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Original Paper

Double Biochemical Modulation of 5-Fluorouracil by Methotrexate and Levo-Folinic Acid in the Treatment of Advanced Digestive Tract Malignancies

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The aim of this study was to evaluate the activity and toxicity of a double biochemical modulation of 5-fluorouracil (5-FU) by means of methotrexate (MTX) and levo-folinic acid (LFA) in patients with advanced carcinoma of the digestive tract, and to assess the prognostic significance of MTX serum concentrations achieved in these patients. 94 patients affected by advanced carcinoma of the colon–rectum, stomach or biliary tract (47 of them previously untreated) received a regimen consisting of MTX 500 mg/m^2 as a 2-h i.v. infusion on day 1, followed by LFA 250 mg/m^2 as a 2-h i.v. infusion and 5-FU 600 mg/m^2 as an i.v. bolus on day 2. Cycles were repeated every 2 weeks. Treatment was administered until tumour progression or for a maximum of 24 courses. MTX serum level was assessed soon after and 24 h (24-h MTXs) after its infusion in 61 patients. One complete and 22 partial responses were obtained, giving an overall activity of 24% (95% confidence interval, 16–34%). Response rate was 30% in chemotherapy-naive patients (colorectal, 26%; gastric, 37%; and biliary-tract, 22%) and 19% in those previously treated (all with fluoropyrimidines). A poor performance status adversely affected the response and survival of patients. The toxicity of treatment was very mild, and occurrence of severe diarrhoea (11% of patients) and mucositis (3%) was lower than that reported with other modulations of 5-FU. A cut-off value of 24-h MTXs was identified as a strong prognostic indicator. Patients with 24-h MTXs $\geq$ 2 μM had a significantly better probability of response (37% versus 5%; $P = 0.032$), longer progression-free survival (5.3 versus 2.3 months; $P = 0.023$) and overall survival (10.8 versus 8.3 months; $P = 0.045$) on multivariate analysis. In chemotherapy-naive colorectal cancer patients, those with 24-h MTXs $\geq$ 2 μM had a response rate of 38% (3/8), with a 19.6-month median survival time, as compared to no responses (0/4) and a 9.9-month median survival in the group with a lower serum concentration. The achievement of such MTX serum levels yielded a 31% (4/13) response rate even in colorectal patients who had previously received a 5-FU–FA treatment.

Key words: methotrexate, 5-fluorouracil, levo-folinic acid, double modulation, pharmacokinetics, gastrointestinal carcinomas

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INTRODUCTION

5-FLUOROURACIL (5-FU) is still one of the most active single agents for gastrointestinal malignancies, but its effectiveness does not exceed a 10–20% objective response rate in both colorectal and gastric cancer [1].

In advanced colorectal carcinoma, many attempts have been made to potentiate the cytotoxicity of 5-FU through its biochemical modulation, and a combination of folinic acid (FA) and 5-FU is considered to be more active than 5-FU alone when given either as a 5-day monthly schedule or a weekly regimen [2, 3]. However, only a few studies have yielded a longer survival for patients treated in this way, and a meta-analysis failed to demonstrate a clear survival advantage for patients treated with 5-FU + FA versus 5-FU alone [4].

From *in vitro* and *in vivo* results [5, 6] it has also been suggested that methotrexate (MTX) potentiates 5-FU cytotoxicity. It is well known that the intracellular accumulation

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of MTX favours its polyglutamation (MTX-glu), which is proportional to the drug concentration and the time of exposure. The MTX-glu inhibits purine metabolism, causing an accumulation of 5-phosphoribosyl-1-pyrophosphate (PRPP) and increased conversion of 5-FU to its active metabolites, 5-fluorouridine triphosphate (5-FUTP) and 5-fluoro-2′-deoxyuridylate (5-FdUMP). MTX-glu may also directly inhibit thymidylate synthase activity. Although some crucial points of this approach, such as the dosage of MTX, the optimal interval between MTX and 5-FU administration, and the role of FA to overcome MTX toxicity, remain uncertain, a recent meta-analysis on several published studies confirmed higher activity with MTX + 5-FU compared with 5-FU alone in terms of response rate, and showed a modest but unequivocal survival advantage for patients receiving the combined regimen [7].

Poon and colleagues [8] have also compared 5-FU modulation by high-dose MTX and FA rescue with a standard 5-FU–FA approach. A moderate survival benefit was found in favour of 5-FU–FA treatment, although response rates were equivalent in the two groups. In view of this, a double modulation of 5-FU by concomitant administration of MTX and high-dose FA (given simultaneously with 5-FU and then in a fractionate schedule as a rescue) has been attempted in order to improve the therapeutical results. Some phase II trials [9, 10] have tested this approach, showing promising, although not consistent, results in terms of both response rate and overall survival. Abad and colleagues [9] administered MTX 200 mg/m^2 24 h before 5-FU (600 mg/m^2) and FA (200 mg/m^2). They observed a 40% objective response rate (20% complete), and an 18-month median survival time, without encountering severe toxicity, while Romero and colleagues [10] reported a 21% overall response rate with a 10-month median survival. In these trials, the MTX dosage and the interval between MTX and 5-FU administration were chosen on the basis of several *in vitro* studies, which suggested that a 24-h exposure to MTXs serum levels ≥ 1 μM is advisable to optimise the synergy with 5-FU [11–13]. The importance of the interval between MTX and 5-FU was also emphasised by Marsh and colleagues [14]. They observed a significantly higher response rate and better survival in patients with a 24-h as compared to those with a 1-h interval between the administration of the two drugs. In a preliminary trial, we tested the combination of 5-FU–FA with escalating doses of MTX. We demonstrated that at least 500 mg/m^2 of MTX administered as a short i.v. infusion was required to maintain a serum concentration ≥ 1 μM for at least 24 h [15]. We also observed that the MTX dosage can be safely escalated up to 750 mg/m^2 without the need to administer additional doses of oral leucovorin as a rescue. In the present paper, we report our expanded experience with this regimen employing MTX at 500 mg/m^2 in patients with advanced gastrointestinal malignancies, both chemotherapy-naive and pretreated.

PATIENTS AND METHODS

Patient characteristics

From January 1992 to April 1994, a series of 94 consecutive patients affected by advanced digestive tract malignancies were enrolled in this multicentric phase II trial. Eligibility criteria were: histologically proven adenocarcinoma of the stomach, biliary tract, pancreas or colon–rectum; presence of measurable lesions not suitable for radical surgery; age ≤ 75 years; ECOG performance status (PS) ≤ 2; life expectancy longer than 3 months. Furthermore, patients had to have normal bone marrow reserve (WBC $\geq 3500/mm^3$ and platelets $\geq 75\,000/mm^3$), and adequate renal and liver functions (serum creatinine $\leq 1.5 \times N$ and serum bilirubin $\leq 2.5 \times N$). Previous chemotherapy was not an exclusion criterion, provided that any treatment had been discontinued for at least one month. Patients gave their informed consent to participate in this investigational study, which was approved by the Ethical Committee for Biomedical Research of the National Tumour Institute of Naples.

Table 1 reports the main characteristics of the series. The majority of patients had PS of 0 or 1. The most frequent primary was colorectal carcinoma, which accounted for 57% of the whole series, followed by gastric (32%) and pancreatic (4%) or biliary tract carcinomas (6%). More than two-thirds of patients had had previous surgical treatment for their disease. 47 out of 94 patients has also received at least one previous chemotheray regimen, which in all but 4 cases included a fluropyrimidine derivative.

Treatment plan

The planned therapy was MTX 500 mg/m^2 diluted in normal saline solution, given as a 2-h i.v. infusion on day 1; levo-folinic acid (LFA) 250 mg/m^2 diluted in normal saline,

Table 1. Main patient characteristics

Characteristics	Number of patients
Total entered	94
Males/females	58/36
Median age (range) in years	62 (36–74)
Performance status (ECOG scale)	
0	24
1	56
2	14
Primary sites	
Stomach	30
Gall bladder/biliary tract	6
Pancreas	4
Colon–rectum	54
Previously treated with surgery	64
Previously treated with chemotherapy	47
With fluoropyrimidines alone	13
With folinic acid + fluoropyrimidine	30
With other drugs	4
Total sites of disease	
Liver	57
Local primary (unresected or recurrent)	59
Lymph nodes	18
Peritoneum	16
Lung	15
Other sites	11
Number of sites/patient	
1	45
2	30
3	15
4	4

administered as a 2-h i.v. infusion on day 2, and 5-FU 600 mg/m^2 given as an i.v. bolus at the end of the LFA infusion. Cycles were repeated every 2 weeks. If a complete response was obtained, two more cycles were given. Otherwise, treatment was continued for a maximum of 24 cycles unless clear tumour progression occurred.

Treatment was postponed for at least one week for WBC count <3000/mm^3 or platelet count <75 000/mm^3. Furthermore, in the presence of gastrointestinal or other non-haematological WHO grade 3 toxicity, all drugs were withheld and, following reversal of toxicity, MTX and 5-FU were resumed with a 25% reduction of dosage, while the dose of LFA remained unmodified.

Evaluation of response and toxicity

Response to therapy was assessed every 2 months by repeating all initially abnormal tests, including endoscopy to evaluate endoluminal lesions. Complete response (CR) was defined as the complete disappearance of all evidence of tumour for at least 1 month. A reduction of more than 50% in the sum of the product of the largest perpendicular diameters of measurable lesions, coupled with an unequivocal reduction of non-measurable lesions, was defined as a partial remission (PR). A less than 50% decrease, or less than 25% increase of tumour burden, was considered as no change (NC), while progressive disease (PD) was defined as an increase of more than 25% of tumour mass, or appearance of new lesions.

Side-effects of treatment were scored according to WHO criteria [16]. Blood cell counts were repeated at each cycle, whereas liver and renal functions were assessed every other cycle during treatment.

The actual dosage of all three drugs administered during the first four courses of treatment was utilised to calculate the dose intensity for each patient [17].

MTX serum concentration assessment

MTX concentration was determined utilising an immunofluorescence technique (Methotrexate Abbott Kit) on patients' sera collected soon after the end of MTX infusion, and 24 h later. Blood was separated by centrifugation at 1500 rpm and sera were stored at −20°C until analysis. A determination on the first three consecutive courses was planned for each patient, and the mean of these three values was used.

Statistical analysis

Progression-free survival, duration of response, and overall survival were calculated from the start of therapy to the occurrence of an event, or to last follow-up. At the time of this analysis (October 1994), the potential follow-up of patients ranged between 6 and 34 months, with a median value of 22 months.

Statistical analyses were carried out using the BMDP statistical software [18]. Association between response and baseline patient characteristics (sex, age, primary site of disease, number of metastatic sites) were tested with the chi-square or Fisher's exact test using two-sided P values. The Wilcoxon rank sum test was used to compare the values of MTX serum level in responder and non-responder patients. Survival curves were generated using the Kaplan–Meier method [19], and differences in survival curves were tested with the log-rank test [20]. Factors independently predictive of response, freedom from progression and overall survival were determined by logistic regression analysis and by multivariate analysis according to the Cox proportional hazards model [21].

The sample size for this study was defined by using the two-stage patient accrual plan of Gehan [22]. According to the reported activity of modulated 5-FU, a minimum response rate of 20% in previously untreated patients was defined to be of some interest. Therefore, if no response was observed in the first 14 treated patients, this hypothesis might be rejected with a 5% β error, and the accrual of patients would be stopped. Otherwise, a number of additional patients, proportional to the responses observed in the first step, would be entered in the second stage to define the true therapeutic activity of the regimen with a 5% α error.

RESULTS

Objective tumour response

A total of 924 cycles were administered, with a median of 8 (range 4–24) courses per patient. Among the 94 treated patients, we observed 23 responses (1 CR and 22 PRs), for an overall activity of 24% (95% CI, 16–34%). Responses were usually observed after four cycles, but in three cases they were registered after eight, and in one patient after 12 cycles of treatment. Duration of responses ranged between 5 and 24 months, with a median of 9.5 months. The patient who achieved a CR was still disease-free 13 months after the start of therapy.

The overall response rate was 20% in colorectal carcinoma patients (chemo-naive, 26%; pretreated, 17%), and 30% in gastric carcinoma patients (chemo-naive, 37%; pretreated, 18%), while 3/10 (2/9 previously untreated) patients with biliary-tract carcinoma responded (Table 2). All 6 patients with pretreated colorectal cancer who responded to our treatment had previously had clearly documented progressive disease while receiving 5-FU–FA chemotherapy.

The probability of response was significantly affected by the PS of patients. In fact, a 29% response rate was observed in patients with PS 0–1 as compared with no response in those with PS 2 ($P = 0.049$). The number of sites of disease did not appear to affect the probability of achieving a response; overall activity rate was 27% in patients with one site of disease compared to 22% in patients with two or more sites. However, no patient with four sites responded to this treatment. No significant difference in probability of response between the different metastatic sites (liver, lung, peritoneum and lymph nodes) was observed. Other pretreatment characteristics, such

Table 2. Responses according to primary site and previous treatment

Primary site	CR + PR(%)	NC	PD	Total
Colon–rectum				
Pretreated	6 (17)	16	13	35
Untreated	5 (26)	11	3	19
Stomach				
Pretreated	2 (18)	4	5	11
Untreated	7 (37)	6	6	19
Gall bladder and pancreas				
Pretreated	1 (100)	0	0	1
Untreated	2 (22)	1	6	9
Total				
Pretreated	9 (19)	20	18	47
Untreated	14 (30)	18	15	47

as age (more or less than 65 years) or sex, were not related to response rate.

The median actual dose intensity of MTX and 5-FU was very close to the planned dose intensity, being 234 mg/m^2/week (94% of that planned) for MTX and 284 mg/m^2/week (95% of that planned) for 5-FU. Furthermore, actual dose intensities of both drugs were not significantly different in responder and non-responder patients.

Survival

At the time of the analysis, 71 patients were dead. Survival of patients ranged between 2 and 26.5 months, and had a median length of 11 months. Median survival time (MST) for advanced colorectal carcinoma was 10.7 months in previously treated and 14.3 months in chemotherapy-naive patients, and the median progression-free survival was 4.2 months and 8.0 months, respectively. Among patients with gastric carcinoma, the MST values were 8.2 months in previously treated and 7.3 months in untreated patients. The corresponding median progression-free survival were 2.3 months and 4.4 months. Survival of the 10 patients with other primaries ranged between 2.5 and 15 months and had a median duration of 6.5 months.

Multivariate analysis confirmed that the PS ($P = 0.015$) and the primary site ($P = 0.023$) were independently predictive of survival of patients. Patients with PS 0 had an MST of 13.4 months, as compared with an MST of 10.4 in PS 1, and 4.1 in PS 2. Patients with colorectal carcinoma had an MST of 11.7 months, with gastric cancer 8.5 and with biliary-tract carcinoma 6.5. Pretreatment was not an independent predictive variable: the MST was 11.5 months in untreated and 10.4 months in pretreated patients.

Significance of MTX serum level

The determination of MTX serum levels (MTXs) at 0 and 24 h from the end of MTX infusion was carried out in only 61 treated patients, and for each of them three measurements were available for analysis. MTXs at time 0 ranged from 40 to 149 μM (median, 89 μM; mean ± S.D., 102 ± 23). At 24 h the mean values of MTXs for each patient ranged from 1.2 to 9.5 μM (median, 2.2 μM; mean ± S.D., 3.2 ± 2.2). The intrapatient variations of the three consecutive determinations ranged between 0.4 and 3 μM. Creatinine clearance determination failed to predict 24-h MTXs (Pearson coefficient = −0.062; $P = 0.711$). 16 patients (26%) in this group responded to treatment. No significant linear correlation was found between response to therapy and 24-h MTXs, since similar mean values of 24-h MTXs were observed in responders (3.4 ± 1.2) and non-responders (3.1 ± 2.4). However, a higher median value of 24-h MTXs was observed in responder patients (3.4 versus 2.0 μM) ($P = 0.012$).

Using the value of 2 μM as a cut-off, we noted that in the 20 patients showing a mean value of less than 2 μM MTXs at 24 h, only 1 (5%) objective response occurred, as compared to 15 (37%) major responses in the remaining 41 patients with higher values ($P = 0.007$). A change in the classification of patients according to this cut-off value was seldom produced by intrapatient variations. In fact, in the 20 patients with a mean value lower than 2 μM, 24-h MTXs was above this cut-off in only 6 of the 60 assessed courses. In the 41 patients with a mean value exceeding this cut-off, only 13 of the 123 evaluated courses showed a value below 2 μM.

In order to identify whether a different categorisation of patients on the basis of 24-h MTXs could better predict the probability of response, we further divided the 41 patients showing ≥2 μM into two groups, each with a similar number of patients. 20 had MTXs at 24 h between 2 and 3 μM, and 21 had concentrations exceeding 3 μM. A direct relationship between the increase of MTXs and response rate was again observed. In fact, 6 (30%) patients responded in the former and 9 (43%) in the latter group. However, this relationship disappeared for 24-h MTXs >5 μM, since only 1 response was obtained in the 9 patients having these serum levels. Since the dichotomous categorisation on the bases of a 2.0 μM value better permitted the split of the patients with a negligible probability of response from the others, we chose to consider only two subgroups of patients defined by this cut-off in all successive analyses. Table 3 details 24-h MTXs values according to primary site and prior chemotherapy, when this cut-off is applied. Prior chemotherapy failed to affect the pharmacokinetics of MTX, while a higher rate of gastric cancer patients had ≥2 μM of MTXs at 24 h, as compared to colorectal cancer patients. In chemotherapy-naive patients with ≥2 μM, 24-h MTXs response rate was 38% (colon 38%, gastric 38%) as compared to no responses in the others. Higher than 2 μM 24-h MTXs were also associated with a good response rate in pretreated patients, regardless of primary. Pretreated colorectal cancer patients achieved a response rate (31%) similar to that shown by those who were chemotherapy-naive (38%) (Table 4). The logistic analysis confirmed the independent prognostic significance of 24-h MTXs on the probability of achieving a response (Table 5).

The 24-h MTXs value also appeared to affect the outcome of our patients. Indeed, median freedom from progression was significantly shorter in patients with lower rather than with higher values (2.3 versus 5.3 months) ($P = 0.001$). At the Cox analysis stratified for primary site, a 24-h MTXs ≥2 μM was

Table 3. Distribution of patients and their mean serum levels according to the 2 μM cut-off value of 24-h MTXs

Characteristics	24-h MTXs ≥ 2 μM	24-h MTXs <2 μM
Colorectal carcinoma	21 patients	13 patients
Median	3.1	1.4
Range	2.0–9.3	1.2–1.9
Gastric carcinoma	16 patients	5 patients
Median	2.4	1.6
Range	2.1–9.5	1.3–1.9
Gall bladder and pancreas	4 patients	2 patients
Median	2.7	–
Range	2.0–7.1	1.6–1.7
Previous chemotherapy	17 patients	12 patients
Median	3.0	1.5
Range	2.0–9.3	1.2–1.9
No previous Chemotherapy	24 patients	8 patients
Median	3.0	1.4
Range	2.0–9.5	1.2–1.9

Table 4. Responses according to 24-h MTX serum levels (total patients = 61)

Primary site	24-h MTXs ≥2 μM	24-h MTXs <2 μM	Total
Colon–rectum			
Unpretreated	3/8 (38%)	0/4	3/12 (25%)
Pretreated	4/13 (31%)	1/9 (11%)	5/22 (23%)
Stomach			
Unpretreated	5/13 (38%)	0/2	5/15 (33%)
Pretreated	1/3 (33%)	0/3	1/6 (17%)
Others			
Unpretreated	1/3 (33%)	0/2	1/5 (20%)
Pretreated	1/1 (100%)	—	1/1 (100%)
Total	15/41 (37%)	1/20 (5%)	16/61 (26%)

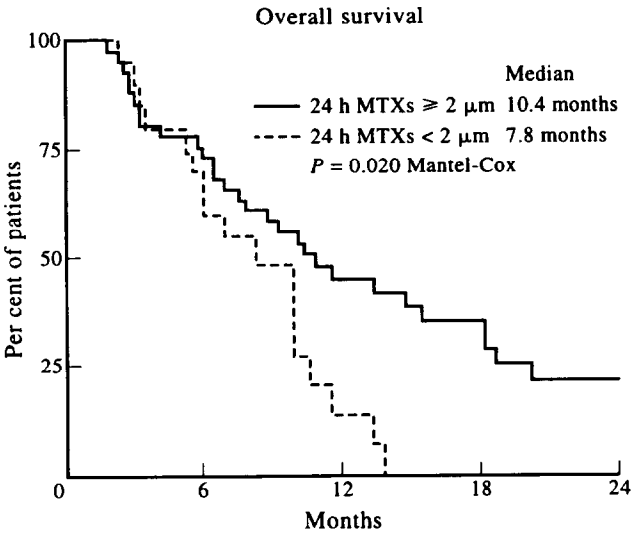

Figure 1. Survival of all treated patients according to MTX serum level assessed at 24 h.

Table 5. Multivariate analysis of factors affecting response rate (RR), progression-free and overall survival of 61 patients with assessed MTX serum level

Variable	RR	P*	Progression-free survival median (months)	P†	Survival median (months)	P†
Primary site		0.358		–		–
Colon–rectum	24%		3.7		10.6	
Stomach	29%		4.2		6.9	
Others	33%		2.0		6.0	
24-h MTXs		0.032		0.023		0.045
≥2 μM	37%		5.3		10.8	
<2 μM	5%		2.3		8.3	
Previous therapy		0.453		0.045		0.070
No	29%		4.6		11.5	
Yes	23%		2.3		8.3	
Performance status		0.617		0.673		0.481
0						
1	30%		6.5		13.4	
2	32%		4.6		9.9	
	0		2.3		4.5	
Number of sites		0.087		0.810		0.795
1	27%		4.2		10.4	
≥2	26%		4.6		9.9	

*P = probability at the logistic regression analysis. †P = probability at the Cox's analysis after stratification for primary site.

the most predictive ($P = 0.023$) characteristic for progression-free survival, followed by previous therapy ($P = 0.045$).

The plotted survival curves of the two groups were also significantly different ($P = 0.020$), showing an increase of more than 2 months in median survival time (8.3 versus 10.8 months), and a 12% probability of being alive at 24 months for patients with 24-h MTXs ≥2 μM, while no patient with the lower value was still alive after 24 months (Figure 1). The 8 chemotherapy-naive colorectal cancer patients achieving a ≥2 μM 24-h MTXs had a 19.6-month median survival time. An encouraging median survival was also observed in pretreated colorectal cancer patients showing 24-h MTXs ≥2 μM (10.2 months). The MST in chemotherapy-naive gastric cancer patients who had 24-h MTX ≥2 μM was 9.2 months. The prognostic impact on survival of 24-h MTXs ≥2 μM also remained significant ($P = 0.045$) at multivariate analysis, while previous treatment lost its significance, probably because of the lower power of the test in this subset of patients (Table 5).

We also analysed the prognostic role of 24-h MTXs as a continuous variable. It failed to affect significantly both progression-free and overall survival at Cox's analysis ($P = 0.283$ and $P = 0.096$, respectively). This was probably due to the non-normal distribution of the 24-h MTXs measurements.

Toxicity

The side-effects observed during treatment are reported in Table 6. The most frequent acute toxicities were diarrhoea and stomatitis, but their intensity was usually mild and manageable, being of grade 3–4 in only 11% and 3% of patients, respectively. Haematological toxicity was negligible if the

Table 6. Main acute toxicities reported during treatment amont 94 patients

Toxicity	WHO grade 1	2	3	4	Total G 3+4 (%)
Diarrhoea	13	8	8	2	10 (11)
Mucositis	15	6	3	0	3 (3)
Nausea/vomiting	12	8	4	0	4 (4)
Anaemia	12	3	3	3	6 (6)
Leucopenia	1	2	2	0	2 (2)
Thrombocytopenia	1	2	0	0	0
Fever	1	4	0	0	0
Hand–foot syndrome	2	3	0	0	0

occurrence of serious anaemia was excluded, which was probably related to the prolonged administration of antifolates in some patients. Occurrence of severe (grade 3–4) diarrhoea was the same among patients with serum levels ≥2 μM (6/41, 15%) as found in patients with levels <2 μM (3/20, 15%). It was experienced by only 1 of the 8 patients (12.5%) that reached a 24-h MTXs level >5 μM.

DISCUSSION

Recently, the double biochemical modulation of 5-FU by MTX and FA has been explored in colorectal carcinoma with positive, but inconsistent results. Response rate and MST were 40% and 18 months, respectively, in the trial conducted by Abad and colleagues [9], as compared to only a 21% response rate and 10-month MST in the study recently carried out by Romero and colleagues [10].

In a previous study using a sequential administration of escalating MTX dosages (250–500 mg/m^2 i.v. on day 1), followed by LFA 250 mg/m^2, and 5-FU 600 mg/m^2 i.v. 24 h later, we observed that a 24-h MTX serum level ≥1 μM was obtained only in a minority of patients receiving a dose of 250 mg/m^2, whereas a short i.v. infusion of MTX 500 mg/m^2 was able to produce a serum level above 1 μM for at least 24 h in all patients [15]. Such a MTX serum concentration and time of exposure seem to be required for an optimal synergy with 5-FU in cell cultures. In fact, Benz and Cadman [13] suggested that one of the postulated intermediates used to explain the interaction between MTX and 5-FU is the PRPP, which reaches maximum levels in colon cancer cells after a 24-h exposure. In view of these considerations we decided to explore this combination further in a phase II drug-oriented trial at the higher dose.

It should be stressed that in this study, we obtained major responses in different digestive tract primaries. Indeed, in previously untreated patients with colorectal carcinoma, our combination had an overall activity rate comparable with other regimens of 5-FU modulated by FA, even taking into account the wide range of activity with the levo-stereoisomer which has recently been reported [23, 24]. Furthermore, in patients with advanced gastric cancer, our treatment showed similar therapeutical activity to the new-generation regimens EAP, FAMTX or PELF, which have obtained no more than a 20–41% overall response rates in recent randomised trials, with a MST generally not exceeding 8–10 months [25–28].

The responses observed in previously treated colorectal patients are, in our opinion, even more important. All responders in this group had previously been found resistant to a 5-FU–FA treatment. Therefore, it would seem that this MTX modulation can reconvert some cases back to sensitivity to an LFA + 5-FU combination. Our results are in agreement with the 20% PR rate reported in pretreated patients with colorectal cancer using a similar regimen in which trimetrexate was used instead of methotrexate [29], and appear clearly better than that reported with other approaches for second-line management of these patients [30, 31].

The most relevant finding in our study was the strong correlation between 24-h MTXs and therapeutical activity of the combination. In fact, the response rate was 37% (15/41) in the patients with ≥2 μM as compared to only 5% (1/20) in the others. Valone and colleagues [32] failed to observe any correlation between 24-h MTXs and response to treatment. However, they pointed out that complete responses were obtained only in patients with higher than 1 μM 24-h MTXs. The authors also hypothesised that the lack of correlation between MTX pharmacokinetics and therapeutical activity could be due to the low MTX serum peaks achieved in their study. In fact, they administered MTX orally at a dose of 50 mg/m^2 every 6 h for 5 doses, so keeping a constant but low MTX serum concentration for 24 h. In contrast, very high MTX serum peaks were reached in our study, ranging between 40 and 149 μM. A long-term exposure to such high MTX serum levels probably induces a higher accumulation of MTX–glu in cancer cells. This might explain the good results we achieved in patients with a favourable pharmacokinetical profile, which look particularly promising in those pretreated. In fact, response rates were similar in pretreated and chemotherapy-naive patients having a ≥2 μM 24-h MTXs (35% versus 38%). This pattern was observed both in colorectal and in gastric cancer patients. The response rate achieved in the 13 pretreated colorectal cancer patients showing higher MTX levels (31%) seems of particular interest, in view of the very low probability of response expected in this group. Also, progression-free and overall survival were strongly related to pharmacokinetics of MTX. At Cox's analysis, the serum levels of MTX at 24 h as a dichotomous variable was the most predictive parameter of progression-free survival, followed by previous treatment. Moreover, it was the only independently predictive parameter of overall survival. A median survival of 19.6 months was observed in 8 chemotherapy-naive colorectal cancer patients who showed 24-h MTXs ≥2 μM. This result compares favourably with those achieved by others, using either MTX–5-FU or 5-FU–FA combinations. In fact, in the two meta-analyses of these combinations, MSTs were 10.7 and 11.5 months, respectively [4, 7]. Moreover, the MST achieved in our pretreated colorectal cancer patients with ≥2 μM 24-h MTXs (10.2 months) seems slightly better than that recently obtained with more aggressive approaches [30, 31]. However, in view of the limited number of patients included in our analysis, we must be very cautious in evaluating these results. Further confirmation on larger series is required prior to concluding that levels of 24-h MTXs ≥2 μM predicts a significant prolongation of overall survival in both pretreated and untreated colorectal cancer patients.

The impact of MTX pharmacokinetics on survival was less relevant in gastric cancer patients. In fact, the MST (9.2 months) observed in chemotherapy-naive patients who had 24-h MTX ≥2 μM was no better than that achievable with recent combinations [25–28]. However, it must be taken into account that our results were obtained at a price of minimal toxicity. Indeed, the occurrence of diarrhoea and stomatitis, that represent the most common toxicities of a FA–5-FU regimen given on a monthly or weekly basis [33], were surprisingly low in our series. This result seems of particular interest if we take into account that a leucovorin rescue for at least 48 h, which is generally recommended for MTX concentrations >1 μM at 24 h, was not performed in this study. In our opinion, the high dose of leucovorin administered together with 5-FU is enough to effectively rescue the MTX toxic effects on normal cells, since it provides a large amount of reduced folates, and so compensates for the inhibition of the dehydrofolate reductase. The mild toxicity profile of our regimen and its feasibility as out-patient treatment make it an interesting alternative to other approaches for elderly patients with metastatic gastrointestinal disease [34]. Moreover, this toxicity profile might also allow a further increase of the dosage of 5-FU. Indeed, although it was not evident in this

trial, a dose–response reltionship for 5-FU has been reported in colorectal carcinoma [35].

In conclusion, this larger series, although quite heterogeneous, confirmed the results observed in our initial study [15]. The double modulation of 5-FU by means of high-dose MTX and LFA is an effective approach to advanced gastrointestinal neoplasms. The therapeutical role of this combination looks particularly promising in colorectal cancer patients previously treated with a 5-FU–FA regimen. The strong therapeutical relevance of MTX pharmacokinetics has been, however, the most important finding of our study. On this basis, some final considerations can be made:

(a) an excessive excretion of MTX, causing a reduction of serum levels to less than 2 μM after 24 h, may impair the efficacy of this regimen;
(b) creatinine clearance does not seem to significantly affect MTX elimination, thus other mechanisms (liver metabolism, biliary excretion etc.) could cause the different pharmacokinetic profiles;
(c) a further increase of the MTX dose should be attempted to achieve the optimal MTX concentration at 24 h; this can be safely made in view of the very mild toxicity encountered in this study, in spite of the lack of leucovorin rescue; and
(d) the achievement of a relevant response rate in colorectal cancer patients refractory to 5-FU–FA suggests that MTX at this high dosage and without leucovorin rescue could potentiate 5-FU activity in a different way from leucovorin, probably through an inhibitory effect on the same 5-FU target (thymidylate synthase).

In view of these considerations, we are now conducting a phase II trial to test a further increase of MTX dosage to 750 mg/m^2 (which our previous phase I study proved to be feasible) in advanced digestive tract malignancies, in order to achieve a sufficient serum concentration in all patients, thereby improving their outcome.

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