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Additional 5-FU-LV significantly increases survival in gastrointestinal cancer

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1. ABSTRACT

Metastatic colorectal and other locally advanced gastrointestinal (G.I.) cancers often recur after curative resection. Many mechanisms of tumor growth and/or immune escape by residual cancer cells may provoke tumor progression. Long-term, cytostatic action with repeated post-adjuvant administration of 5-fluorouracil (FU)-leucovorin (LV) cycles may interrupt or downregulate these mechanisms and favor the recovery and/or increase the immune system activity. Seventy patients were considered. An active prospective cohort including 21 patients (study group) was matched in a 1:1 ratio with a retrospective parallel control group of 21 patients. The study group received long-term repeated post-adjuvant administration of 5-FU-LV cycles, while the matched control group was conventionally treated. Statistical analysis was performed by Kaplan-Meier method and Cox's proportional hazard regression model. The five-year disease-free survival (DFS) was 77.0 + 10.1% and 31.7 + 10.6% ($p = 0.001$; hazard ratio (HR) 5.3, 95% C.I.: 1.7-16.1, $p = 0.003$), while the five-year overall survival (OS) was 88.0 + 8.1% and 37.0 + 10.7% ($p = 0.001$; HR 8.9, 95% C.I.: 2.0-39.9, $p = 0.004$) in the study group and in matched controls respectively. These findings suggest a relevant improvement in the outcome of this population by an intermittent and prolonged cytostatic effect with 5-FU-LV.

2. INTRODUCTION

Metastatic colorectal and other locally advanced gastrointestinal (G.I.) cancers submitted to potentially curative resection often recur. Prognosis remains poor and median 5 year disease-free survival (DFS) and overall survival (OS) range from 10% to 50% (1-7). Many studies have described different mechanisms promoting tumor growth and/or immune escape (8). Based upon these well described mechanisms, we have proposed an interpretative model to explain a significantly improved clinical benefit and OS observed in endocrine dependent metastatic breast cancer patients (8-10). Undetectable cancer cells likely residual after curative resection, with the same mechanisms may provoke a tumor progression to clinically detectable metastatic disease. 5-fluorouracil (5-FU) and leucovorin (LV) are among the most active drugs in the different kinds of G.I. cancer. In this exploratory study, we have hypothesized that in patients at high risk of relapse the post-adjuvant intermittent and long-term administration of 5-FU-LV may reduce the activities of residual cancer cells and halt tumor progression (11). Therefore, the working hypothesis was to observe in the entire studied population a significant decrease in relapses and a prolonged OS. In 19 recently reported of these patients tested with this new pharmacological approach, 5-year DFS and OS were

significantly better than in similar patients as expected from literature data (11). In the present report, we updated the study group and collected data from all similar G.I. cancer patients who in the same interval time were admitted for operation at the same Surgical Department in our University Hospital. In the entire population, control patients were matched and selected in a 1:1 ratio to our 21 studied patients who in addition had received intermittent long-term post-adjuvant 5-FU-LV cycles.

3. MATERIALS AND METHODS

3.1 Patients

From April 1997 to November 2009, 117 consecutive patients were admitted for a single synchrone or metachrone metastatic lesion from colorectal cancer or other G.I. cancers at a high risk of relapse at General Surgery Department of our Pisa University Hospital. The G.I. patients were with stage IIA-IIB-III esophagus, stage IB-IIB-III gallbladder, stage IB-IIA-IIB-III pancreatic and biliary duct, and stage IB-II-III-IV gastric cancers. Forty-seven (40%) of these 117 patients were ruled out. In fact, of those 47, 9 were 80 years or older, 8 had and "early" (pTis-pT1N0M0) gastric (n = 7) or pancreatic (n = 1) cancers, 11 had a gastric (n = 10) or pancreatic (n = 1) cancer with infiltrated margins of resection (R1), 12 did not undergo an operation due to metastatic cancer, 3 had gastric lymphoma and were sent to the Hematology/Oncology Unit, and 4 other patients had periampullary cancer with a relatively favorable prognosis. All the 70 remaining patients with a Karnofsky PS 0-1 were submitted to conventional potentially curative resection at the same General Surgery Department from the same surgeons and had to be disease-free at a complete, post-operative radiological study. They were eligible for this study and were sent for post-operative follow-up at one of two oncologic centers in our University Hospital. Center availability and patient preference were the main criteria for the choice of center. Twenty one of the 35 patients who came to our center were the study group. The remaining 14 were entered into the control group. Thirty-five patients who were comparable to the study group for their principal prognostic characteristics (pTNM, post-operative stage, grading and type of curative resection) were sent to the other oncological center and were also included in the control group. The 49 controls were matched; 21 of them were selected to form the matched control group while the remaining 28 were included in the unmatched control group. The study end date was July 31, 2010, that was last observation. No study or control patient was lost to follow-up. All study patients gave witnessed informed consent and the study was approved by the Council of the Department of Internal Medicine of Pisa University.

3.2. Clinical and pathological features

3.2.1. Study and matched control groups

The principal clinical and pathological characteristics of the study group and matched control group are shown in Table 1. In all but the one study group patient with peripheral cholangiocarcinoma (patient n. 21), the resection margins were free. In this patient with infiltration of the deep margins the additive treatment was also evaluated in this condition of minimally detectable disease.

3.2.2. Unmatched control group

One of the 28 eligible patients not included in the matched controls was diagnosed with esophagus cancer, 23 with gastric cancer (stage IB (8), II (5), IIIA (5), IIIB (1), IV (4)), 2 with pancreatic cancer (stage IB (1) and IIB (1)), 1 with gallbladder cancer (stage IIA/IIB) and the last one with peripheral cholangiocarcinoma (stage IIIA/C). The mean age was 66.7 $\pm$ 9.0 years (32-76 range). All evaluated patients were staged according to the 2009 UICC Staging System (12).

3.3. Study design

A matched retrospective cohort study was utilized, in which an active prospective cohort (study group) was matched with a control retrospective parallel cohort. The controls were patients who had received conventional treatment. These control patients were matched with study patients according to cancer type, stage and age, which are baseline variables that have a major impact on DFS and OS. Patients in the control group were selected in a 1:1 ratio to patients in the study group who, in addition, received intermittent, long-term, post-adjuvant 5-FU-LV. Data on the study group were derived from a prospectively collected database, while those on control patients were obtained from their case reports. In a few controls, the date of recurrence, date of death and further information were derived from an accurate, structured interview of relatives and from the death certificate.

3.4. Adjuvant chemotherapy (CT) after curative resection

3.4.1. Study group

Among the 21 patients in the study group, all 10 with gastric cancer after gastrectomy received a docetaxel and 5-FU regimen (13) for six (n = 9) or eight (n = 1) cycles. However, 1 of the 10 gastric cancer patients (n. 9, Table 1) who had refused recruitment at the time of gastrectomy, entered the trial 46 months later, soon after curative resection of a single secondary nodule of right lung. This patient was not given further adjuvant therapy after excision of the lung nodule. Five cycles with the above-mentioned docetaxel-5-FU schedule (5-FU c.i. 350 mg/day concomitant with radiation therapy for 6 weeks was administred between the first and second cycles) were given to 1 patient with pancreatic cancer. The other 2 patients with pancreatic cancer received six cycles of a gemcitabine and capecitabine regimen (14). The patient with esophageal cancer received the first cycle with cisplatin 40 mg/ m^2 on days 1 and 2 and 5-FU 1000 mg/ m^2 on days 1 to 4 c.i. After four weeks, the second cycle of this adjuvant CT was interrupted due to heavy side effects. Three out of the four colorectal cancer patients received six cycles of FOLFOX-4 regimen (15). The 3 patients with gallbladder (n = 2) or peripheral cholangiocarcinoma (n = 1) did not receive any conventional adjuvant treatment.

3.4.2. Matched control group

One patient with stage IIIA gastric cancer received six cycles of etoposide-5-FU-LV regimen (16).

Table 1. Principal clinical and pathological characteristics of the study group and matched control patients.

Study group (n = 21)								
Pt (n)	Age (yrs)	Sex	Cancer type	Type of curative resection	pTNM classification	Stage	Grade	Adjuvant CT
1	51	F	Gastric	Total gastrectomy with D1D2	T2bN0M0	IB	3	Yes
2	77	M	Gastric	Subtotal gastrectomy with D1D2	T2bN0M0	IB	3	Yes
3	51	M	Gastric	Subtotal gastrectomy with D1D2	T3N0M0	II	3	Yes
4	68	M	Gastric	Total gastrectomy with D1D2 and R.P.E.	T2bN1(4/53)M0	II	3	Yes
5	69	M	Gastric	Subtotal gastrectomy with D1D2	T2aN1(4/12)M0	II	2	Yes
6	73	M	Gastric	Subtotal gastrectomy with D1 and hepatic segmentectomy	T2bN2(7/14)M0	IIIA	2	Yes
7	59	F	Gastric	Subtotal gastrectomy with D1	T2bN2(10/14)M0	IIIA	1	Yes
8	74	M	Gastric	Subtotal gastrectomy with D1D2	T2aN3(18/42)M0	IV	3	Yes
9	45	M	Gastric	Total gastrectomy with D1D2 and R.P.E then right lung lobectomy	T2bN1(2/27)M1	IV	2	No
10	64	F	Gastric	Total gastrectomy with D1	T3N3(23/42)M0	IV	3	Yes
11	58	F	Esophageal	Subtotal esophagus removal	T3N1(6/48)M0	III	2	No
12	71	M	Colorectal	Right emicolectomy and hepatic segmentectomy (V-VI)	T4N0M1*	IV	3[a]	Yes
13	66	M	Colorectal	Anterior rectum removal and hepatic segmentectomy (V)	T3N1(3/20)M0[a]	IV	2[a]	Yes
14	49	F	Colorectal	Anterior rectum removal then atypical resection of the right inferior lung lobe	T4N1(1/27)M0[a]	IV	2[a]	Yes
15	64	M	Colorectal	Anterior rectum removal then hepatic segmentectomy (VII)	T3N2(10/14)M0[a]	IV	2[a]	No
16	53	F	Pancreatic	Duodenocephalopancreasectomy	T3N0M0	IIA	2	Yes
17	66	M	Pancreatic	Duodenocephalopancreasectomy	T3N1b(6/24)M0	IIB	2	Yes
18	66	M	Pancreatic	Duodenocephalopancreasectomy	T3N1b(1/31)M0	IIB	2	Yes
19	67	F	Gallbladder	Gallbladder removal	T2NxM0	IB/IIB	2	No
20	55	M	Gallbladder	Gallbladder removal	T1bNxM0	IA/IIB	1	No
21	72	M	Peripheral cholangioca.	Atypical resection of the V and VII hepatic segments	T3NxM0	IIIA/C	3	No
Matched control group (n = 21)								
1	63	F	Gastric	Subtotal gastrectomy with D1D2	T2aN0M0	IB	2	Yes
2	72	M	Gastric	Subtotal gastrectomy with D1D2	T2bN0M0	IB	2	No
3	65	M	Gastric	Subtotal gastrectomy with D1	T2bN1(2/5)M0	II	2	Yes
4	58	M	Gastric	Subtotal gastrectomy with D1	T2aN1(1/23)M0	II	3	Yes
5	73	M	Gastric	Total gastrectomy with D1D2	T2bN1(5/48)M0	II	3	Yes
6	74	M	Gastric	Total gastrectomy with D1 and R.P.E.	T2bN2(9/18)M0	IIIA	3	No
7	56	M	Gastric	Subtotal gastrectomy with D1D2	T3N1(4/11)M0	IIIA	3	Yes
8	61	F	Gastric	Total gastrectomy with D1	T3N3(17/21)	IV	3	Yes
9	51	M	Gastric	Subtotal gastrectomy with D1D2	T3N3(28/28)M0	IV	3	Yes
10	74	F	Gastric	Subotal gastrectomy with D1D2	T2bN3(39/47)	IV	3	No
11	69	M	Esophageal	Resection of distal esophagus	T3N1(8/12)M0	III	2	No
12	70	M	Colorectal	Resection of sigma and hepatic segments (II-III)	T3N0M1[a]	IV	2[a]	Yes
13	45	M	Colorectal	Resection of sigma and hepatic segment (IV)	T3N1(2/13)M1[a]	IV	2[a]	Yes
14	63	M	Colorectal	Left emicolectomy then resection of right inferior lung lobe	T3N2(5/10)M0[a]	IV	3[a]	No
15	77	M	Colorectal	Resection of sigma then hepatic segmentectomy (I)	T3N0M0[a]	IV	2[a]	Yes
16	62	M	Pancreatic	Duodenocephalopancreasectomy	T2N0M0	IB	2	Yes
17	76	F	Pancreatic	Duodenocephalopancreasectomy	T3N1(1/14)M0	IIB	3	No
18	62	M	Pancreatic	Duodenocephalopancreasectomy	T2N1M0	IIB	2	Yes
19	73	F	Gallbladder	Gallbladder removal	T3NxM0	IIA/IIB	3	No
20	65	M	Gallbladder	Gallbladder removal	T2N0M0	IB	1	No
21	63	F	Peripheral cholangioca.	Resection of hepatic segments (II-II-III-IV) and gallbladder removal	T3N1(1/7)M0	IIIC	2	Yes

For details also see text; *pTNM classification and grading refers to primary colorectal cancer; CT: chemotherapy; R.P.E. = resection of proximal esophagus.

Two other patients with stage II (1) and IV (1) gastric cancer received 4 cycles of docetaxel and 5-FU (13) followed by four cycles with M-FAMTX (17). The last two M-FAMTX cycles were given without doxorubicin due to heavy side effects. Four other control patients with stage II (3) and IV (1) gastric cancer received six cycles of the PELF (18) regimen. One of three patients with primary carcinoma at the sigma was given eight cycles of the FOLFOX-4 regimen; the two other patients received six cycles of 5-FU 500 mg/m^2 c.i. on days 1 to 5 and LV 200 mg/m^2 every 4 weeks. These three patients received this adjuvant treatment soon after their potentially curative resection of single synchrone (n = 2) or metachrone (n = 1) metastatic lesion. Two of the 3 patients with pancreatic cancer were given six cycles of gemcitabine and capecitabine (14). The patient with peripheral cholangiocarcinoma received 6 cycles of 5-FU-leucovorin consisted with the just reported schedule.

3.4.3. Unmatched control group

Adjuvant CT was administered to the esophagus cancer patient, 10 (43%) of the 23 patients with gastric cancer (stage IB, n = 1; stage II, n = 2; stage IIIA, n = 2; stage IIIB, n = 2; and stage IV, n = 3), to both patients with stage IB-IIB pancreatic cancer, and to that with stage IIA/IIB gallbladder cancer. In 1 further patient with stage IIIA gastric cancer, CT was interrupted at the second cycle.

3.5. Additive post-adjuvant 5-FU-LV cycles in the study group

The series of additive 5-FU-LV cycles was started in July 1998 as already reported (see reference 11).

Table 2. Comparison of the main characteristics between study group and matched controls

Parameter	Study group (n = 21) mean±sd	Matched controls (n = 21) mean±sd	Total controls (n = 49) mean±sd	P value*
Number of patients	21	21	49	
Age	62.7±9.2 (range 45-77)	65.3±8.4 (range 45-77)	66.1±8.9 (range 32-77)	0.473
Male	14 (66.7%)	15 (71.4%)	32 (65.3%)	1.00
Cancer type				1.00
Colorectal	4 (19.0%)	4 (19.0%)	4 (8.2%)	
Esophagus	1 (4.8%)	1 (4.8%)	2 (4.1%)	
Gallbladder	2 (9.5%)	2 (9.5%)	3 (6.1%)	
Pancreas	3 (14.3%)	3 (14.3%)	5 (10.2%)	
Peripheral cholangiocarcinoma	1 (4.8%)	1 (4.8%)	2 (4.1%)	
Stomach	10 (47.6%)	10 (47.6%)	33 (67.3%)	
Stage				1.00
I	4 (19.0%)	4 (19.0%)	13 (26.5%)	
II	6 (28.6%)	6 (28.6%)	13 (26.5%)	
III	4 (19.0%)	4 (19.0%)	12 (24.5%)	
IV	7 (33.3%)	7 (33.3%)	11 (22.4%)	
Grade				0.809
1	2 (9.5%)	1 (4.8%)	1 (2.0%)	
2	11 (52.4%)	10 (47.6%)	20 (40.8%)	
3	8 (38.1%)	10 (47.6%)	28 (57.1%)	
Adjuvant CT				
yes	†15 (71.4%)	13 (61.9%)	27 (55.1%)	0.744
Follow-up time				
Mean (months)	79.9	91.5	87.0	
Median (months)	77.0	87.0	92.0	

CT: chemotherapy; *Fisher's exact test was used with categorical data, and Mann-Whitney test was used with continuous variables; †patient n. 9 (see Table 1) was not included as he did not receive further adjuvant therapy after excision of the lung nodule.

3.6. Intensive post-operative monitoring

In both centers, control visits were performed at two to three months intervals during the first three years, and every four to six months thereafter. Serum tumor markers (TM), in particular SCC for patients who had squamous esophageal carcinoma, CA19.9 for those who had pancreatic cancer and the CEA-TPA-CA19.9-CA72.4 tumor marker panel for all other evaluated cancers were measured (19). Abdominal and chest computed tomography aimed at the suspected site and, when necessary, magnetic resonance imaging, esophagus-gastroduodenoscopy (EGDS), colonoscopy (CS) and cyto-histology were used to confirm the relapse.

3.7. Statistical analysis

Differences between the study group, the matched control group and the total controls in demographic, clinical and pathological features were tested using non-parametric analysis. Only patients who at least had completed 2 cycles of the chosen regimen were considered to have received adjuvant CT. Fisher's exact test and the Mann-Whitney test were used for categorical and continuous variables respectively (Table 2).

DFS and OS curves were estimated using the Kaplan-Meyer method. A 5 years survival analysis was performed and the log-rank test (unadjusted analysis) was used to compare survival curves. As the expected cumulative 5 year OS was 40%, a matched control group of 21 patients was able to detect a statistical significance with an □ = 0.05 and a power = 0.80. The two study arms were also compared using the Cox's proportional hazard regression model allowing for the covariates (adjusted analysis) of: age, stage, grade and adjuvant CT as principal risk factors for recurrences and mortality of the different studied G.I. cancers (1-7). Schoenfeld residuals were used to verify the proportional hazard assumption. The hazard ratio (HR) including 95% confidence interval was presented for the matched control group. To compare 5-year DFS and OS rates in the two groups a chi-square test based on the log(-log()) transformation for the survival function was used (20). An analysis including all 49 controls as a comparison group was conducted in order to evaluate whether a potential selection bias due to the matching had any effect on the results. A 2-sided $p < 0.05$ was considered to be statistically significant. All statistical analysis were performed using SPSS 13.0 software (SPSS Inc., Chicago, Illinois, USA).

4. RESULTS

The study group was well comparable to the matched control group for the main characteristics (Table 2).

4.1. Follow-up

All 21 patients received additional 5-FU-LV cycles. In these 21 patients, no WHO grade 3 or 4 side effects occurred. Therefore, neither an interruption nor any decrease in the planned dosing of CT was necessary. Nineteen of these 21 patients are alive and 17 are disease-free. The 4 remaining, of whom 2 had gastric cancer (patients n. 7 and n. 10), one who had peripheral cholangiocarcinoma (patient n. 21), and the last one with esophageal cancer (patient n. 11) relapsed 29, 16, 25 and 26 months after curative resection of the primary cancer, respectively. These four patients had only completed the first (patient n. 10) and the first and second (patients n. 7, n. 11 and n. 21) series of the additive 5-FU-LV therapy as well as other two patients (patients n. 18 and n. 17 respectively). Another patient (patient n. 8) is receiving the second series of post-adjuvant CT. The 13 remaining

Table 3. Relapses and deaths within five years following curative resection of different G.I. cancers at high risk of relapse according to stage in the study group, and matched and unmatched controls; cumulative five-year DFS and OS in the groups

Cancer type	Stage	Pts n	Study group (n = 21)		Matched controls (n = 21)		Unmatched controls (n = 28)		
			Relapse (n)	Death (n)	Relapse (n)	Death (n)	Pts (n)	Relapse (n)	Death (n)
Gastric	IB	2	0	0	1	0	8	1	0
	II	3	0	0	2	2	5	3	3
	IIIA/B*	2	1	1	1	1	6	5	5
	IV	3	1	1	3	3	4	4	4
Esophageal	III	1	1	0	1	1	1	0	0
Metastatic colorectal†	IV	4	0	0	3	3	-	-	-
Pancreas	IB, IIA/B‡	3	0	0	2	2	2	1	1
Gallbladder	IA/B, IIA/B	2	0	0	0	0	1	1	1
Peripheral cholangiocarcinoma	IIIA/C	1§	1	0	1	1	1	1	1
Total		21	4	2	14	13	28	16	15
Cumulative 5-year survival (%)			77.0 ±1 0.1	88.0 ± 8.1	31.7 ± 10.6	37.0 ± 10.7		37.9 ± 9.9	40.1 ± 10.1

*In the unmatched controls, 4 patients were with stage IIIA and the 2 remaining were with stage IIIB; †With curative resection of single metastasis; ‡The 3 study group patients and the 3 matched controls were staged IIA (1) and IIB (2); in the unmatched controls 1 of the 2 patients was staged IB and the other IIB; §With positive margins of resection. Comparison between study group and matched controls: p = 0.001 for 5-year DFS; p = 0.001 for 5-year OS.

patients completed all four series of additional chemotherapy. Table 3 shows the outcome within five years following curative resection of different G.I. cancers at high risk of relapse according to stage in the study group, and matched and unmatched controls. In the study group, the potential follow-up after primary or metastatic curative resection was 79.9+ 53 months; 7 patients had a follow-up less than 5 years. In the 21 matched controls, the potential follow-up was 91.5 + 30 months; two patients had a follow-up less than 5 years. Seven (33.3%) patients are alive and disease-free. Three of these 7 patients were operated for either stage IB (n = 1), stage II (n = 1) or stage IIIA (n = 1) gastric cancer. Another patient underwent anterior rectum removal concomitant with hepatic segmentectomy for a single metastatic lesion from rectal cancer. Two were operated for gallbladder cancer and the remaining one for stage IB pancreatic cancer. In the 28 unmatched controls, the potential follow-up was 83.7 + 32.5 months. Eleven (39.2%) patients are alive and 10 (35.7%) are disease-free. These 11 patients were operated for stage IB (n = 7) or stage II (n = 2) gastric cancer, for esophagus cancer (n = 1) and for pancreatic cancer (n = 1). Two patients died from primary lung cancer and from hemorrhagic stroke, respectively, and were censored.

4.2. Five-year OS and DFS

The 5-year OS and DFS curves of the 21 study patients, the 21 matched controls, and the 28 unmatched controls after curative resection are shown in Figures 1-2. The number of relapses and deaths that occurred within the five years following curative resection and the cumulative five-year DFS and OS rates for all three groups are shown in Table 3. The cumulative 5-year DFS and OS rates in the 21 matched and in the 28 unmatched controls were similar to those expected and previously reported (11). So far, no relapse and only one death due to metastatic disease occurred after five years (control n. 1, matched controls). The five years DFS and OS curves were significantly different between the study group and the matched control group (p = 0.001 and p = 0.001, respectively) (Figures 1-2). The two groups also significantly differed in the DFS and OS rates at five-years (p = 0.001 and p = 0.001, respectively). In the study group, the DFS and OS rates at five years (77.0 ± 10.1 % and 88.0 ± 8.1 %, respectively) were 2.4 times higher than those in the matched control group (31.7 ± 10.6 % and 37.0 ± 10.7 %, respectively). The univariate Cox proportional hazard model showed that the matched controls had a risk of relapse and death that was higher than that of the study patients (HR = 5.3, 95% C.I.: 1.7-16.1, p = 0.003; HR = 8.9, 95% C.I.: 2.0-39.9, p = 0.004, respectively). Once all confounding variables (age, stage, grade and adjuvant CT) were entered into the Cox model, the risk of relapse and death remained significantly higher in the matched control group (p = 0.000 and p = 0.004, respectively). Schoenfeld residuals did not show any violation of the proportional hazard assumption. The sensitivity analysis, which was performed using all 49 controls as a control group, confirmed a significant difference between the study patients and controls in the DFS and OS rates (p = 0.001 and p = 0.000, respectively). In the control group, the DFS and OS rates at five years were 35.1 ± 7.2% and 38.7 ± 7.4%, respectively.

5. DISCUSSION

5.1. Improved outcome and shortcomings

In the study patients the estimated five-year OS and DFS were 88% and 77%, respectively. Therefore, they were doubled compared to those observed in matched and unmatched controls (Table 3). Our group included different

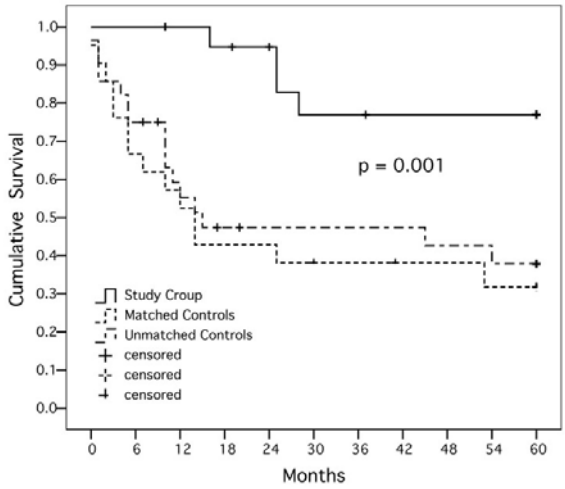

Figure 1. Unadjusted Kaplan-Meier curves showing the disease free survival in study group, matched control group and unmatched control group (study group v.s. matched control group: Log-Rank test p = 0.001).

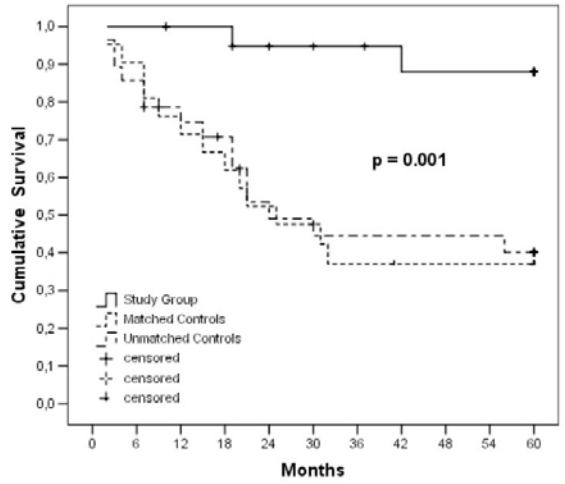

Figure 2. Unadjusted Kaplan-Meier curves showing the overall survival in study group, matched control group and unmatched control group (study group v.s. matched control group: Log-Rank test p = 0.001)

types of G.I. cancer. However, all of these cancers share an unfavorable prognosis. Furthermore, patients who received prolonged and intermittent post-adjuvant 5-FU-LV therapy (study group) were strictly matched according to cancer type, stage and age in a ratio of 1:1 with patients who received conventional therapy methods. Therefore, we compared two groups in which most characteristics were equalized. Moreover, we also statistically controlled for the more important predictors and demographic variables, including stage, grade, adjuvant CT and age. As to adjuvant CT, it has to be considered that in most locally advanced GI cancers and in colorectal cancer after curative resection of liver metastases, no recommended standard adjuvant therapy does exist, because no certain DFS and OS benefit has been reported (21-26). In this study, the five-year DFS and OS of our matched and unmatched control groups were in line with those reported in the literature (11). A significant difference was also confirmed when all matched and unmatched control patients were used as a control group.

5.2. The study reasons and interpretation

Tumor growth and spread are the final results of carcinogenesis by intermediate mechanisms and tumor cells are the principal source of most factors responsible of tumor growth and spread, as well as of inhibited cell mediated immunity at the tumoral microenvironment, where mainly they act by autocrine and paracrine loops (8) Likely these mechanisms are at work for relatively long time before clinical and/or radiological signs of primary cancer or the relapse occur. 5-fluorouracil arrests tumor cell replication at the G0 or G1-early S phase and inhibit protein synthesis. Therefore, if the above mentioned mechanisms are at work in the residual G.I. tumor microenvironment, we have hypothesized that the prolonged and intermittent administration of 5-FU is expected to produce a clinical benefit. In fact, following the repeated transient downregulation of the many detrimental tumor cell activities via 5-FU-LV the immune system could maintain or recover its control and ultimately avoid cancer progression. Interestingly, high 5-year OS and DFS rates have been reported in breast cancer patients following cytostatic effect with 5-FU and tamoxifen given for 2 years as adjuvant therapy (27). In the last years we have started to regularly submit all our high risk GI cancer patients to an extensive immunological assessment including cytokines and growth factor evaluation, to further document our working hypothesis and initial data.

In the overall, these data confirm that prolonged and intermittent cytostatic effect with 5-FU-leucovorin combination improves the outcome of this population of G.I. cancer patients at high risk of relapse. A large, prospective, multicenter, randomized trial to definitively validate the use of the proposed therapeutic protocol is expected.

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Abbreviations, G.I.: gastrointestinal, DFS: disease-free survival, OS: overall survival, 5-FU: 5-fluorouracil, LV: leucovorin, AJCC: American Joint Cancer Committee, CT: chemotherapy, FOLFOX: oxaliplatin plus 5-fluorouracil/leucovorin, M-FAMTX: modified therapy with 5-fluorouracil, doxorubicin and methotrexate, EGDS: esophagus-gastroduodenoscopy, CS: colonoscopy, SCC: squamous cell carcinoma

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