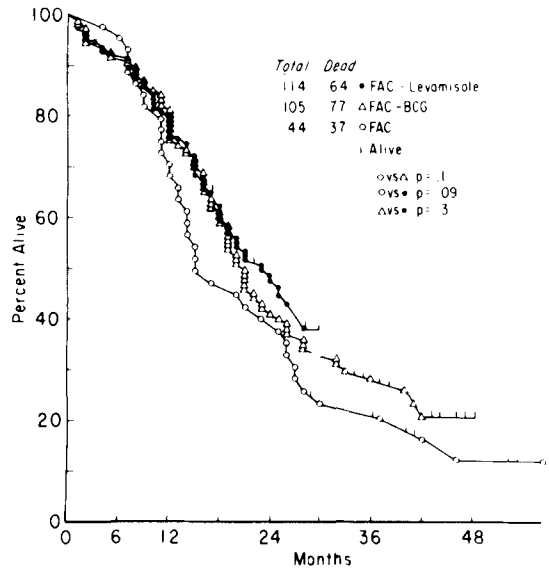

FIG. 3. Survival of all patients, regardless of response, for all three treatment programs in Stage IV breast cancer.

While there was a significant difference in duration of remission between the chemotherapy alone and chemoimmunotherapy programs, the duration of stable disease (8 months) as well as the survival of patients with stable disease (12 months) was identical in all programs. At the time of this report, the median follow-up for FAC is 42 months, for FAC-BCG is 36 months and for FAC-Levamisole, 28 months.

Figure 2 shows the length of survival of patients achieving an objective response on all 3 regimens. The median survival of responders was 16 months for FAC, 24.9 months for FAC-BCG and 25.2 months for FAC-Levamisole. Both FAC-BCG and FAC-Levamisole were significantly superior to FAC ($p = 0.008$). The survival of all patients, including those with stable and progressive disease is shown on Fig. 3.

Since the time elapsed between the documentation of first evidence of recurrent disease and initiation of chemotherapy is variable, we looked at the survival of all patients from the onset of first recurrence until death or last contact. The median survival time from first recurrence was 31.5 months for all patients regardless of response status (Fig. 4). The median survival time from diagnosis of primary breast cancer until death or last follow-up was 57.6 months.

Pattern of Recurrence

We analyzed this patient population to determine the first site of progressive disease when this current chemoimmunotherapy regimen failed. Sixty percent of relapses occurred at the site of original disease and an additional 20% relapsed at both the original metastatic sites and new metastatic areas not previously present; 16% of relapses were in entirely new areas without progression or relapse in previously noted metastatic sites. Included in this group, 6% of all relapses were first in the central nervous system. No in-

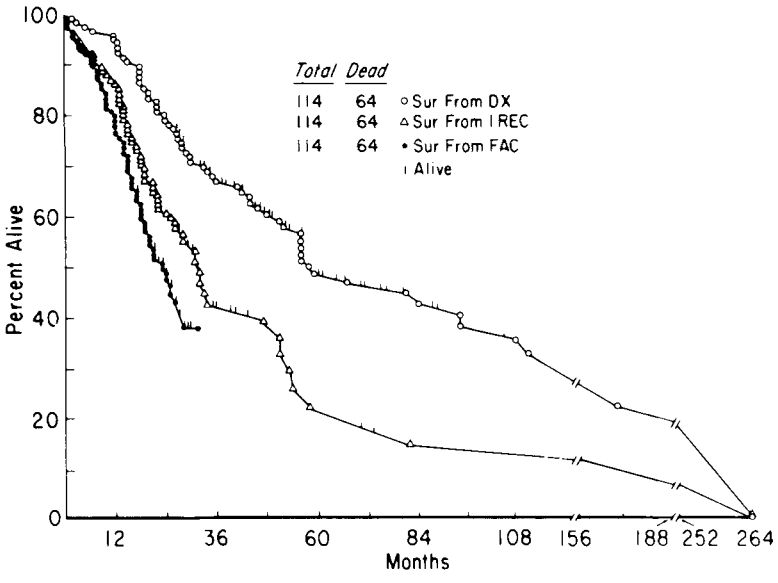

FIG. 4. Survival from initiation of FAC-Levamisole, from the time of first recurrence and from the first diagnosis of primary breast cancer for all patients treated with the FAC-Levamisole program.

formation regarding the site of relapse was available for 3% of the patients.

Causes of Death

Sixty-four patients have died on the FAC-LMS program. Forty-nine of them died as a direct consequence of progressive disease (78%); three of these 49 patients died from progressive central nervous system disease. The remaining deaths were caused by infection (4 patients), cardiovascular disease (5 patients), bowel obstruction unrelated to tumor (1 patient), hypercalcemia without evidence of progressive disease (1 patient) and the cause of death was unknown for 4 patients.

TOXICITY

Most side-effects or toxic manifestations of the programs described in this paper were secondary to chemotherapy with FAC. Gastrointestinal side-effects (nausea, vomiting), alopecia and myelosuppression were the most commonly observed effects (Table 3). The incidence and severity of these side-effects was not different in the three programs reported here. Nausea and vomiting usually started 3 to 4 hours after the infusion of day 1 chemotherapy and lasted for anywhere between 4 and 12 hours. The use of antiemetics met with variable success. Alopecia was always reversible at the end of ADM therapy and sometimes even while ADM was being continued.

Granulocytopenia was the most noticeable evidence of bone marrow toxicity. The lowest counts were encountered between days 10 and 14 with prompt recovery by the third week of treatment. Thrombocytopenia was not observed in the majority of patients and in those who developed a low platelet count this was mild and of no clinical significance. The few patients who presented with severe bone marrow involvement by tumor predictably had much more severe episodes of myelosuppression during the first few courses of chemotherapy. Myelosuppression was slightly cumulative and dose reductions were necessary

TABLE 3. Nonhematologic Toxicity Attributable to Chemotherapy

Symptom	FAC-BCG % Patients	FAC-Levamisole % Patients
Nausea	92	77
Vomiting	86	74
Alopecia	98	95
Mucositis	22	14
Fever unknown origin	10	14
Minor infections	8	12
Weakness	4	9
Weight loss >5 lbs.	5	8
Anorexia	11	8
Diarrhea	16	7
Sepsis	5	7
Congestive heart failure	4	3
Skin pigmentation	—	2
Adriamycin infiltration	4	2
Septic death	—	1
Hematuria	1	1

TABLE 4. Hematologic Toxicity

	FAC		FAC BCG		FAC LEV	
	1	6	1	6	1	6
Lowest WBC						
Median	2.4	2.7	1.1	1.5	2.0	2.2
Range	(.1–5.0)	(.8–6.2)	(.5–3.1)	(.8–3.0)	(.4–5.2)	(1.0–6.1)
Lowest PMN						
Median	.86	1.4	.8	1.1	.8	1.0
Range	(0–3.8)	(0.2–3.1)	(0.3–2.6)	(0.5–2.4)	(0–3.4)	(0–3.1)
Lowest platelet						
Median	180	152	180	225	209	160
Range	(41–345)	(65–265)	(45–345)	(70–480)	(10–574)	(55–356)
% Protocol dose						
Median	100	80	100	80	100	80
Mean	93	81	91	77	95	86

Hematologic toxicity for all three regimens for the first and sixth courses of treatment. Percentage of planned protocol doses administered for the first and sixth courses. No statistically significant differences were detected.

in most patients during the course of therapy. Minor infections were observed in 14% of patients. Bacteriologically proven sepsis was detected in 7% of patients, one of whom died as a direct consequence of infection. No hemorrhagic complications were observed (Table 4). When the incidence and severity of infections in the three treatment programs was compared, no apparent differences were found.

Side-effects related to Levamisole and BCG were quite acceptable. Local tenderness, pruritis and fever were reported by the majority of patients treated with BCG.[11] Excessive local side-effects requiring a reduction in dose of BCG were seen in 13% of patients and immunotherapy had to be discontinued in 3% of patients on account of toxicity. The side-effects observed with LMS have been described before.[12] Nausea, the commonest side-effect, was reported by 21% of patients, followed in frequency by fever (13%), vomiting (12%), bitter taste (5%), anorexia, headache, emotional lability and maculo-papular rash. The dose of LMS was decreased for 7% of patients and discontinued in 5%. The overall tolerance of this drug was excellent in the great majority of patients. One patient in a partial remission interrupted chemotherapy against medical advice and continued taking Levamisole. Two months after the last course of chemotherapy, the patient developed an acute episode characterized by fever, sore throat and malaise. She was admitted to her local hospital with moderately severe granulocytopenia and mucositis. No source of infection was detected and the syndrome disappeared after discontinuing Levamisole. No specific treatment was given although broad spectrum coverage with antibiotics was started at the time of admission.

TABLE 5. Chemoimmunotherapy Studies with Levamisole in Metastatic Breast Cancer

Author	No. pts.	Chemo	LMS	CR + PR (%)	MST
Stephens[27]	24	FAC	–	37*	10
	21	FAC	+	67	21*
Klefstrom[17]	18	VAC	–	39	—
	20	VAC	+	70*	—
	18	CMFVb	–	22	—
	22	CMFVb	+	50*	—
Hortobagyi	44	FAC	–	73	15
	114	FAC	+	72	20.5*

V = Vincristine, F = 5-FU, A = Adriamycin, C = cyclophosphamide, M = Methotrexate, Vb = Vinblastin, MST = median survival time.
* $p < 0.05$.

DISCUSSION

The results described in this paper show that Levamisole given in combination with aggressive cytoreductive chemotherapy is able to prolong remissions induced by such chemotherapy as well as the survival of responding patients. While the overall survival of all patients treated with this combination was prolonged, this was due to the change in survival in patients who achieved a partial or complete response. As in our previous reports, patients with stable disease or progressive tumor growth in spite of therapy, achieved no benefit by the addition of immunotherapy with Levamisole to the program. When the FAC-LMS program was compared to FAC-BCG, they were virtually identical in effectiveness and there was no significant difference in response rate or duration of remission and survival, while both were significantly superior in duration of response and survival to FAC chemotherapy alone.

BCG is considered an immunopotentiator capable of inducing a non-specific enhancement of the immune reaction. Its major influence is over the reticulo-endothelial system, *i.e.*, the macrophages, although it is also known to act on B and T lymphocytes. It can stimulate the immune reaction above "normal levels".[14] In contrast, LMS is an immunorestorative agent active only on the compromised host and able to "restore" the immune reaction up to, but not beyond, normal levels.[27] The major target of LMS is the T cell-dependent immune reaction although it is known to influence macrophages and other phagocytes too.

A considerable fraction of patients with metastatic breast cancer is known to be immunodeficient.[7] Since immunocompetent patients have a better prognosis,[9] it is likely that immunorestoration by LMS alters considerably the outcome of antineoplastic therapy, thus explaining the prolongation of response and survival observed in this study.

Other factors that might explain this difference have to be considered. Improvement in supportive care may have influenced the survival of the chemoimmunotherapy groups, although the minimal number of infectious or hemorrhagic deaths in all three groups could not account for the sizable differences observed. Development of more effective treatment regimens after relapse may have prolonged survival in the latter two groups; this would not explain the prolongation of remission and no differences in response to secondary forms of treatment were observed in this analysis. Finally, undetected maldistribution of prognostic factors might have contributed to the differences seen; comparison of the three groups by known prognostic factors failed to show such maldistribution.

Comparing the toxicity of the two programs, it is evident that neither immunotherapeutic agent modified side-effects or toxicity due to chemotherapy. However, in contrast to previous reports,[20] myelosuppression was not different in the three programs nor was the incidence of infection or hemorrhagic complications.[33] Thus, it appears that neither BCG nor LMS, at the doses and schedule administered here, was able to modify the effect of chemotherapy on the bone marrow, and therefore, the observed differences were not due to better tolerance of chemotherapy or the administration of higher doses of drugs. It also appears that the effects of the nonspecific immunotherapeutic agents over the immune system were unable to modify host defenses sufficiently enough to decrease the incidence of common bacterial or viral infections.

While the list of side-effects attributed to LMS is extensive, the incidence of each one of these symptoms was negligible except for nausea, vomiting and a low-grade fever. Overall, LMS was much better tolerated than BCG and certainly the administration of this drug by the oral route was far easier than the use of BCG by scarification. Agranulocytosis related to LMS, in some cases fatal, has been reported[19,22,32,35,36]; only one of the patients included in this report developed agranulocytosis while on LMS. In this case the agranulocytosis was reversible upon discontinuing LMS and an uneventful recovery followed. However, the pattern of development of agranulocytosis coincided with that described by other investigators: a syndrome of fever, mucositis and weakness developed two months after discontinuing chemotherapy, while LMS immunotherapy alone was continued. It is conceivable that the immunological rebound that follows discontinuation of chemotherapy, is enhanced by LMS thus precipitating an immunologically mediated agranulocytosis. It is also possible that LMS acts as a hapten on the leukocytes in sensitized patients. This syndrome was not observed in any of the pa-

tients treated by Rojas[24] or in those reported by Amery.[28]

Following the initial report of the effectiveness of LMS in prolonging disease-free interval and survival of patients with breast cancer treated with radiotherapy,[24] there have been a number of reports in other tumors such as lung cancer,[28] squamous carcinoma of the head and neck area[34] and melanoma,[5] where LMS alone or in combination with chemotherapy achieved superior results than those observed with standard therapy. Recently two independent investigators, using combination chemotherapy with and without LMS confirmed our results (Table 5). Stephens, in a prospective randomized study, showed an increased overall remission rate with FAC/LMS as compared to FAC chemotherapy alone; the duration of remission and survival of chemoimmunotherapy treated patients was statistically superior to that observed with FAC chemotherapy alone.[27]

Klefstrom recently reported two sequential randomized studies comparing chemotherapy with and without LMS.[17] In the first study (Table 5) patients were treated with CTX, Methotrexate, 5-FU and Vinblastine with and without LMS. As shown on the table, patients treated with chemoimmunotherapy had a higher response rate. In a subsequent study he used a combination consisting of Vincristine, ADM and CTX, also with and without LMS. Again the response rate in the combination containing LMS was superior to the response rate with chemotherapy alone.[17] The median survival time for the different groups was not mentioned but graphs presented showed a clear trend in favor of the Levamisole treated patients.[17] Both Stephens and Klefstrom reported that only patients who achieved a remission had a longer survival. This is consistent with our findings using LMS or BCG.[11] Both Klefstrom and Stephens claimed a higher objective response rate for patients treated with chemotherapy and Levamisole. This was not observed in our study. Significant differences in the administration of chemotherapy existed between our study and the other two. Stephens administered FAC at the same doses we used but the frequency of administration was of 28 days in his study versus 21 days in ours. Furthermore, after achieving a remission, maintenance was administered every other month while in our study we continued every 21 days. Preliminary results of the analysis of a large group of patients treated at M. D. Anderson Hospital with chemotherapy suggested that the frequency of administration of chemotherapy appears to be important for achieving a higher remission rate and longer survival.[29] Klefstrom's chemotherapeutic programs used relatively low doses of each chemotherapeutic agent. His chemotherapy was also administered every 28 days which might also account for the differences. These differences suggest that LMS enhances the therapeutic efficacy of chemotherapy even when suboptimal dose-scheduling is used. This could enhance the therapeutic ratio of combination chemoimmunotherapy. Another significant difference between these studies and ours is the dose of LMS used. Stephens used a fixed dose of Levamisole and the differences reported between his two patient groups were markedly significant in those patients weighing less than 70 kg, but were less dramatic in those patients weighing greater than 70 kg. In contrast, based on an earlier report,[28] we used a dose of 100 mg/m^2 to maximize efficacy in all patients.

A number of recent reports have suggested that nonspecific immunotherapy with a variety of agents (BCG, MER, Levamisole, *C. parvum*) has a definite role in the treatment of breast cancer.[11,12,23,27,31] These results with FAC-Levamisole in advanced breast cancer will be reproducible by others as the papers by Stephens and Klefstrom show. Since in our experience (and that of others) Levamisole is easier to administer and better tolerated than BCG or *C. parvum*, we consider that this drug should take preference over the bacterial immunoadjuvants since their therapeutic effectiveness appears similar. The small but real risk of Levamisole related agranulocytosis requires that caution be observed and a better knowledge of the pathogenesis of this phenomenon. Given the fact that BCG and LMS alter the immune response by different mechanisms of action and both appear to prolong remission and survival in patients responding to chemotherapy, it is tempting to speculate that the combined use of the two agents might produce additive or synergistic effects. However, preliminary results failed to show an advantage of the combination over each agent used individually.[13]

While nonspecific immunotherapy with either BCG or LMS appeared to prolong the

duration of remission, the pattern of relapse did not change and eventually after a longer duration of remission, patients started relapsing at the same rate observed on the chemotherapy program alone. The pattern of development of progressive disease suggested that in more than 80% of patients, the beneficial effect of chemotherapy or chemoimmunotherapy was transient at best and the previously present site of bulky tumor contained cells resistant to the present combination. This suggests the usefulness of a non-cross-resistant form of consolidation therapy, such as a different combination of drugs, radiation therapy or surgery when applicable, to erradicate residual disease.

REFERENCES

1. Bull, J. M., Tormey, D. C., Li, S.-H., Carbone, T. P., Falkson, G., Blom, J., Perlin, E., and Simon, R.: A randomized comparative trial of Adriamycin vs. methotrexate in combination drug therapy. *Cancer* 41:1649–1657, 1978.

2. Blumenschein, G. R., Cardenas, J. O., Freireich, E. J., and Gottlieb, J. A.: FAC chemotherapy for breast cancer. *Proc. Am. Assoc. Clin. Oncol.* 15:193, 1974.

3. Carter, S. K.: Integration of chemotherapy into combined modality treatment of solid tumors. VI. Adenocarcinoma of the breast. *Cancer Treat. Rev.* 3:141–174, 1976.

4. Gehan, E. A.: A generalized Wilcoxon test for comparing arbitrarily singly-censored samples. *Biometrica* 52: 203–223, 1965.

5. Gonzalez, R., and Spitler, L.: Effect of Levamisole as a surgical adjuvant therapy on malignant melanoma. *In* Proceedings of the Fourth Conference on Immune Modulation and Control of Neoplasia by Adjuvant Therapy, M. A. Chirigos, Ed. *Cancer Treat. Rep. (Suppl.)* 1978 (In press).

6. Gutterman, J. U., Cardenas, J. O., Blumenschein, G. R., Hortobagyi, G. N., Livingston, R. B., Mavligit, G. M., Freireich, E. J., Gottlieb, J. A., and Hersh, E. M.: Chemoimmunotherapy of disseminated breast cancer: prolongation of remission and survival. *Br. Med. J.* 2:1222–1225, 1976.

7. Gutterman, J. V., Mavligit, G. M., Hersh, E. M., Hortobagyi, G. N., and Blumenschein, G. R.: Immunology and immunotherapy of human breast cancer. Recent developments and prospects for the future. *In* Current Approaches to Therapy. 1. Breast Cancer: Advances in Research and Treatment, W. McGuire, Ed. New York, Plenum Publishing 1977; pp. 313–373.

8. Hersh, E. M.: Immunosuppressive agents. *In* Handbook of Experimental Pharmacology, Vol. 38-1, A. C. Sartorelli and D. G. Johns, Eds. New York, Springer-Verlag, 1974; pp. 577–617.

9. Hersh, E. M., Gutterman, J. U., Mavligit, G. M., Mountain, C. W., McBride, C. M., Burgess, M. A., Lurie, P. M., Zelen, M., Takita, H., and Vincent, R. G.: Immunocompetence, immunodeficiency, and prognosis in cancer. *Ann. NY Acad. Sci.* 276:386–406, 1976.

10. Hortobagyi, G. N., Blumenschein, G. R., Tashima, C. K., Buzdar, A. U., Gutterman, J. U., and Hersh, E. M.: Multidisciplinary treatment of locally advanced (stage III) breast cancer. *Proc. Am. Soc. Clin. Oncol.* 19:361, 1978.

11. Hortobagyi, G. N., Gutterman, J. U., Blumenschein, G. R., Tashima, C. K., Burgess, M. A., Einhorn, L., Buzdar, A. U., Richman, S. P., Livingston, R. B., and Hersh, E. M.: Combination chemoimmunotherapy of metastatic breast cancer with 5-fluorouracil, doxorubicin, cyclophosphamide and BCG. *Cancer* (In press).

12. Hortobagyi, G. N., Gutterman, J. U., Blumenschein, G. R., Tashima, C. K., Buzdar, A. U., and Hersh, E. M.: Levamisole in the treatment of breast cancer. *In* Immune Modulation and Control of Neoplasia by Adjuvant Therapy, M. A. Chirigos, Ed. New York, Raven Press, 1978; pp. 131–140.

13. Hortobagyi, G. N., Yap, H. Y., Blumenschein, G. R., Gutterman, J. U., Buzdar, A. U., Tashima, C. K., and Hersh, E. M.: Response to disseminated breast cancer to combined modality treatment with chemotherapy and Levamisole with or without BCG. *In* Proceedings of the Fourth Conference on Immune Modulation and Control of Neoplasia by Adjuvant Therapy, M. A. Chirigos, Ed. *Cancer Treat. Rep. (Suppl.)* 1978 (In press).

14. Huchet, R., and Florentin, I.: Studies on the mechanism of action of BCG. *In* BCG in Cancer Immunotherapy, G. Lamoureux, R. Turcotte, V. Portelance, Eds. New York, Grune and Stratton, 1976; pp. 85–96.

15. Jones, S. E., Durie, B. G. M., and Salmon, S. E.: Combination chemotherapy with Adriamycin and cyclophosphamide for advanced breast cancer. *Cancer* 36:90–97, 1975.

16. Kaplan, E. L., and Meier, P.: Nonparametric estimation from incomplete observations. *J. Am. Stat. Assoc.* 53:457–481, 1958.

17. Klefstrom, P.: Levamisole in addition to chemotherapy in advanced breast cancer. *In* Symposium on Immunotherapy of Malignant Disease (In press).

18. Krutchik, A. N., Buzdar, A. U., Barker, J. L., Montague, E. T., Blumenschein, G. R., Tashima, C. K., Hortobagyi, G. N., Yap, H. Y., Gutterman, J. U., Benjamin, R. S., and Hersh, E. M.: Prolongation of disease-free interval and survival with combined chemoimmunotherapy and irradiation of inflammatory breast carcinoma. *Proc. Am. Soc. Clin. Oncol.* 19:346, 1978.

19. Lichtenfeld, J. L., and Wiernik, P. H.: Levamisole in a Phase I trial. *Cancer Treat. Rep.* 60:963–964, 1976.

20. Lods, J. C., Dujardin, P., and Halpern, C. M.: Levamisole and bone marrow restoration after chemotherapy. *Lancet* 1:548, 1976.

21. McCredie, J. A., Inch, R., and Southerland, R. M.: Effect of postoperative radiotherapy on peripheral blood lymphocytes in patients with carcinoma of the breast. *Cancer* 29:349–356, 1972.

22. Parkinson, D. R., Jerry, L. M., Shibata, H. R., Lewis, M. G., Cano, P. O., Capek, A., Mansell, P. W., and Marquis, G.: Complications of cancer immunotherapy with Levamisole. *Lancet* 1:1129–1132, 1977.

23. Pinsky, C. M., Dejager, R. L., Wittes, R. E., Wong, E. P., Kaufman, R. J., Mike, V., Hansen, J. A., Oettgen, H. F., and Krakoff, I. H.: *Corynebacterium parvum* as adjuvant to combination chemotherapy in patients with advanced breast cancer: preliminary results of a prospective randomized trial. *In* Immunotherapy of Cancer:

Present Status of Trials in Man, W. D. Terry and D. Windhorst, Eds. New York, Raven Press, 1978; pp. 647–654.

24. Rojas, A. F., Michiewicz, E., Feierstein, J. N., Glatt, H., and Olivari, A. J.: Levamisole in advanced human breast cancer. *Lancet* 1:211–215, 1976.

25. Roth, J. A., Golub, S. H., Grimm, E. A., Eilber, F. R., and Morton, D. L.: Effects of operation on immune response in cancer patients: Sequential evaluation of in vitro lymphocyte function. *Surgery* 79:46–51, 1976.

26. Smalley, R. V., Carpenter, J., Bartolucci, A., Vogel, C., and Krauss, S.: A comparison of cyclophosphamide, Adriamycin, 5-fluorouracil (CAF) and cyclophosphamide, methotrexate, 5-fluorouracil, vincristine, prednisone (CMFCP) in patients with metastatic breast cancer: A Southeastern Cancer Study Group Project. *Cancer* 40: 625–632, 1977.

27. Stephens, E.: Levamisole and FAC treatment in disseminated breast cancer. *In* Proceedings of the Fourth Conference on Immune Modulation and Control of Neoplasia by Adjuvant Therapy, M. A. Chirigos, Ed. *Cancer Treat. Rep. (Suppl.)* 1978 (In press).

28. Study group for Bronchogenic Carcinoma: Immunopotentiation with Levamisole in resectable bronchogenic carcinoma: A double blind controlled trial. *Br. Med. J.* 3:461–464, 1975.

29. Swenerton, K. E., Legha, S. S., Smith, T., Hortobagyi, G. N., Gehan, E. A., Gutterman, J. U., and Blumenschein, G. R.: Prognostic factors of metastatic breast cancer treated with intensive chemoimmunotherapy. *Cancer Res.* 1979 (In press).

30. Symoens, J.: Treatment of the compromised host with Levamisole, a synthetic immunotherapeutic agent. *In* Immune Modulation and Control of Neoplasia by Adjuvant Therapy, M. A. Chirigos, Ed. New York, Raven Press, 1978; pp. 1–9.

31. Tashima, C. K., Blumenschein, G. R., and Gutterman, J. U.: Comparison of Adriamycin combination drug program with BCG immunotherapy vs. MER immunotherapy for metastatic breast cancer. *Proc. Am. Soc. Clin. Oncol.* 17:288, 1976.

32. Thornes, R. D.: Interpretation of management of Levamisole-associated side-effects. *In* Immune Modulation and Control of Neoplasia by Adjuvant Therapy, M. A. Chirigos, Ed. New York, Raven Press, 1978; pp. 157–164.

33. Van Eygen, M., Zanmensky, P. Y., Heck, E., and Raymalkers, I.: Levamisole in prevention of recurrent upper respiratory tract infection in children. *Lancet* 1: 382–385, 1976.

34. Wanebo, H. J., Hilal, E. Y., Strong, E. W., Oettgen, H. F., Pinsky, C. M.: Randomized trial of Levamisole in patients with squamous cancer of head and neck. *Proc. Am. Soc. Clin. Oncol.* 19:354, 1978.

35. Wilkins, S. A., and Olkowski, Z. L.: Immunocompetence of cancer patients treated with Levamisole. *Cancer* 39:487–493, 1977.

36. Willoughby, M. L. N., Baird, G. M., and Campbell, A. M.: Levamisole and neutropenia. *Lancet* 1:657, 1977.