

## A Randomized Trial Comparing Cisplatin Plus 5-fluorouracil With or Without Levamisole in Operable Gastric Cancer

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**Objectives :** *To determine the effectiveness and toxicity when levamisole was added to the adjuvant combination chemotherapy in patients with operable gastric cancer.*

**Methods :** *After en bloc resection of gastric cancer without gross or microscopic evidence of residual disease from April 1991 to December 1992, 100 patients were randomized to 6 months of 5-fluorouracil 1,000mg/m<sup>2</sup>/day administered as continuous infusion for 5 days, cisplatin 60mg/m<sup>2</sup>/day as intravenous infusion for 1 day with or without levamisole (50mg every eight hours P.O for a period of three days every 2 weeks for 6 months). This chemotherapy treatment was begun within 2 to 4 weeks after the surgery. The chemotherapy consisted of discrete 5-day courses administered at 4-weeks intervals. All 100 patients are assessable.*

**Results :** *The fifty patients were assigned to each treatment group. There was no statistical difference and no bias in the distribution of characteristics of the 100 evaluable patients between the two groups. A total of 274 courses of treatment were given in the levamisole group and 260 courses of treatment in non-levamisole group. Eleven patients in each group did not finish planned 6 courses of treatment mainly due to non-compliance. At median follow up of 39 months, 32 patients relapsed 19 in the levamisole group and 13 in the non-levamisole group ( $p=0.284$ ). Twenty five patients died of relapsed diseases, 15 in the levamisole group and 10 in the non-levamisole group. The levamisole group tended to show more risk of overall death rate and recurrence than the non-levamisole group. However, this result was not statistically significant at 3 years. The treatment was well tolerated in both treatment groups. The grade 2-3 toxicities were nausea/vomiting (levamisole, non-levamisole group: 31.7%, 29.3% of treatment courses respectively), diarrhea (7.6%, 8.4%), mucositis (11.6%, 12.3%), and leukopenia (9.8%, 9.6%).*

**Conclusion :** *Levamisole had negative effects on disease-free survival and overall survival when added to adjuvant combination chemotherapy of cisplatin and 5-fluorouracil in patients with operable gastric cancer. Both treatment arms were generally well tolerated and the toxicity profile was similar with or without levamisole.*

**Key Words :** *Gastric cancer, Levamisole*

## INTRODUCTION

Although surgeical treatment is the most effective treatment for gastric cancer, the adjuvant chemotherapy is of prime importance for the prevention of postoperative recurrence and the prolongation of survival.

Advances in surgical techniques improved the therapeutic results for gastric cancer, yet it reached its limit for the patients who had advanced disease. Radiotherapy has no effect

against gastric cancer. Chemotherapy is not yet satisfactory as a treatment for advanced gastric cancer.

Recently Immunotherapy has shown promised as another therapeutic modality by improving the survival of the patients with gastric cancer.

Levamisole was originally developed as an anthelmintic. Since Renoux et al<sup>1)</sup> reported increased immune response of the host after receiving levamisole, Levamisole has been known to have a broad range of immunomodulatory effects. It includes the enhancement of antibody production to

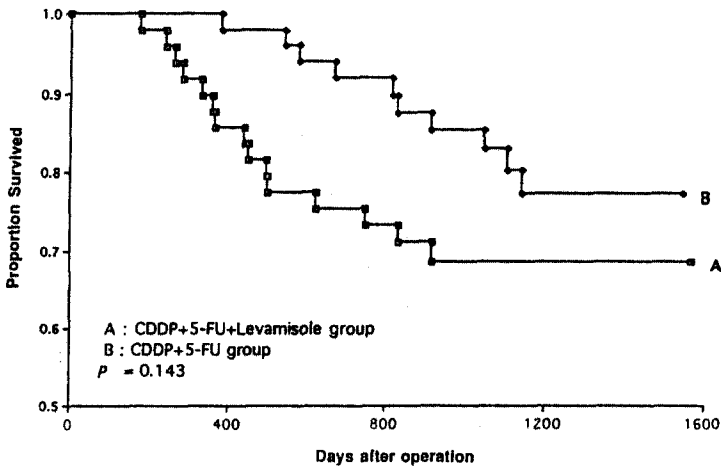


Fig. 1. Survival curves of patients with gastric cancer in all cases. Levamisole Versus Chemotherapy alone group.

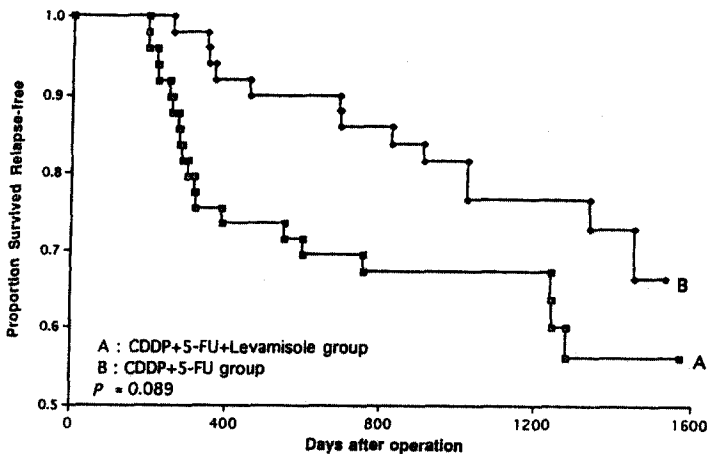


Fig. 2. Relapse-free interval following curative resection in patients with gastric cancer.

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several different antigens, augmentation of a variety of cellular immune responses, synergistic activity with T-lymphocyte mitogens and increasing those immune responses depressed by disease or immunosuppressive therapy.

The ability of levamisole to restore normal functions of phagocytes and T lymphocytes in compromised hosts has suggested its potential usefulness in immunotherapy<sup>2)</sup> for malignancy. Preliminary results in lung cancer and breast cancer has shown a survival gain in patients treated with levamisole<sup>3, 4)</sup>. Treatment with levamisole plus 5-fluorouracil reduced recurrence of colon cancer and death rate in patients with resected stage C colon cancer<sup>5)</sup>. Furthermore, early reports indicated that side-effects were relatively uncommon and mild, leading to discontinuation of treatment in only 4.9% of 1179 colon cancer patients.

Effectiveness in the other types of tumors, including stomach cancer, has not been established.

We wish to report the results of a prospective, randomized, controlled study to determine the effectiveness of levamisole when it was added to conventional chemotherapy as an adjuvant therapy to surgery in patients with operable gastric cancer. The goals were to confirm the preliminary observations of the disease free survival, overall survival and toxicity of levamisole.

### PATIENTS AND METHODS

From April 1991 to December 1992, 100 patients with gastric cancer who underwent curative resection in the Department of Surgery, University of Ulsan, Asan Medical Center, were assigned randomly to either group, one with or one without levamisole. Patient eligibility was established at the time of the study entry.

The criteria for patients selection were as follows: 1) histologic documentation of adenocarcinoma of the stomach; 2) no previous treatment for cancer; 3) TNM stage IB or higher; 4) a potentially curative en bloc resection of tumors without gross or microscopic evidence of residual disease; 5) adequate bone marrow, renal and hepatic functions. Entry into the study was allowed

no earlier than one week and no later than five weeks after surgery. All patients entered into the study gave informed consent and were randomly assigned to either chemotherapy with levamisole or chemotherapy alone. Both group had periodic evaluations. All patients were followed for total postoperative survival period. The patients attended the follow-up clinic at monthly intervals. On each visit, they were examined for evidence of recurrent tumor. Relevant investigations were performed if tumor presence was suspected. Unless the side-effects were considered unacceptable, each treatment group received treatment for 6 months.

Hematologic testing and blood-chemistry panels were repeated every four weeks. The levamisole group received levamisole by mouth at a dose of 50mg every eight hours for a period of three days: this was repeated every two weeks for 6 months. Chemotherapy were consisted of 5-FU 1,000mg/m<sup>2</sup>/day administered as a continuous intravenous infusion for 5 days and cisplatin 60mg/m<sup>2</sup>/day as intravenous infusion for 1 day. This chemotherapy regimen repeated every 4 weeks for 6 cycles. Patients were required to have a white blood cell count (WBC) greater than 4,000/mm and a platelet counts(plateletes) greater than 150,000/mm. to receive therapy.

To receive midcycle of chemotherapy, the WBC had to be above 3,000/mm and platelets greater than 100,000/mm.

Criteria for recurrence included documentation by histologic or cytologic examination, or by evidence of metastases on the chest and bone x-ray.

Survival was the primary end point of this study. The time of the recurrence was also determined. Statistical analysis were carried out according to the procedures of the Statistical Analysis System. The survival curves were generated by the Kaplan-Meier method. The log-rank statistic was used to compare the distributions of survival times. The Cox proportional-hazards model was used to determine the ratios of relapse and survival rates and to perform all multivariate analyses.

**Table 1. Patient Characteristics**

	CDDP+5-FU +levamisole (%) (n=50)	CDDP+5 -FU(%) (n=50)	p-value*
Age(years)			
<55yr	29(58)	22(44)	0.230
>55yr	21(42)	28(56)	
Sex			
Male	34(68)	39(78)	0.386
Female	16(32)	11(22)	
Tumor location			
Upper third	7(14)	5(10)	0.575
Middle third	7(14)	11(22)	
Lower third	36(72)	34(68)	
Tumor size(Cm)			
<5.5	27(54)	23(46)	0.549
>5.5	23(26)	27(54)	
T stage			
T1	2( 4)	1( 2)	0.687
T2	4( 8)	4( 8)	
T3	42(84)	40(80)	
T4	2( 4)	5(10)	
N stage			
N0	8(16)	9(18)	0.242
N1	21(42)	28(56)	
N2	21(42)	13(26)	
TNM stage			
IB	2( 4)	1( 2)	0.510
II	8(16)	11(22)	
IIIA	18(36)	22(44)	
IIIB	22(44)	15(15)	
IV	0( 0)	1( 2)	
Histologic grade			
Well differentiated	2( 4)	3( 6)	0.314
Mod. differentiated	10(20)	8(16)	
Poorly differentiated	33(66)	38(76)	
Undifferentiated	5(10)	1( 2)	

\* : by Fisher's exact test

## RESULTS

### 1. Characteristics of the Patients

Out of 100 patients registered, fifty patients were assigned to each treatment group. There was no statistical difference in the distribution of ineligible, inevaluable, or dropout patients between the two groups.

The two groups were comparable in age, sex, tumor size, tumor location in the stomach, TNM stage and histologic differentiation(Table 1).

### 2. Side-effects

The majority of patients tolerated both treatment relatively well. Eleven patients in each group did not finish planned 6 courses of treatment due to non-compliance. No patient withdrew from the study due to side-effect of the drug in both group. There was no significant difference in the side-effects of the drugs between chemotherapy alone and levamisole group. Mild nausea and vomiting (Grade II) were encountered in both treatment groups at the same rate (62 of 274 courses in the levamisole group and 55 of 260 courses in the chemotherapy alone group). Nausea, vomiting, leukopenia, fatigue, weakness were slightly more frequent in the levamisole group than in the chemotherapy alone group, but the difference was not statistically significant (p<0.05)(Table 2).

### 3. Duration of survival

Table 3 shows the disease-free survival and overall survival between the two groups. There was

**Table 2. Side-effects by Treatment Arm**

	CDDP+5-FU+levamisole(%) 274 Courses		CDDP+5-FU(%) 260 Courses	
	Grade II	Grade II	Grade II	Grade II
Nausea/vomiting	62(22.6)	25(9.1)	55(21.2)	21(8.1)
Diarrhea	17( 6.2)	4(1.5)	15( 5.8)	7(2.7)
Mucositis	18( 6.6)	15(5.5)	17( 6.5)	15(5.8)
Anemia	38(13.9)	2(0.7)	38(14.6)	0( 0)
Leukopenia	23( 8.4)	4(1.5)	21( 8.1)	1(0.4)
Thrombocytopenia	0( 0)	1(0.4)	1( 0.4)	1(0.4)
Fever/infection	0( 0)	2(0.7)	1( 0.4)	0( 0)
Fatigue/weakness	15( 5.5)	5(1.9)	12( 4.6)	6(2.3)
Dizziness	1( 0.4)	0( 0)	5( 1.9)	0( 0)

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**Table 3. Disease Free Survival and Overall Survival\***

	CDDP+5-FU +levamisole	CDDP+ 5-FU	p value*
Total No. of patients			
Patients with relapse	50	50	
Local	19	13	
Systemic	3	2	
Both	10	8	0.284
Unknown	4	1	
Patients who died	3	2	
NED*+died	19	10	
Relapse + died	4	0	
Patients who lived	15	10	
in relapse	4	3	0.077

\* : median duration of follow-up : (39 months)

\*\* : by Fishers exact test

\* NED : No evidence of disease

no significant difference in duration of survival between the two groups. At median follow up of 39 months, 32 patients relapsed with their disease. (19 in levamisole group and 13 in chemotherapy alone group)(p=0.284). 26 patients died after relapse, (16 in levamisole group and 10 in chemotherapy alone group)(p=0.077). Three patients expired without evidence of recurrence of gastric cancer in levamisole group. Listed causes of death were myocardial infarction (1 patient), sepsis (1 patient), and sudden death(1 patient). 4 patients in levamosloe group, 3 patients in chemotherapy alone group were alive with relapsed disease.

Among the patients with curative en bloc resection of gastric cancer, adjuvant chemotherapy with levamisole group increased the risk of overall death rate. At 3 years the levamisole-treated

**Table 4. Univariate Analysis of Disease-free Survival and Survival According to Prognostic Variables**

Variable	No. of patients	NED	P-value*	Survival rate at 3yr	p-value*
Chemotherapy					
Chemo +Leva	50	67%	0.089	69	0.143
Chemo alone	50	77%		83	
Sex					
Male	73	74%	0.144	77	0.382
Female	27	66%		74	
Age(years)					
<55yr	51	70%	0.247	74	0.914
>55yr	49	74%		79	
Tumor location					
Upper third	12	67	0.485	67	0.182
Middle thrid	18	83		94	
Lower third	70	70		73	
Tumor size(Cm)			0.189		
<5.5	50	67		73	0.315
>5.5	50	76		79	
N. stage					
N0	17	87	0.176	87	0.161
N1	49	74		79	
N2	34	61		67	
TNM stage**					
A	22	81	0.077	81	0.065
B	40	79		84	
C	38	60		64	
Histologic grade*					
Well & Mod.	23	78	0.938	83	0.783
poorly & undiff	77	70		74	

\* : by log-rank test

\*\* A : Ib+II stage, B : IIa stage, C : IIb + IV

\* : well & mod : well and moderately differentiation groups poorly & undiff : poorly and undifferentiation groups

**Table 5. Multivariate Analysis of Disease-free Survival and Survival According to Prognostic Variables by Coxs Proportional Hazards Model**

Variable	NED		Survival Rate	
	Relative Risk	P-value	Relative Risk	P-value
TNM stage				
A	1	—	1	—
B	1.36	0.57	1.06	0.92
C	2.49	0.08	2.43	0.12
Treatment				
Chemo+Leva	1	—	1	—
Chemo alone	0.59	0.15	0.62	0.24

The relative risk is the increased risk of dying after chemotherapy per unit of measurement of the covariate

patients had a higher recurrence than the chemotherapy-only group. But these result was not significantly different between the levamisole and the chemotherapy alone groups.

Table 4 shows univariate analysis of disease-free survival and survival according to prognosis variables. In logistic regression using univariate analysis, chemotherapy with the levamisole group or the chemotherapy alone group( $p=0.089$ ), TNM stage ( $p=0.077$ ) were trended to significant factors associated. In the analysis of survival and time to treatment failure, the key prognostic factors were the chemotherapy with or without levamisole and TNM stage. By a backward-regression selection procedure, chemotherapy, sex, age, tumor location, tumor size, N. stage, TNM stage and histologic grade were not all found to be independent determinants of recurrence( $p>0.05$ ). After correction for the influence of prognostic factors with Cox regression model, the difference in 3-year survival probabilities of the levamisole group(69%) and chemotherapy alone group(83%) was 14%. But these result was not significant ( $p=0.143$ ). Table 5 shows multivariate analysis of disease-free survival and survival according to prognostic variables by Cox's proportional hazards model. According to increased TNM stage ( $p=0.57$ ,  $p=0.08$ ) and after chemotherapy with levamisole rather than chemotherapy alone( $p=0.15$ ), the relative risk is increased.

## DISCUSSION

Levamisole has been extensively evaluated in clinical trials for various cancer therapy, particularly in the minimal disease as a adjuvant therapy after surgical treatment. There have been many reports showing a significant improvement in duration of remission and survival by actuarial analysis in the levamisole-treated group in the cases of cancers of the lung, colon and rectum<sup>5</sup>. The addition of levamisole to combination chemotherapy was found to increase both the response rate and survival in a randomized double-blind trial in patients with breast cancer and gastric cancer in one study<sup>6</sup>.

Another study failed to show a beneficial effect of additional levamisole<sup>7</sup>. In an experimental model system of KHT sarcoma in mice, the addition of levamisole did not improve local tumor control. It did not decrease the incidence of metastases<sup>8,9</sup>. Levamisole was found to have a negative influence on survival in a multihospital randomized trial in patients with extensive lung cancer. The does-related toxicity of levamisole was also found to be significant<sup>7</sup>. The results from a large series of 720 patients with breast cancer showed that the patients with N+M0 disease developed a significantly higher recurrence rate after total mastectomy with partially axillary lymph node biopsy when levamisole was combined with radiotherapy<sup>10</sup>. In malignant melanoma, the National Cancer Institute of Canada, Clinical Trials Group, conducted a similar study and reported benefit in the groups of patients receiving levamisole<sup>11</sup>. However, in this same study, when levamisole was combined with BCG, no advantage whatever was obtained and in a comparable melanoma adjuvant trial, Spittler et al found levamisole therapy to produce no advantage over placebo in either time to recurrence or survival<sup>12</sup>. In individual experiments with Moloney virus-induced rhabdomyosarcomas in BALB/c mice, levamisole treatment seemed to be associated with an accelerated growth of the primary tumor, but this was not significant and even counterbalanced by a greater number of animals which showed inhibited tumor growth with levamisole<sup>7</sup>.

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The investigators, therefore, concluded that levamisole had no effect on the primary growth of these tumors. In an allogeneic model using L 1210 leukemia transplanted to C3H mice, an increased number of tumor takes was observed with levamisole treatment. This effect was associated with an elevated level of serum blocking activity and possible immunologic enhancement of tumor growth by levamisole. In light of the findings of Amery et al., levamisole is more effective when given prior to surgery. Japan Levamisole Research Association Group reported<sup>13)</sup>, at operable gastric cancer patients receiving 600 mg Tegafur daily were then divided into two groups according to whether levamisole was given or not. The 2-year follow-up demonstrated that in stage III patients, the levamisole group was superior to the non-levamisole group, since the former prolonged the relapse-free interval significantly. The survival rate for stage III disease was also significantly higher in the levamisole group than in the non-levamisole group.

In our study, levamisole was added to the basic combination of cisplatin and 5-FU because the combination of cisplatin and 5-FU seems to be significantly more effective than the single agent treatment. However, in our trial, a poorer survival was found for en bloc resection of gastric cancer patients treated with levamisole than for non-levamisole groups. Our current findings would indicate that the adjuvant use of levamisole to conventional chemotherapy in patients with gastric cancer after surgery did not provide an additional beneficial effect on duration or survival rate; in fact, in operable gastric cancer patients, a negative influence on survival over a 3-year follow-up period was observed. In 1980, Brincker<sup>10)</sup> published his results of an interim analysis of a study very similar to ours and stated that levamisole was leading to a higher incidence of recurrences.

Our study shows no evidence of a favorable or unfavorable effect of levamisole on prognosis. The size of the study does not exclude the possibility of the existence of a small positive or negative effect. Our conclusion is that levamisole does not have any clinically significant influence on prognosis of patients with resectable gastric cancer.

The both treatment arms were generally well tolerated and the toxicity profile was similar with or without levamisole.

## REFERENCES

1. Renoux G. *The general immunopharmacology of levamisole. Drugs 1980; 20:89-99.*
2. By Henry C. Stevenson, Ira Green, J. Michael Hamilton, Barbara A. Calabro, and R. Parkinson. *Levamisole: Known Effects on the Immune System, Clinical Results and Future Applications to the Treatment of Cancer. J Clin Oncol 1991; 9:2052-2066.*
3. Amery WK. *Final results of a multicenter placebo-controlled levamisole study of resectable lung cancer. Cancer Treat Rep 1978; 62:1677-1683.*
4. C. Kamby, C. Rose, B. Ejlersen, J. Andersen, N.E. Brikler, L. Rytter, K.W. Andersen, K. Zedeler. *Adjuvant Systemic Treatment and the Pattern of Recurrences in Patients with Breast Cancer. Eur J Cancer Clin Oncol 1988; 24:439-447.*
5. Charles G. Mortel, Thomas R. Fleming, John S. Macdonald, Daniel G. Haller, John A. Laurie, Catherine M. Tangen, James S. Ungerleider, William A. Emerson, Douglass C. Tormey, John H. Glick, Michael H. Veeder, James A. Mailliard. *Fluorouracil plus Levamisole as Effective Adjuvant Therapy after Resection of Stage III Colon Carcinoma: A Final Report. Ann Intern Med 1995; 122:321-326.*
6. Hiroaki Miwa, Fumio Ono, Minoru Moriyama, Tsutomu, Kobayashi, Tetsuhide Oka, Kenji Nakamura, Kunzo Orita. *Immunochemotherapy of gastric cancer with levamisole. Acta Med. Okayama 1980; 34, (4): 275-281.*
7. Stephen Davis, William Mielowski, Jay J. Rohwedder, John P. Griffin, Amir A. Neshat. *Levamisole as an Adjuvant to Chemotherapy in Extensive Bronchogenic Carcinoma. Cancer 1982; 50:646-651.*
8. Willem K. Amery, Federico Spreafico, Alejandro F. Rojas, Emiel Denissen, Michael A. Chrigos. *Adjuvant treatment with levamisole in cancer A review of experimental and clinical data. Cancer Treat Rev 1977; 4:167-194.*
9. Baker D, Constable W, Elkon D, Rinehart L. *The influence of ICRF-159 and levamisole on the incidence of metastases following local irradiation of a solid tumor. Cancer 1981; 48:2179-2183.*
10. Executive Committee of the Danish breast Cancer Cooperative Group. *Increased Breast-cancer Recurrence Rate After Adjuvant Therapy With Levamisole: A preliminary Report. Lancet October 1980; 16:824-827.*
11. By Ian C. Quirt, Wendy E. Shelly, Joseph L. Pater,

- Audley J. Bodurtha, Peter B. McCulloch, Thomas A. McPherson, Alexander H.G. Paterson, Robert Prentice, Hulbert K.B. Silver, Andrew R. Wilan, Kenneth Wilson, Benny Zee. *Improved Survival in Patients With Poor-Prognosis Malignant Melanoma Treated With Adjuvant Levamisole: A Phase III Study by the National Cancer Institute of Canada Clinical Trials Group.* *J Clin Oncol* 1991; 9:729-735.
12. Spitzer LE. *A randomized trial of levamisole versus placebo as adjuvant therapy in malignant melanoma.* *J Clin Oncol* 1991; 9:736-740.
13. Minoru Niimoto, Takao Hattori, Ichiji Ito, Ryuichiro Tamada, Kiyoshi Inokuchi, Kunzo Orita, Hisashi Furue, Nobuya Ogawa, Tomohiro Toda, Motonosuke Furusawa, Shigemasa Koga, Isamu Hashimoto, Tatsuhei Kondo, Shigeru Fujimoto, Yuzuru Sugiyama, Osahiko Abe, Masaaki Oya. *Levamisole in postoperative adjuvant immunochemotherapy for gastric cancer.* *Cancer Immunol Immunother* 1984; 18:13-18.
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