Journal of BUON **10:** 499-504, 2005

ORIGINAL ARTICLE

Leucovorin and 5-fluorouracil *versus* levamisole and 5-fluorouracil as adjuvant chemotherapy in rectal cancer

N. Tsavaris[1], K. Genatas[2], C. Kosmas[3], H. M. Skopelitis[1], A. Dimitrakopoulos[1], M. Zacharakis[4], G. Kouraklis[5], G. Zografos[6], A. Macheras[7], N. Diamantis[1], Th. Diamantis[4], S. Chalkias[1], S. Tsaousi[1], G. Papastratis[6], G. Karatzas[7], E. Papalambros[4]

[1]Department of Pathophysiology, "Laikon" General Hospital, Athens; [2]Second Surgical Unit, "Areteion" University Hospital, Athens; [3]Second Department of Medical Oncology, "Metaxa" Cancer Hospital, Piraeus; [4]Second Department of Propedeutic Surgery, "Laikon" General Hospital, Athens; [5]Fist Department of Propedeutic Surgery, "Laikon" General Hospital, Athens; [6]Third Department of Surgery, "G. Gennimatas" General Hospital, Athens; [7]Third Department of Surgery, "Attikon" General Hospital, Athens, Greece

Summary

Purpose: *To evaluate the effectiveness of 6-month therapy with leucovorin (LV)+5-fluorouracil (5-FU) versus 12-month therapy with levamisole (LVS)+5-FU, as adjuvant chemotherapy in patients with completely resected Astler-Coller stage B_2 or C_1/C_2 rectal cancer (RC).*

Patients and methods: *One hundred and fifty patients with surgically resected RC were enrolled. Seventy patients with stage B_2 and 80 with stage C were randomly assigned to adjuvant chemotherapy with 5-FU+LV×6 months or 5-FU+LVS×12 months. Patient characteristics were equally balanced between the examined groups. Adjuvant chemotherapy consisted of LV 20 mg/m^2 intravenously (i.v.) plus 5-FU 450 mg/m^2 i.v. bolus, on days 1-5 every 4 weeks for 6 cycles or 5-FU 450 mg/m^2 i.v. bolus every week plus LVS tablets 50 mg t.i.d×3 days every 2 weeks for 1 year.*

Results: *After a median follow up for survivors of 8.7 years (range 1.8-10.5), all of the patients were evaluable. There were no significant differences between the two treatment groups with respect to the recurrence rates (p=0.821). Moreover, there were no significant differences between the two treatment groups in disease-free survival (DFS) (p=0.84) [B_2 (p=0.805) and C (p=0.978)] and overall survival (OS) rates for patients of either stage B_2 or C (p=0.78). Toxicities were more frequent in the 5-FU+LVS versus 5-FU+LV group: myelosuppression (grade 3 leucopenia, 12 versus 4%, p <0.04), diarrhea (grade 0, 60 versus 76%, p <0.02), and liver toxicity (increase of transaminases >3-fold, 12 patients versus 2, p <0.03). No patient stopped chemotherapy because of toxicity, and there were no treatment-related deaths.*

Conclusion: *Adjuvant chemotherapy in RC with LV +5-FU for 6 months is equally effective and less toxic than LVS+5-FU for 12 months.*

Key words: adjuvant chemotherapy, 5-fluorouracil, leucovorin, levamisole, rectal cancer

Introduction

The goals of adjuvant chemotherapy administered in conjunction with surgery are to delay or prevent tumor recurrence and to improve survival by eliminating micrometastases. The failure of surgical therapy alone is probably due to the presence of residual occult disease and regional or distant micrometastases, and there has been much interest in developing adjuvant treatments that will improve prognosis in these patients. The benefit from adjuvant chemotherapy in colorectal cancer has been clearly established [1,2].

In colon cancer, the pathological stage of the resected tumor is the most important determinant in predicting outcome [3], and Dukes classification (or one of its modifications) is a commonly used staging system. About 90-95% of patients with Astler-Coller

Received 03-03-2005; Accepted 28-05-2005

Author and address for correspondence:

Nicolas Tsavaris, MD
Department of Pathophysiology
Athens University School of Medicine
"Laikon" General Hospital
17 Agiou Thoma street
115 27 Athens
Greece
Tel/Fax: +30 210 6463191
E-mail: tsavari1@otenet.gr

stage A and B_1 colon cancer are cured by surgical resection alone, but the great majority of patients with stage B_2 (invasion of the serosa or the pericolonic fat) or C (metastasis to regional lymph nodes) have a significantly higher risk of disease recurrence and metastases [4-6]. A large study from the National Intergroup led by Moertel et al. [1], showed an unequivocal significant advantage for the treatment with 5-FU plus LVS of surgically resected Dukes C colon cancer patients. The same authors did not recommend any specific adjuvant therapy for patients with Dukes B colon cancer [1]. However, other studies demonstrated that Dukes B colon cancer patients can benefit from adjuvant chemotherapy [7-11].

5-FU is an active drug for this disease and its action is potentiated when it is combined with LV [12, 13]. After the good results obtained in advanced colon carcinoma with LV plus 5-FU [14, 15], preclinical and clinical trials support the use of LV and 5-FU in adjuvant chemotherapy [13,16].

The aim of the present study was to evaluate the effectiveness of 6-month therapy with LV/5-FU *versus* 12-month therapy with LVS/5-FU, in reducing the recurrence rate in patients with surgically resected Astler-Coller stage B_2 or C_1/C_2 RC. Only patients with RC were studied, because there are important differences between RC and colon cancer, due to the different natural histories of these two localizations.

Patients and methods

Inclusion criteria

These criteria included histologically confirmed adenocarcinoma of the rectum and complete *en block* resection of the primary tumor, with no gross evidence of residual disease. The primary RC had to show at least one of the following predictors of poor prognosis: Astler-Coller stage B_2 (transmural penetration of the muscular wall with tumor involvement into or through the serosa) provided there was evidence of bowel obstruction or perforation; adherence to or invasion of adjacent organ(s) or bowel perforation by the tumor but with all visible disease resected; Astler-Coller stage C_1/C_2 (regional lymph node metastases). Additional inclusion criteria were regional peritoneal or mesenteric tumor implants resected *en block*; no evidence of distant metastasis; gross inferior margin of the primary tumor located above the peritoneal reflection. Patients with RC whose inferior tumor margin was at or below the peritoneal reflection were not eligible.

Exclusion criteria

These criteria included concurrent radiation, prior exposure to 5-FU, prior radiation or chemotherapy for RC, any concurrent malignant tumor in the previous 3 years except superficial squamous or basal cell carcinoma of the skin or carcinoma *in situ* of the cervix, evidence of unresected regional or distant metastases, pregnancy or lactation, and incomplete surgical resection.

Included patients were between 18-70 years old and had a WHO performance status of 0 or 1. Their principal characteristics are summarized in Table 1. There was a good balance between the groups and subgroups. The two groups were equally balanced with respect to pre-treatment disease characteristics. They were required to be able to give signed informed consent, were stratified as stage B_2 or C and randomized to the study between 21-30 days postoperatively. Adjuvant chemotherapy (5-FU plus LV or 5-FU plus LVS) started as soon as they were considered able to tolerate treatment, but not later than 5 weeks after surgery.

Pretreatment and follow-up evaluation

Within 72 hours before randomization the medi-

Table 1. Patients characteristics and groups of patients according to therapy

Characteristic	*Treatment*		
	5FU+LV	*5FU+LVS*	*p-value*
Age, years, mean (median; range)	60.53 (62; 27-70)	59.58 (61.5; 38-70)	1.0
Sex (males/females)	41/34	43/32	1.0
Stage (patients, n)			
B2	35	35	1.0
C	40	40	
Type of operation, n (%)			
end-to-end	49 (65)	54 (72)	0.404
colostomy	26 (35)	21 (28)	
Grade, n (%)			
I	1 (1)	2 (3)	0.897
II	68 (91)	65 (87)	
III	6 (68)	8 (10)	
Tumor diameter (cm), n (%)			
< 5	47 (63)	44 (59)	1.0
> 5	28 (37)	31 (41)	
Positive lymph nodes, n (%)			
0	35	35	0.706
1-2	17 (47)	19 (53)	
> 3	23 (52)	21 (48)	

cal history was taken and the patients underwent physical examination, complete blood cell count, serum biochemistry and measurement of CEA and CA 19-9 serum tumor markers. Before each cycle, all patients had physical examination, complete blood count, serum biochemistry, measurement of CEA and CA 19-9 serum tumor markers, and chest x-ray. Full blood count was taken weekly for toxicity monitoring. Chest and abdominal computerized tomography (CT) were performed every 3 cycles and at the end of treatment. After completing adjuvant chemotherapy, follow-up included complete blood count, serum biochemistry, serum CEA and CA 19-9 estimation, chest x-ray and CT of the abdomen every 6 months as well as colonoscopy once a year for 5 years. Follow-up after 5 years continued for life without formal protocol requirements.

Treatment schedule

Patients were randomized to one of the two treatment groups: (A) LV 20 mg/m^2 i.v. bolus and 5-FU 425 mg/m^2 i.v. bolus (immediately after LV) on days 1-5; treatment was repeated every 4 weeks for 6 cycles; (B) 5-FU 425 mg/m^2 i.v. bolus on days 1-5 and after 4 weeks weekly 5-FU 425 mg/m^2 i.v. bolus plus LVS tablets 50 mg t.i.d for 3 days every 2 weeks (starting with days 1-5 of 5-FU administration) for 12 months.

Toxicity evaluation

Toxicity was recorded according to the WHO criteria [17]. In case of multiple toxicities the dose administered was based on the most severe toxicity experienced. Dose reductions applied to the dose of chemotherapy given in the preceding treatment cycle and were based on toxicities observed from the previous chemotherapy cycle, with 20% dose reduction applied only to 5-FU for any ≥ grade 3 toxicity (excluding nausea/vomiting, anemia and alopecia). LV and LVS doses were not modified for chemotherapy toxicity.

Statistical analysis

The study was designed to accrue 120 patients, 60 in each group. This number was selected in order to complete the study within a reasonable period of time, considering our usual annual accrual rate. OS and DFS were the primary study endpoints. The Pearson x^2 model was used for comparison of the two groups for stage and the number of recurrences and deaths. All p-values were two-sided and the level of significance was set at α=0.05. A p-value < 0.05 was considered statistically significant. Survival curves were generated using the Kaplan-Meier method and comparison between treatment groups were carried-out by the log-rank test [18, 19].

Results

Patient characteristics

Patient enrolment begun in November 1993 and was completed in October 1998. A total of 150 patients entered the study, 75 in each group. All were considered eligible for the final analysis. The principal characteristics of the 150 patients are shown in Table 1. Randomization yielded two well-balanced groups with respect to age, sex, stage, number of involved lymph nodes for stage C patients, etc (Table 1). All patients were assessable for DFS and OS.

Recurrence rate

There were 10 (14.3%) recurrences and 10 (14.3%) deaths in stage B_2 patients and 35 (43.8%) recurrences and 35 (43.8%) deaths in stage C patients. No differences whatsoever were noticed between the examined groups. With regard to survival status, there were 45 deaths, 22 in group A and 23 in group B. In relation to stage, in stage B_2 there were 9 (25%) recurrences and deaths in LV arm and 10 (29%) in LVS arm, whereas in stage C patients there were 14 (42%) recurrences and deaths in the LV arm and 17 (45%) in the LVS arm. No statistically significant difference was found between the two groups in relation to recurrences (x^2 = 0.051, degree of freedom=1, p=0.821) or deaths (x^2 =0.202, degree of freedom = 1, p=0.654) (Tables 2 and 3).

Survival analysis

The median follow-up for all enrolled patients was 8 years (range 1.8-11.7) and for survivors it was 8.7 years (range 1.8-10.5). DFS and OS were calculated from the day of randomization. There were no

Table 2. Recurrence rates of both treatments and both stages (B_2 or C)

Stage	*Treatment*	*Recurrence*		*Total*
		No, n (%)	*Yes, n (%)*	
B_2	5-FU + LV	30 (74)	5 (26)	35
	5-FU + LVS	30 (71)	5 (29)	35
C	5-FU + LV	23 (58)	22 (42)	40
	5-FU + LVS	22 (55)	26 (45)	40

Table 3. Survival rates of both treatments and both stages (B_2 or C)

Stage	*Treatment*	*Survival status*		*Total*
		Alive, n (%)	*Dead, n (%)*	
B_2	5-FU + LV	26 (74)	9 (25)	35
	5-FU + LVS	25 (71)	10 (29)	35
C	5-FU + LV	26 (65)	14 (42)	40
	5-FU + LVS	23 (58)	17 (45)	40

differences between the two groups in relation to OS (p=0.75; Figure 1). Also, no difference between the two treatments with regard to DFS was observed (p=0.84; Figure 2).

Multivariate analysis

The proportional hazard model analysis for DFS and OS was applied with age, treatment, sex, stage, operation, histology, tumor diameter (≤ 5 or >5 cm), and number of involved lymph nodes as explanatory variables.

The analysis was carried out in a stepwise (backward unconditional) fashion. The results indicated a near statistically significant effect for the number of positive nodes (OS p=0.09, DFS p=0.14). Tumor diameter (≤ 5 cm) predicted for a trend for better OS only in patients with stage B_2 (p=0.095), but not for patients with stage C tumors (p=0.47).

Toxicity

All patients were assessable for toxicity. The observed toxic reactions are presented in Table 4. There were no toxicity-associated deaths. Myelotoxicity (grade 3 leucopenia, 12 *versus* 4%, p < 0.04), diarrhea (grade 0, 60 *versus* 76%, p < 0.02), and liver toxicity (increase of transaminases >3-fold, 12 *versus* 2 patients, p < 0.03) were more frequent in the LVS group. There was no difference in the remaining examined toxicity parameters between the two groups. No patient discontinued treatment because of toxicity. In two pa-

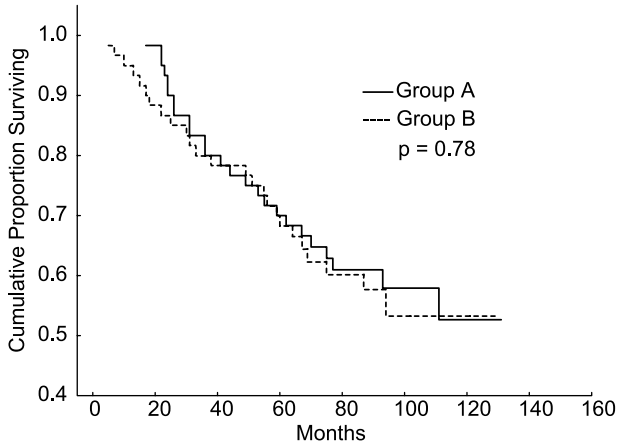

Figure 1. OS between the examined groups (median OS: 77.5 months; Group A median OS: 79.5 months, Group B median OS: 70 months).

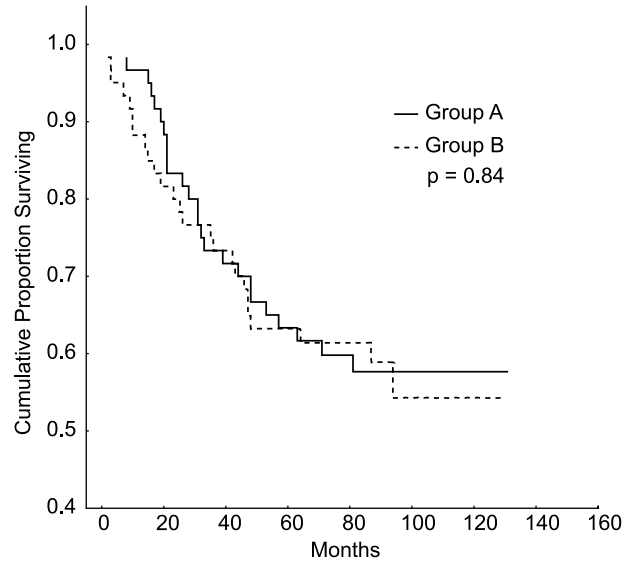

Figure 2. DFS between the examined groups (median DFS: 72.5 months; Group A median DFS: 78.5 months, Group B median DFS: 66 months).

Table 4. Percent WHO toxicity for the study arms for the total number of administered cycles

Parameter	*Grade*	*5-FU + LV*	*5-FU + LVS*	*p-value*
Leucopenia	0	87	74	0.03
	1-2	9	14	0.37
	3	4	12	0.04
Anemia	0	88	80	0.26
	1-2	12	20	0.17
Thrombo-cytopenia	0	97	90	0.78
	1	3	10	0.08
Diarrhea	0	76	60	0.02
	1-2	14	21	0.26
	3	10	14	0.57
	4	0	5	0.07
Mucositis - stomatitis	0	86	72	0.02
	1-2	10	16	0.29
	3	4	9	0.25
	4	0	3	0.24
Nausea - vomiting	0	85	82	0.70
	1-2	15	18	0.70
Liver toxicity		12	2	0.03
Neurotoxicity*		14	10	0.50

*evaluated according to the number of patients

tients from each group the 5-FU dose was reduced by 20% because of leucopenia. In 10 and 8 patients treated with LVS and LV, respectively, 5-FU was reduced by 20% because of diarrhea.

Discussion

The present study was conducted exclusively in patients with RC, prospectively comparing two acceptable treatment regimens: 5-FU+LV administered over 6 months *versus* 5-FU+LVS administered over 12 months. Moreover, an aspect of the present study was based on randomization that might be impacted by the surgeon operating these patients. It is already known that surgeons specializing in colorectal surgery may provide more radical operations and more accurate stage determination, that should be taken seriously into account in randomization [20].

Comparison between the two regimens in terms of DFS and OS did not reveal any statistically significant difference. This has already been known by other multi-institutional prospective randomized trials [21-25].

Toxicity was acceptable and both regimens were well tolerated, and we found that the incidence of the various toxicity parameters were similar between the examined groups, with more severe myelosuppression, diarrhea, and liver toxicity in the LVS group. This was in agreement with other studies [26-30].

It can be concluded that both adjuvant treatments (5-FU+LV×6 months *versus* 5-FU+LVS×12 months) do not yield significant differences in terms of DFS and OS [31-33].

Although many studies have stressed a significant survival advantage in patients receiving LVS, more recent studies found that the inclusion of LVS in adjuvant chemotherapy regimens for colorectal cancer does not delay recurrence or improve survival [26-28, 34-36].

The prognostic trend connected with the number of positive of lymph nodes found in our study is in agreement with results reported by other authors [37]; however, in this study the marginally significant difference was probably due to the small number of patients. Other studies referred to prognostic factors such as age [37], sex and location of the tumor, which were found to predict in a statistically significant manner for DFS and OS [38].

A factor which might affect the quality of life is the duration of therapy, particularly when this is addressed to patients in good physical condition, as those rendered free of macroscopic local disease and with no evidence of distant metastases treated in the adjuvant setting. Based on this, the arm treated with 5-FU+LV for 6 months is preferable. Given the equivalent outcome between the two treatment arms, either with 5-FU+LV (×6 months) or with 5-FU+LVS (×12 months), treatment with LV is more tolerable and convenient, and should therefore be recommended as standard adjuvant chemotherapy for patients with RC.

We conclude that adjuvant chemotherapy in RC should be offered for Astler-Coller stage B_2 and C patients with LV+5-FU for 6 months. An adverse prognostic factor in this study emerged to be the number of positive regional lymph nodes.

References

1. Moertel CG, Fleming TR, Macdonald JS et al. Fluorouracil plus levamisole as effective adjuvant therapy after resection of stage III colon carcinoma: a final report. Ann Int Med 1995; 122: 321-326.
2. Moertel CG, Fleming TR, McDonald JS et al. Levamisole and fluorouracil for adjuvant therapy of resected colon carcinoma. N Engl J Med 1990; 322: 352-358.
3. Dukes CE, Bussey HJR. The spread of colorectal cancer and its effect on prognosis. Br J Cancer 1958; 12: 309-320.
4. Olson RM, Perencevich NP, Malcolm AW, Chaffey JT, Wilson RE. Patterns of recurrence following curative resection of adenocarcinoma of the colon and rectum. Cancer 1980; 45: 2969-2974.
5. Gunderson LL, Sosin H. Areas of failure found at reoperation (second or symptomatic look) following "curative surgery" for adenocarcinoma of the rectum: clinicopathologic correlation and implications for adjuvant therapy. Cancer 1974;34: 1278-1289.
6. Minsky BD, Mies C, Recht A, Rich TA, Chaffey JT. Resectable adenocarcinoma of the rectosigmoid and rectum. 1. Patterns of failure and survival. Cancer 1988; 61: 1408-1414.
7. Zaniboni A. Adjuvant chemotherapy in colorectal cancer with high-dose leucovorin and fluorouracil: impact on disease-free survival and overall survival. J Clin Oncol 1997; 15: 2432-2441.
8. Zaniboni A, Labianca R, Marsoni S, Torri V, Mosconi P, Grilli R, Apolone G, Cifani S, Tinazzi A for the SITAC-01 Investigators. A randomized trial of adjuvant 5-fluorouracil and folinic acid administered to patients with colon carcinoma-long term results and evaluation of the indicators of health-related quality of life. Cancer 1998; 82: 2135-2144.
9. Mamounas E, Wieand S, Wolmark N et al. Comparative efficacy of adjuvant chemotherapy in patients with Dukes C colon cancer: Results from four National Surgical Adjuvant Breast and Bowel project adjuvant studies (C-01, C-02, C-03, and C-04). J Clin Oncol 1999; 17: 1349-1355.
10. Mamounas EP, Rockette H, Jones J et al. Comparative efficacy of adjuvant chemotherapy in patients with Dukes' B a Dukes' C colon cancer : Results from four NSABP adjuvant studies (C-01, C-02, C-03, C-04). Proc Am Soc Clin Oncol 1996 ; 15 : 205a (abstr # 461).
11. Wolmark N, Fisher B, Rockette H et al. Postoperative adjuvant chemotherapy or BCG for colon cancer: Results from NSABP protocol C-01. J Natl Cancer Inst 1988; 80: 30-36.

12. Pinedo HM, Peters GJ. 5-fluorouracil: biochemistry and pharmacology. J Clin Oncol 1988; 6: 1653-1664.
13. Evans RM, Laskin JD, Hakala MT. Effect of excess folates and deoxyinosine on the activity and site of action of 5-fluorouracil. Cancer Res 1981; 41: 3288-3295.
14. Machover D, Schwarzenberg L, Goldschmidt E et al. Treatment of advanced colorectal and gastric adenocarcinomas with 5FU combined with high-dose folinic acid: a pilot study. Cancer Treat Rep 1982; 66: 1803-1807.
15. Madajewicz S, Petrelli N, Rustum YN et al. Phase I-II trial of high-dose calcium leucovorin and 5-fluorouracil in advanced colorectal cancer. Cancer Res 1984; 44: 4667-4669.
16. IMPACT. Efficacy of adjuvant fluorouracil and folinic acid in B2 colon cancer. J Clin Oncol 1999; 17: 1356-1363.
17. Miller A, Hoogstraten B, Staquet M, Winkler A. Reporting results of cancer treatment. Cancer 1981; 47: 207-217.
18. Armitage P, Berry G (eds). Statistical Methods in Medical Research, (2nd edn). Oxford : Blackwell, 1994.
19. Everitt BS (ed). Statistical Methods for Medical Investigations. New York; Oxford University Press, 1989.
20. Hermanek P, Mansmann U, Staimmer DS, Riedl S, Hermanek P. The German experience: the surgeon as a factor in colon and rectal cancer surgery. Surg Oncol Clin North Am 2000; 9: 33-49.
21. IMPACT. Efficacy of adjuvant fluorouracil and folinic acid in colon cancer. Lancet 1995; 345: 939-944.
22. Hassan S, Turner P. Systolic time intervals: a review of the method in the non-invasive investigation of cardiac function in health, disease and clinical pharmacology. Post Grad Med J 1983; 59: 423-434.
23. Wolmark N, Rockette H, Mamounas E et al. Clinical trial to assess the relative efficacy of fluorouracil and leucovorin, fluorouracil and levamisole, and fluorouracil, leucovorin and levamisole in patients with Dukes' B and C carcinoma of the colon: results from National Surgical Adjuvant Breast and Bowel Project C-04. J Clin Oncol 1999; 17: 3553-3559.
24. Sastre J, Navarro M, Aranda E et al. Retrospective evaluation of toxicity in three different schedules of adjuvant chemotherapy for patients with resected colorectal cancer. TTD Spanish Cooperative Group. Oncol Rep 1999; 6: 1421-1424.
25. Cascinu S, Georgoulias V, Kerr D, Maughan T, Labianca R, Ychou M. Colorectal cancer in the adjuvant setting: perspectives on treatment and role of prognostic factors. Ann Oncol 2003; 14: 25-29.
26. Laurie J, Moertel C, Fleming T et al. Surgical adjuvant therapy of large bowel carcioma: an evaluation of levamisole and combination of levamisole and fluorouracil. The North Central Cancer Treatment Group and Mayo Clinic. J Clin Oncol 1989; 7: 1447-1456.
27. QUASAR Collaborative Group. Comparison of fluorouracil with additional levamisole, high dose folinic acid, or both, as adjuvant chemotherapy in colorectal cancer: a randomized trial. Lancet 2000; 355: 1588-1596.
28. Cascinu S, Catalano V, Piga A et al. The role levamisole in the adjuvant treatment of stage III colon cancer patients: randomized trial of 5-fluorouracil and levamisole versus 5-fluorouracil alone. Cancer Invest 2003; 21: 701-707.
29. Andre T, Colin P, Louvet C et al. Randomized adjuvant study comparing two schemes of 5-fluorouracil and leucovorin in stage B2 and C colon adenocarcinoma: study design and preliminary safety results. Groupe d'Etude et de Recherche Clinique en Oncologie Radiotherapies. Semin Oncol 2001; 28 (Suppl 1): 35-40.
30. McDonald J. Adjuvant chemotherapy of colon cancer. CA Cancer J Clin 1999; 49: 202-219.
31. Arkenau HT, Bermann A, Rettig K, Strohmeyer G, Porschen R. 5-Fluorouracil plus leucovorin is an effective adjuvant chemotherapy in curatively resected stage III colon cancer: long-term follow-up results of the adjCCA-01 trial. Ann Oncol 2003; 14: 395-399.
32. Dencausse Y, Hartung G, Sturm J et al. Adjuvant chemotherapy in stage III colon cancer with 5-fluorouracil and levamisole versus 5-fluorouracil and leucovorin. Onkologie 2002; 25: 426-430.
33. Porschen R, Bermann A, Loffler T et al. Fluorouracil plus leucovorin as effective adjuvant chemotherapy in curatively resected stage III colon cancer: results of the trial adjCCA-01. J Clin Oncol 2001; 19: 1787-1794.
34. Lavery IC, Lopez-Kostner F, Pelley RJ, Fine RM. Treatment of colon and rectal cancer. Surg Clin North Am 2000; 80: 535-569.
35. Windle R, Bell PR, Shaw D. Five years results of a randomized trial of adjuvant 5-fluorouracil and levamisole in colorectal cancer. Br J Surgery 1987; 74: 569-572.
36. Rougier P, Nordlinger B. Large scale trial for adjuvant treatment in high risk resected colorectal cancers. Rationale to test the combination of loco-regional and systemic chemotherapy and to compare l-leucovorin + 5-FU to levamisole+ 5-FU. Ann Oncol 1993; 2: 21-28.
37. Luna-Perez P, Trejo-Valdivia B, Labastida S, Arcia-Alvarado S, Rodriguez D, Delgado S. Prognostic factors in patients with locally advanced rectal adenocarcinoma treated with preoperative radiotherapy and surgery. World J Surg 1999; 23: 1069-1074.
38. Elsaleh H, Joseph D, Grieu F, Zeps N, Spry N, Lacopetta B. Association of tumor site and sex with survival benefit from adjuvant chemotherapy in colorectal cancer. Lancet 2000; 355: 1745-1750.