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Jan A.M. Van Laar · Youcef M. Rustum
Clasina L. Van der Wilt · Kees Smid · Catharina M. Kuiper
Herbert M. Pinedo · Godefridus J. Peters

Tumor size and origin determine the antitumor activity of cisplatin or 5-fluorouracil and its modulation by leucovorin in murine colon carcinomas

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Abstract 5-Fluorouracil (FUra) is one of the few effective agents in the treatment of patients with colorectal cancer. Its effects on the target enzyme thymidylate synthase (TS) can be modulated by leucovorin (LV) or cisplatin (CDDP). Tumor size and differentiation of tumor characteristics can influence therapeutic efficacy. We therefore studied the relationship between tumor size (cutoff point 200 mm^3) and the antitumor activity of FUra and its modulation by LV in murine Colon 26 and Colon 38 tumors. The doubling time of tumors measuring $> 200\ mm^3$ was about 160% longer. The antitumor effect of FUra in these large tumors was decreased and could not be modulated by LV. In addition, three subtypes of Colon 26 (Colon 26-A, Colon 26-B, and Colon 26-10) were identified and characterized for tumor-induced weight loss, TS activity, response to chemotherapy, and histological features. Mice bearing Colon 38 and Colon 26-10 did not lose weight as a result of tumor growth. Colon 26-A caused a weight loss of up to 19%, whereas mice with Colon 26-B tumors remained within 10% of their initial weight and tolerated at least 2.5 times more tumor load than did mice bearing Colon 26-A, which induces cachexia. Among untreated tumors, TS catalytic activity was highest in Colon 26-B (5536 pmol mg protein^{-1} h^{-1}) and lowest in Colon 38 (799 pmol mg protein^{-1} h^{-1}); Colon 26-A and Colon 26-10 had intermediate activities (about 2500 pmol mg protein^{-1} h^{-1}). 5-Fluoro-2′-deoxyuridine monophosphate (FdUMP) binding was comparable in the three Colon 26 subtypes but was lower in Colon 38. The antitumor activity of FUra could be modulated by LV in Colon 38, Colon 26-10, and Colon 26-A but could not in Colon 26-B, with complete responses (CR) being obtained in Colon 26-10 and Colon 38. The latter two were highly sensitive to CDDP, followed by Colon 26-A and Colon 26-B (CRs: 50%, 40%, 25%, and 0, respectively). Furthermore, necrosis was noted in Colon 26-B and Colon 38 but not in Colon 26-A. In conclusion, (1) the antitumor activity of FUra in large tumors is decreased and cannot be modulated by LV and (2) characteristics and sensitivity to chemotherapeutics can vary substantially in closely related tumors of the same origin.

Key words Tumor size · Tumor origin · Cisplatin · 5-Fluorouracil · Leucovorin modulation · Antitumor activity

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J.A.M. Van Laar · C.L. Van der Wilt · K. Smid · C.M. Kuiper
H.M. Pinedo · G.J. Peters (✉)
Department of Oncology, Free University Hospital,
P.O. Box 7057, 1007 MB Amsterdam, The Netherlands

J.A.M. Van Laar · Y.M. Rustum
Grace Cancer Drug Center, Roswell Park Cancer Institute,
Buffalo, NY 14263, USA

Introduction

5-Fluorouracil (FUra) in combination with the modulator leucovorin (LV) is now recommended for treatment of patients with advanced colorectal cancer, but the effect in terms of survival remains limited [1, 2]. FUra has to be metabolized to the nucleotide level and acts by incorporation of its metabolite 5-fluorouridine-5′-triphosphate (FUTP) into RNA [3] and by decreased DNA synthesis caused by 5-fluoro-2′-deoxyuridine monophosphate (FdUMP)-mediated inhibition of thymidylate synthase (TS) activity [4, 5]. The latter effect can be increased by LV [5–7]. Cisplatin (CDDP) has an unclear inhibitory effect on TS and can produce a synergistic antitumor effect when combined with FUra [8, 9]. Addition of CCDP to fluoropyrimidine therapy has increased responses in patients with advanced colorectal cancer [10]. Combination of FUra, LV, and CDDP has resulted in a 51% response rate in advanced colorectal cancer [11]. Other

combinations of fluoropyrimidines with platinum or platinum analogues (oxaliplatin) are currently being investigated for treatment of patients with colorectal cancer [12].

A large tumor load is associated with decreased therapeutic efficacy by mechanisms such as decreased accessibility for drugs, metastases with altered characteristics, and development of heterogeneity in the tumor itself [13, 14]. It is suspected that modulation of cytotoxic drugs and their cytostatic action is decreased in large tumors; however, this has never been described. Therefore, we investigated the effectiveness of FUra and LV modulation of FUra in murine Colon 26 and Colon 38 tumors with a large volume as compared with moderately sized tumors. These preclinical tumor models are commonly used as a panel to test novel chemotherapeutics against colorectal cancer, to study the process of metastasis, and also for mechanistic studies of chemotherapeutics [3, 7, 9, 15–43]. These two tumors have been induced at the Southern Research Institute (SRI, Birmingham, Ala.) with *N*-methyl-*N*-nitrosourethan or 1,2-dimethylhydrazine, respectively [15]. For example, though originating from one source, Colon 26 can develop different characteristics, such as a decreased growth rate, different responses to cytostatics, or histological structural changes after various passages [15–17, 34, 44]. For instance, Sugimoto et al. [34] have demonstrated that in vitro adaptation of Colon 26 to serum-free conditions leads to development of tumors with new pheno- and genotypes. The effect of these mutations could result in changes in metastatic features or altered enzyme activities.

Tanaka et al. [35, 36] related weight loss and tumor-induced cachexia with metastases in Colon 26. Differentiation of the metastatic abilities of the Colon 26 tumor results in altered cachexic features [35, 36]. Furthermore, if mutations lead to increased target enzyme activity, it is likely that these changes could account for the ineffectiveness of chemotherapeutics as seen in the clinic, where tumor heterogeneity for TS activity has been observed [5].

We therefore also evaluated the various properties and TS activity of three subtypes of Colon 26 (Colon 26-A, Colon 26-B, and Colon 26-10) derived from the same parent but maintained in different laboratories. An attempt was made to correlate possible differences with the therapeutic efficacy of FUra $\pm$ LV and CDDP.

Materials and methods

Mice and drugs

In the Netherlands, FUra was purchased from Hoffman-La Roche (Mijdrecht, The Netherlands) and diluted with sterilized and pyrogen-free phosphate-buffered saline (PBS); LV was obtained from Lederle Cyanamid (Etten Leur, The Netherlands); and CDDP was supplied by Bristol-Myers Squibb (Woerden, The Netherlands). At Buffalo, FUra and LV were obtained from Sigma Chemical Co. (St. Louis, Mo., USA). FUra was dissolved in sterile 0.9% saline. CDDP was purchased from Bristol-Myers (Syracuse, N.Y., USA) and dissolved in 0.9% saline. BALB/c and C57B1/6 mice in Amsterdam were obtained from Harlan/Cpb (Zeist, The Netherlands), and Harlan (Prattvile, Ala.) delivered BALB/c mice in Buffalo and kept them at five to six animals per cage with access to water and food ad libitum. Animal experiments were performed according to the rules of the local Animal Ethics Committees.

Drugs were given i.p. on weekly schedules. In both Amsterdam and Buffalo the maximum tolerable dose (MTD) was defined as the dose producing a maximal drug-related weight loss of 10% but not causing drug-related deaths. In Amsterdam, treatment with FUra alone could be given at a dose of 100 mg/kg [9, 23, 24, 26–28,, 30, 31, 37, 39]. In combination with LV the optimal schedule was 50 mg/kg LV given at 1 h before and simultaneously with FUra [23, 24, 39]. In Buffalo, 100 mg/kg FUra caused 80% deaths (data not shown) and FUra alone was thus given at a safe dose of 80 mg/kg [38]. CDDP was used at a dose of 9 mg/kg.

Histological evaluation

Tumors were removed from untreated mice. Formalin-fixed paraffin samples were stained with hematoxylin-eosin for further histological evaluation. Necrosis was assessed by histological evaluation of five to ten tumor samples.

Tumor and antitumor activity

Colon 26 and Colon 38 are murine (adeno-) carcinomas. For experiments in Amsterdam, Colon 38 and a Colon 26 tumor (later named Colon 26-A) were originally provided by Dr. P. Lelieveld of the REPGO-TNO Institute (later named Medical Biological Laboratory, TNO; Rijswijk, The Netherlands). This institute received the tumors from SRI [44]. Colon 26-A samples (passage 42, originally frozen on 17 November 1977) and Colon 38 samples (passage 28, frozen on 25 Mach 1980) were implanted into mice at the laboratories of the Free University on 05 January 1984 and subsequently maintained by transplantation. Samples from the original passages were kept in liquid nitrogen, frozen with 10% dimethylsulfoxide (DMSO), and transplanted before the 20th transplantation round to avoid differentiation from the original tumor. Colon 26-10 cells (provided by Dr. W.D. Klohs, Ann Arbor, Mich., USA) [21] were originally derived from a Colon 26 tumor and established as a cell line. In Amsterdam, Colon 26-10 cells were injected into the flanks of BALB/c mice, which led to a new tumor line named Colon 26-10. Colon 26-B is another Colon 26 tumor, which was also initially obtained from Dr. T.H. Corbett during his stay at SRI and subsequently propagated at the Roswell Park Cancer Institute in the laboratory of Dr. Y.M. Rustum. Approximately every 3 months, tumors were taken out of the bank and retransplanted s.c. into BALB/c mice. Prior to banking, tumor fragments were tested for mycoplasma. For further evaluation, Colon 26-B was transported to Amsterdam, where experiments were repeated to compare the two tumors, which originated from the same source but were maintained in different laboratories. The characteristics are summarized in Table 1.

Tumors were maintained in female BALB/c (all Colon 26 variants) and C56B1/6 (Colon 38) mice and were transplanted in fragments of 1–5 mm^3 into both flanks of 2-month-old female mice, when experiments were initiated. Mice were weighed at least three times a week to monitor toxicity and to determine the amount of drugs to be given. Tumor volumes were obtained by caliper measurement using the formula height $\times$ length $\times$ width $\times$ 0.5, which was shown to be one of the most reliable and reproducible methods

Table 1 Characteristics of murine colon tumors

Tumor	Colon 26-A	Colon 26-B	Colon 26-10	Colon 38
Type	Undifferentiated colon carcinoma, local fibrosarcoma	Undifferentiated colon carcinoma	Undifferentiated colon carcinoma	Differentiated colon adenocarcinoma
Source	SRI[f]/TNO, Rijswijk	SRI/Buffalo	SRI/Ann Arbor	SRI/TNO, Rijswijk
Host	BALB/c	BALB/c	BALB/c	C57B1/6
Take rate[a]	100%	95–100%	95–100%	90–95%
Necrosis	−	+	±	+ +
TD[b]	3.0 days	2.9 days	4.1 days	4.9 days
CWL[c]	19.2%	8.5%	0	0
MLS[d]	20 days	20 days	25 days	> 40 days
FUra sens.[e]	±	±	+ + +	+ +

[a] Percentage of successfully transplanted tumors
[b] Tumor-doubling time
[c] Cachexic weight loss, defined as the loss of weight just before anticipated death
[d] Median life span after transplantation of a tumor measuring 1–5 mm^3; only Colon 26-A acounted 100% of tumor-induced deaths, whereas mice with other tumors were often killed after tumors had reached a diameter of 2 cm
[e] Sensitivity to FUra treatment: $\pm$ 0.25 > GDF < 1.0, + + median GDF > 2.0, + + + meidan GDF > 2.0 and complete response rate > 50%
[f] Obtained from Dr. D.P. Griswold and from Dr. T.H. Corbett during their stay at SRI. Dr. Corbett is currently at Wayne State University, Detroit, Michigan

in both our laboratories and others. In addition, irregularities in tumor shape, such as those often observed in Colon 38, can more easily be corrected with this method by the use of three instead of two axes [46]. When tumors had reached 50–200 mm^3 in size, treatment was started and this day was designated as day 0. Mice bearing tumors with an initial size of > 200 mm^3 were excluded from standard evaluation, although treatment was nonetheless given. This cutoff point was based on an agreement made with other laboratories [26, 42, 47] at the Free University and with several collaborative groups within Europe [including the European Organization for Research and Treatment of Cancer (EORTC) Screening and Pharmacology Group] to ensure that data would be comparable. As a general policy, mice bearing tumors with a diameter of > 2 cm were euthanized. Tumor volumes were obtained every 3–4 days and were expressed relative to volumes measured on day 0. Since most of these data were obtained retrospectively and survival analysis was allowed at the time at which these experiments were carried out, a median life span (MLS) could be calculated. The MLS was defined as the day after transplantation on which the median part of a group had died.

The antitumor activity was evaluated by analyzing (a) the tumor-doubling time (TD), representing the time in which the tumors doubled their size after the start of treatment; (b) the growth-delay factor [GDF = (TD treated mice − TD untreated mice)/TD untreated mice]; (c) the maximal mean tumor volume of treated animals/tumor volume of untreated animals (T/C max); and (d) the percentage of complete regressions (CR) as defined by Van Laar et al. [38]. More than one T/C value recorded for mice with normal-sized tumors was included in the tables so as to compare these T/C values with those noted for mice bearing large tumors, who died earlier. Four different antitumor-activity scoring systems were used, since each type of tumor responds in a different way. For instance, the GDF of sensitive tumors cannot be calculated if many CRs are achieved; thus, the T/C max is a better alternative. However, for mice who would die very early due to cachexia, the T/C value would not be reliable and would cover only a short period.

The influence of tumor size on antitumor activity was studied by analyzing the data from previous experiments [7, 9, 23, 24, 26–31, 37, 38]. This approach was chosen because the growth of Colon 38 tumors is quite heterogenous and many mice would be required to produce enough large tumors in one experiment; this was not considered ethical. Therefore, it was felt that a retrospective study involving a large number of tumors would be adequate to provide a sufficient number of large tumors. The data from published experiments involving animals with a tumor size of > 200 mm^3, which were initially excluded, were now reanalyzed and compared with data compiled from previously published experiments. The analysis was not done in Colon 26-10 and in experiments with the LV-FUra combination in Colon 26-B, because tumor growth in these models is more homogeneous and many more experiments would be required to produce a sufficient number of large tumors.

Enzyme assays

Tumors (size 100–200 mm^3) were removed from untreated mice and immediately frozen in liquid nitrogen. TS activity was measured both with a ligand-binding assay to determine the number of free FdUMP-binding sites of TS and with a ^3H-release assay to determine the rate of conversion of dUMP into deoxythymidine monophosphate (dTMP) and, thereby, the catalytic activity of TS. The latter assays were performed at two substrate concentrations: 1 μM dUMP (nonsaturated) and 10 μM dUMP (saturation concentration). Potential inhibition of TS was determined by the addition of 10 nM FdUMP; dissociation constants (K_i values) for FdUMP were calculated using Dixon plots for competitive inhibition. Both assays were performed with saturating folate concentrations according to published methods [7, 39, 45].

Statistical analysis

Student's t-tests for unpaired data were used for statistical analysis. A P value of < 0.05 was considered significant.

Results

Histological evaluation

The amount of necrosis and tumor differentiation were evaluated by examination of histological cross sections

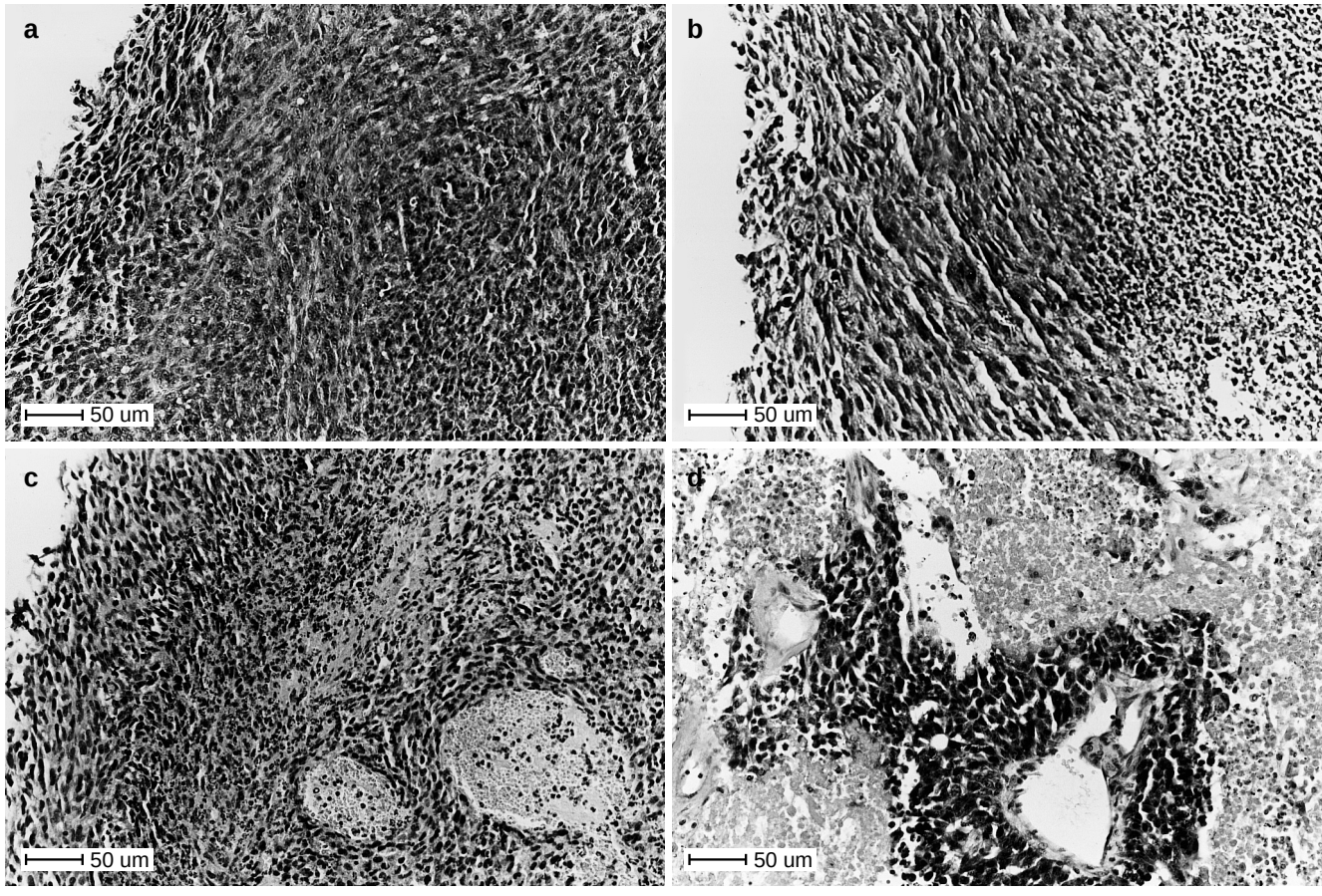
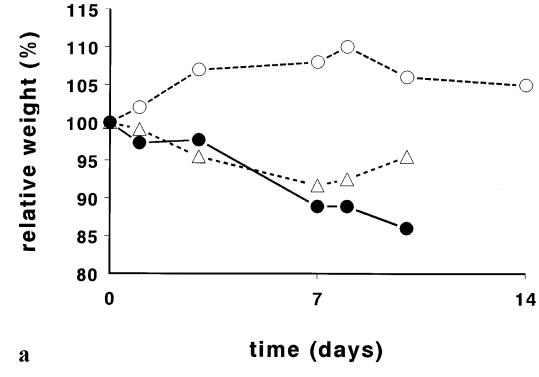


Fig. 1a–d Histological examples (HE staining) of **a** Colon 26-A, **b** Colon 26-B, **c** Colon 26-10, and **d** Colon 38. Note that the amount of necrosis between Colon 26-A and Colon 26-B differs. The adenocarcinoma Colon 38 is well differentiated, but the Colon 26 subtypes are poorly differentiated carcinomas as described by Corbett et al. [15, 16] and Van Kraanenburg-Voogd et al. [44]

(Fig. 1). Colon 38 is a well-differentiated adenocarcinoma as described by Corbett et al. [15, 16]. This tumor has the largest amount of necrosis (> 80%). All Colon 26 tumors are poorly differentiated. Colon 26-A does not show necrosis; both Colon 26-10 and Colon 26-B show some extent of necrosis, with Colon 26-B showing a somewhat higher extent (Table 1, Fig. 1).

Effect of tumor origin on life span and weight loss

In Table 1 the histological characteristics and features of the tumors studied are summarized. In Fig. 2,



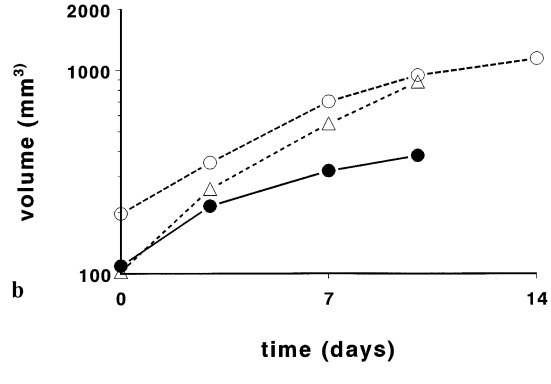


Fig. 2a Relative weight changes and **b** absolute tumor volumes determined in untreated Colon 26-subtype-bearing mice. Data represent mean values for 3–7 experiments. Graphs start at 10 days after transplantation and are continued to the MLS. SD values are omitted for purposes of legibility but remain with a 20% limit. (*black circles* Colon 26-A, *white triangle* Colon 26-B, *white circles* Colon 26-10.) Note that mice bearing Colon 26-A ultimately tolerate a lower tumor load than mice bearing Colon 26-B, but they show a 2-fold greater weight loss

growth characteristics and tumor-induced cachexia from all Colon 26 subtypes are shown. Figure 2a illustrates that mice inoculated with Colon 26-B or Colon 26-10 could tolerate larger tumors than could mice inoculated with Colon 26-A, which developed more severe cachexia and died as a consequence (Fig. 2b). At the time of death, each mouse bearing Colon 26-A lost about 20% of its initial weight (data of these individual mice are not shown), whereas mice bearing Colon 26-B or Colon 26-10 tolerated much larger tumor volumes and had to be euthanized only when tumor diameters reached the permitted 2 cm. The 14% weight loss shown by Colon 26-A-bearing mice at day 10 indicates that not all mice died at the MLS. At this point, Colon 26-10 did not induce any weight loss and mice with Colon 26-B tumors lost only a mean of 8.5% of their initial weight. In addition, when Colon 26-B tumors had reached a volume of 400 mm^3 (the volume at which Colon 26-A-bearing mice generally died), the weight loss in these mice did not exceed 5% (data not shown). At this point, mice bearing Colon 26-A were close to anticipated death and were euthanized before they could die of tumor-induced cachexia.

Effect of tumor size on TD, the antitumor activity of FUra, and modulation with LV

To relate tumor size with therapy failure, we retrospectively analyzed the results of experiments performed previously [7, 9, 23, 24, 26–31, 37–39]. At day 0, when treatment started, volumes of large Colon 26-A, Colon 26-B, and Colon 38 tumors were 273, 361, and 457 mm^3, respectively. Tumor volumes in the normal-sized groups were about 100–125 mm^3. The growth behavior of large tumors differed from that of normal-sized tumors; a decline of up to 50% of the normal growth rate was seen in both Colon 26 and Colon 38. A lower deceleration of growth rate was observed in treated groups with large tumors (Table 2; Figs. 2, 3). In terms of GDF and T/C, all groups of mice with normal-sized tumors responded better than those with large tumors (Table 2). Mice treated with FUra showed a dose-dependent response in large Colon 38 tumors that was with the results of compiled experiments and the trend seen in previous studies [23, 28, 30, 31].

In the compiled group of Colon 38 and Colon 26-A tumors with a normal volume the addition of LV of FUra improved all the antitumor parameters (Table 2;

Table 2 Antitumor activity of FUra ± LV on normal (*N*) and large (*L*) murine colon carcinomas. Data represent mean values ± SD (*NS* Not significant)

Tumors (*n*)	Treatment (size)[a]	TD (days)	GDF (mean/median)	T/C in % (day)
Colon 26-A:				
207	– (N)	$3.0 \pm 0.8^{*4}$		
35	– (L)	4.5 ± 1.7		
107	FUra 100 (N)	$5.2 \pm 1.6^{*4}$	$0.7^{NS}/0.8$	63^{*3}(7), 43(10)
22	FUra 100 (L)	9.2 ± 3.5	1.0/1.1	76(7)
106	LV-FUra 100 (N)	10.9 ± 3.8^{NS}	$2.6^{*4}/3.2$	46^{*4}(7), 31(10)
11	LV-FUra 100 (L)	10.4 ± 4.2	1.2/0.9	63(7)
Colon 26-B:				
101	– (N)	$2.9 \pm 1.1^{*4}$		
22	– (L)	4.7 ± 1.2		
15	FUra 80 (N)	7.5 ± 4.6^{NS}	$1.6^{NS}/1.1$	28^{*1}(10)
5	FUra 80 (L)	8.7 ± 4.4	0.9/0.7	54(10)
Colon 38:				
115	– (N)	$4.9 \pm 1.1^{*4}$		
41	– (L)	7.9 ± 3.1		
47	FUra 60 (N)	$16.3 \pm 2.6^{*2}$	$2.3^{*4}/2.5$	18^{*4}(17)
22	FUra 60 (L)	19.3 ± 6.7	1.4/1.6	27(17)
57	FUra 100 (N)	26.5 ± 1.2^{NS}	$4.4^{*4}/4.3$	14^{*4}(17)
28	FUra 100 (L)	24.0 ± 5.2	2.0/2.0	19(17)
72	LV-FUra 100 (N)	$31.7 \pm 1.9^{*4}$	$5.5^{*4}/5.4$	8^{*4}(17)
16	LV-FUra 100 (L)	26.4 ± 6.5	2.3/2.6	18(17)

$^{*1}P < 0.05$; $^{*2}P < 0.02$, $^{*3}P < 0.01$, $^{*4}P < 0.001$. Additional calculations: FUra 100 (N) as compared with LV-FUra 100 (N), $P < 0.001$ for all parameters in both Colon 26-A and Colon 38; FUra 100 (L) as compared with LV-FUra 100 (L), NS for all parameters in Colon 26-A and Colon 38. Colon 38: FUra 60 (N) as compared with FUra 100 (N)–TD $P < 0.01$, GDF $P < 0.02$, T/C $P < 0.05$; FUra 60 (L) as compared with FUra 100 (L) – TD, GDF, T/C $P < 0.001$

[a] (*N*) Tumor volume of 50–200 mm^3, (*L*) tumor volume of $> 200\ mm^3$. Each specific antitumor parameter of the normal-sized group is compared with the corresponding parameter of larger tumors. For example, the T/C of FUra 100 (N) is compared with that of FUra 100 (L)

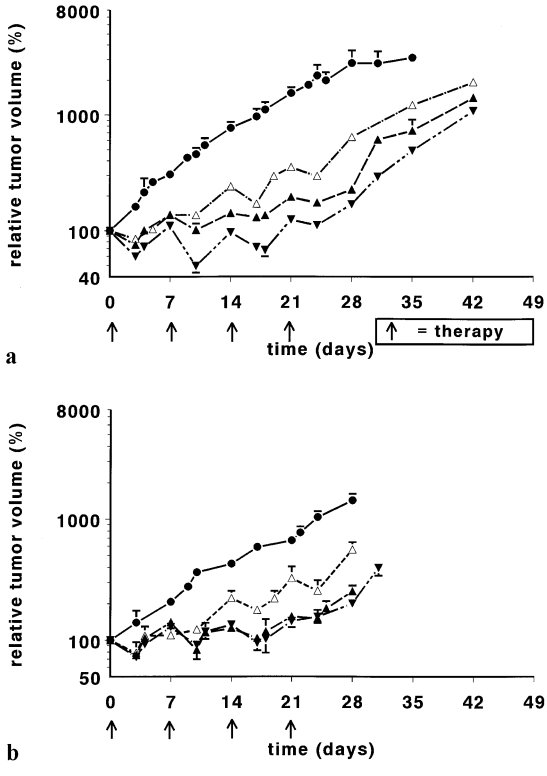


Fig. 3a, b Antitumor activity of weekly $\times 4$ FUra $\pm$ LV given to mice with **a** normal and **b** large Colon 38 tumors. Graphs are continued to the MLS, values are expressed $\pm$ SEM and might therefore not be visible, and numbers can be derived from Table 2. (*Black circle* Untreated, *white triangles* 60 mg/kg FUra, *black triangels* 100 mg/kg FUra, *black inverted triangles* 50 mg/kg LV given at 1 h before and with 100 mg/kg FUra.) Normal-sized tumors: 100 mg/kg FUra as compared with 100 mg/kg FUra + LV at days 7 and 42 $P < 0.05$, days 3 and 14 $P < 0.01$, other days $P < 0.001$. FUra 100 mg/kg as compared to 60 mg/kg at days 3 and 7 $P > 0.05$ (not significantly different = NS), day 10 $P < 0.05$, day 17 $P < 0.01$, other days $P < 0.001$. Large tumors: FUra as compared with FUra + LV = NS all days. 100 mg/kg FUra as compared with 60 mg/kg at days 10, 14, and 28 $P < 0.01$, day 21 $P < 0.05$, day 24 $P < 0.02$, day 17 $P < 0.001$

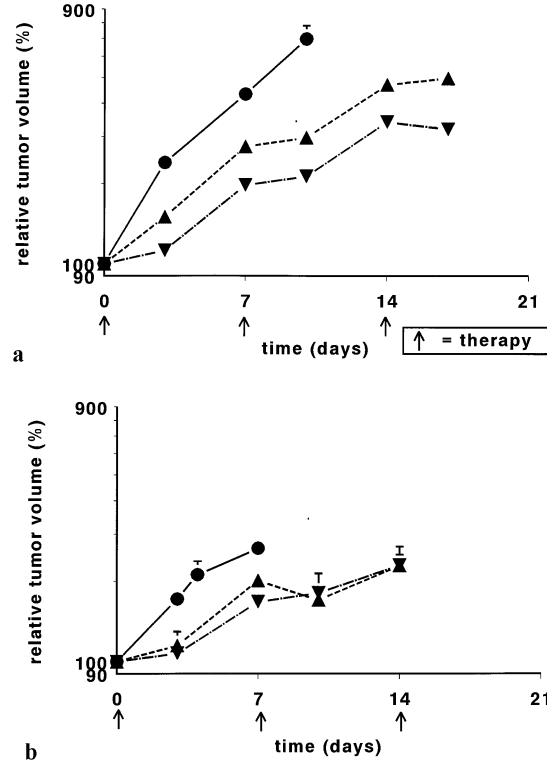


Fig. 4a, b Antitumor activity of weekly $\times 4$ FUra $\pm$ LV given to mice with **a** normal and **b** large Colon 26-A tumors. Graphs are continued to the MLS and values are expressed $\pm$ SEM. (*Black circles* Untreated, *black triangles* 100 mg/kg FUra, black inverted triangles 50 mg/kg LV given at 1 h before and together with 100 mg/kg FUra.) Normal-sized tumors: FUra + LV significantly ($P < 0.001$) better than FUra alone; large tumors: no significant difference

Figs. 3a, 4a) as previously describe in separate experiments [7, 23, 24, 39]. However, the modulating effect of LV on FUra therapy was not seen when tumors were larger than 200 mm^3; all antitumor parameters of the FUra + LV group were the same as those determined for FUra alone (Table 2; Figs. 2b, 3b).

Effect of tumor origin on antitumor activity and LV modulation

These data derive from normal-sized tumors. Of the Colon 26 subtypes, only Colon 26-A and Colon 26-10 were sensitive to LV modulation (Tables 2, 3). The data from various experiments conducted over an 8-year period were compiled and are shown in Fig. 4a. In this figure the antitumor effect of FUra against Colon 26-A was significantly improved by LV ($P < 0.001$). Also, in the separate published experiments performed over an 8-year period, these differences were reproducible and significant [7, 23, 24, 39]. The MTD of FUra determined in Buffalo was slightly lower (80%) than that established in Amsterdam. Because a dose dependency might be causing a lack of modulation of the antitumor activity of FUra by LV in mice bearing Colon 26-B (T/C at day 11 of 0.31 and 0.35 for FUra with and without the addition of LV, respectively), we transported Colon 26-B to Amsterdam. In Dutch laboratories we could give 100 mg/kg FUra to Colon 26-B bearing mice. However, also under these circumstances an antitumor effect of FUra could not be enhanced significantly by LV (Table 3). The antitumor effect of 100 mg/kg FUra could also be enhanced by LV in mice with Colon 38 tumors (Table 2, Fig. 3a).

All Colon 26 subtypes were relatively resistant to FUra, except for Colon 26-10, in which CRs of even up to 50% were seen. FUra given to mice with Colon 38 and Colon 26-A tumors (data from [7, 27, 28, 30]) showed a dose-dependent response (Table 2, Fig. 3). No CR was observed when 60 mg/kg FUra was used. Only higher doses of FUra (100 mg/kg) $\pm$ LV could achieve CRs in Colon 38-bearing mice (12.5% and 15%, respectively).

Table 3 Antitumor activity of FUra ± LV in various murine Colon 26 ademocarcinomas. Data represent mean values ± SD (*NS* Not significant, NE not evaluable since tumors did not regrow)

Tumor	Therapy[a]	TD (days)[b]	GDF[c]	T/C (%)[d]	*n*[e]	CR (%)[f]
Colon 26-A	–	3.0 ± 0.8			207	0
	FUra 100	5.2 ± 1.6	0.7/0.8	43 ± 19 (10)	102	0
	LV-FUra 100	10.9 ± 3.8*	2.6*/3.2	31 ± 11 (10)*	106	0
Colon 26-B[g]	–	3.4 ± 0.8			10	0
	FUra 100	6.1 ± 1.7	0.8/0.9	22 ± 11 (11)	12	0
	LV-FUra 100	9.8 ± 5.2[NS]	1.9[NS]/1.7	15 ± 5 (11)[NS]	13	0
Colon 26-10	–	4.1 ± 2.6			10	0
	FUra 100	NE	NE	9.5 ± 5 (10)	12	100
	LV-FUra 100	NE	NE	2.3 ± 3 (10)*	12	100

*$P < 0.001$
[a] Doses are expressed in mg/kg
[b] Tumor-doubling time
[c] Growth-delay factor; mean and median values are given
[d] Maximal T/C values are given in parentheses; day after the start of treatment
[e] Total number of tumors analyzed
[f] Complete response (in only 1 experiment where FUra and FUra + LV were compared in Colon 26-10. The CR rate of single-agent FUra was 53% in 4 subsequent experiments, total $n = 48$)
[g] Colon 26-B treated in Amsterdam. Colon 26-B in Buffalo: TD of 80 mg/kg FUra ± LV, 8.4 and 8.0 days, respectively. Modulation of FUra by LV was analyzed per tumor subtype

Table 4 Antitumor activity of CDDP in various murine Colon 26 carcinomas. Mice were treated with 9 mg/kg CDDP at weekly intervals. Data represent mean values ± SD (*CR* complete response, *NE* not evaluable)

Tumor	TD (Days)[a]	GDF (median)	T/C %[b] (day)	*n*[c]	CR (%)
Colon 26-A	33.6 ± 3.8*	8.3*	17 ± 10* (10)	12	25
Colon 26-B[d]	6.9 ± 4.7	0.8	37 ± 17 (10)	13	0
Colon 26-10	NE[e]	NE	13 (14)	12	50
Colon 38	33 ± 2.1	3.8	4 (21)	6	40

*$P < 0.001$ versus Colon 26-B in Amsterdam
[a] Tumor-doubling time
[b] Maximal percentage of T/C
[c] Total number of tumors analyzed
[d] Colon 26-B treated in Amsterdam
[e] Not evaluable as insufficient numbers of treated tumors doubled their initial volume

Effect of tumor origin on CDDP sensitivity

CDDP was included in these experiments, since it had previously been shown that if the right dose and schedule are given, the compound can be active [9, 16, 31, 37] in colon cancer [11]. The experiments with CDDP showed marked differences in the CDDP sensitivity of the three Colon 26 subtypes, which initially derived from the same source (Table 4). Colon 26-10 was the most CDDP-sensitive tumor, followed by Colon 38. In all, 25% of all mice with Colon 26-A tumors could be cured, whereas no CR was observed in mice bearing Colon 26-B. Also, assessment by GDF and T/C max revealed that Colon 26-A was more sensitive than Colon 26-B to CDDP. To exclude circumstantial influences such as the dissolving of drugs, mouse breed, or food, Colon 26-B was tested in The Netherlands. Although a variation in CDDP sensitivity in these two experiments with Colon 26-B was seen, Colon 26-B remained significantly less sensitive to CDDP than the other two Colon 26 subtypes (Table 4).

Effect of tumor origin on TS activity

The differences in response to LV modulation of FUra observed between various tumors might be explained by differences in the initial TS levels. To delineate the outcome of these experiments we included the TS activities of Colon 26-A and Colon 38 (tumors in which the antitumor activity of FUra could be modulated by LV and that are resistant and sensitive to FUra, respectively [7, 23, 24, 39]) and related them to the TS levels of Colon 26-B and Colon 26-10.

No significant variation in the number of free FdUMP-binding sites was observed among the three different subtypes of Colon 26 (Table 5). This might indicate that the number of FdUMP-binding sites is not an important parameter of the failure of LV modulation in Colon 26-B. In Colon 38, lower numbers of FdUMP-binding sites were found, which might partly explain its sensitivity to FUra therapy and the successful LV modulation of FUra. The catalytic activity of TS was 2 times higher in Colon 26-B as compared

Table 5 FdUMP binding and TS catalytic activity in different murine colon carcinomas. Data represent mean values ± SD for 3–8 tumors (*ww* wet weight)

Tumor	FdUMP binding (fmol/mg ww)	TS catalytic activity (pmol/mg protein^{-1} h^{-1})[a]		Ratio TSCA/FB[b]	Ratio 10/1[c]
		1 μ*M* dUMP	10 μ*M* dUMP		
Colon 26-A [39]	126 ± 55	935 ± 337	2324 ± 720	18.4	2.5
+ 10 n*M* FdUMP		249 ± 144 (27)	1267 ± 547 (55)		5.1
Colon 26-10	90 ± 8	922 ± 180	2852 ± 339	31.7	3.1
+ 10 n*M* FdUMP		229 ± 28 (25)	1597 ± 173 (56)		7.0
Colon 26-B	122 ± 33	1744 ± 127	5536 ± 387	45.4	3.2
+ 10 n*M* FdUMP		271 ± 70 (16)	2745 ± 307 (50)		10.1
Colon 38 [39]	54 ± 5	335 ± 138	799 ± 228	14.3	2.4
+ 10 n*M* FdUMP		49 ± 19 (14)	376 ± 104 (47)		7.7

[a] Numbers in parentheses represent percentages of control values
[b] Ratio of TS catalytic activity at 10 μ*M* dUMP/FdUMP binding
[c] Ratio of TS catalytic activity at 10 versus 1 μ*M* dUMP

with Colon 26-A and Colon 26-10 at both low (nonsaturated) and high (saturated) dUMP concentrations. The ratio of catalytic activity to free FdUMP-binding sites was calculated to determine whether enzymic properties of TS also differ among the tumors. This ratio was again significantly higher in Colon 26-B (Table 5). The possibility that protein concentration in tumors would account for the difference was ruled out as these were comparable for all tumors (data not shown).

The catalytic activity detected in Colon 38 tumors was much lower than that observed in the different Colon 26 subtypes. This is possibly related to the sensitivity FUra and LV-modulation of Colon 38. The ratio of TS catalytic activity at 10 versus 1 μ*M* dUMP was calculated to demonstrate possible variations in enzyme kinetics. In the case of a high Michaelis constant (K_m), a lower ratio would be found. This ratio, however, was comparable for all tumors. The inhibition of TS activity that could be achieved by the addition of 10 n*M* FdUMP in the assay was comparable with respect to absolute TS activity for Colon 26 tumors. Indeed, the K_i values found for FdUMP among the four different tumors were in the same range, varying from 1 to 2.5 n*M* FdUMP. In Colon 26-B tumors the TS catalytic activity could be decreased to 16% by 10 n*M* FdUMP but remained the highest value found among all tumors analyzed. This accounted for the slightly higher ratio found between inhibited TS catalytic activity at 10 and 1 μ*M* dUMP in Colon 26-B. The ratios mentioned were comparable with those obtained in previous experiments and with those found in human colorectal cancer [7, 9, 39, 45] and indicate that enzyme kinetics did not vary significantly.

Discussion

The present study shows that a large tumor size negatively influences the therapeutic efficacy of FUra modulation by LV in murine tumor models. Furthermore, subtypes of Colon 26 that derived from the same source developed variable toxic effects in mice and showed distinct variability in the tumor response to FUra modulation by LV.

Tumor mass has been associated with resistance to chemotherapy [13, 14]. Vascularity, cell-kinetic changes, and genetic mutations are held responsible for these phenomena. The reduced growth rate of all large tumors might be held responsible for some of the observations in this study, but it is clear that other factors must cause the decreased sensitivity of FUra modulation by LV. Drug availability in the tumor center could be decreased in large tumors. For instance, in the center of large tumors, increased intenstitial pressure gradients cause radial fluid flow from the center to the periphery [14]. As a consequence, larger molecules such as LV might have difficulties in reaching central tumor cells, possibly explaining the lower modulation achieved in large tumors. Significant FUra uptake in Colon 26-A has been observed by Visser et al. [40]. The presence of FUra has also been demonstrated in the center of Colon 38 tumors [32], although at lower levels than in the viable rim of tumors. Since the mechanism of LV uptake differs from the of FUra [48–50], the smaller FUra molecule would reach the tumor center better than LV; thus, the efficacy of modulation could be diminished in large tumors. This phenomenon might be supported by the observation that the dose-dependent relationship of FUra persisted in large tumors, whereas the LV modulation had disappeared in large tumors.

Analysis of the tumor characteristics of three Colon 26 subtypes indicated marked differences in the present study, the most pronounced being tumor-induced cachexia and the amount of necrosis. Different characteristics for a Colon 26 variant have been published by Tanaka et al. [35, 36], who investigated weight loss and cachexia in mice bearing this Colon 26 tumor. This group found Colon 26 metastasis to be accountable for tumor-induced cachexia without anorexia. The

cachexia was associated with wasting of muscle and adipose tissue accompanied by hypoglycemia, hypercorticism, and disorders of hepatic function. As compared with age-matched controls, these ($CD2F_1$) mice showed about 30% loss of weight associated with tumors of approximately 6 g from 28 days until death at 45 days after inoculation of 10^6 parent Colon 26 cells.

In our experiments, Colon 26-A-bearing mice lost about 20% of their initial weight at the time just before anticipated death at a median of 20 days after tumor implantation, when tumors weighed about 500 mg each. The difference in MLS as compared with that reported by Tanaka et al., can be related to the difference in mouse strain, as BALB/c mice were used in our study. These mice did not tolerate more than 1 g of total Colon 26-A burden. Another factor might be that the cell lines used by Tanaka et al. [35, 36] had developed other genetic mutations and therefore lost some metastatic features. Corbett et al. [15–17] implanted 10^7 cells in BALB/c mice and the MLS ranged from 27 to 35 days. Our data are more consistent with those observations, as the additional period required to develop a solid tumor from a cell suspension will also cause a time delay. The differences in MLS could also be explained by an alteration of tumor characteristics. The SRI group of Corbett [16] described Colon 26 as being highly invasive and metastatic with $> 90\%$ lung involvement, whereas Colon 38 was a lowly metastatic ($< 5\%$ lung metastasis) and less invasive tumor. Table 1 and Fig. 1 reveal less weight loss and relatively long survival for mice with Colon 38, Colon 26-10, and Colon 26-B tumors. For mice with those tumors the absence of weight loss might be explained by acquired lower metastatic potential or by lower induction of interleukin 1 (IL-1) or tumor necrosis factor (TNF), factors known to cause cachexia [51]. Colon 26-10 was selected by creation of a cell line from a solid tumor; cells capable of surviving in vitro cell-growth conditions might not have the metastatic abilities of their parent Colon 26 cells.

An explanation for the difference in FUra sensitivity of the various Colon 26 variants might be related to differences in uptake possibly influenced by cachexia. Differences in [^{18}F]-FUra uptake had previously been reported for Colon 26 and Colon 38 [40]. Although the distribution of FUra differs within different mouse strains, this cannot explain the variation of responses seen among the Colon 26 subtypes, as all of these were transplanted into BALB/c mice. In recent experiments comparing Colon 26-A and Colon 26-10, both tumors were transplanted into the same mouse [52]. The same sensitivity profile and drug distribution was observed for both tumors, whether the tumors were in the same mouse or in different mice. From this observation we can conclude that during the treatment period, Colon 26-A (which causes cachexia) did not affect drug uptake and efflux in Colon 26-10.

The exact degree of metastasis in Colon 26-10 is unknown, but it is low, as we did not observe weight loss or tumors in other organs such as the lungs or the liver. Colon 26-B is another alternation or selection of Colon 26 that is intermediate to Colon 26-A and Colon 26-10 in terms of causing weight loss. It is known that the phenotypic and genotypic characteristics of Colon 26 can alter, depending on the site of metastasis, the metastatic potential, and culture under serum-free conditions [34, 43]. Yusa and co-workers [43] also described that the metastatic potential of a variant of Colon 26 could be increased by transfection of the activated *c-erbB-2* gene and that tumors would show different morphological characteristics after this transfection. Variations observed in Colon 26-10 and Colon 26-B might be caused by mutation(s) from the original tumor (Colon 26-A).

The potential of Colon 26 to develop variants has been described by Sugimoto et al. [34], who correlated in vitro serum-free cell-culture conditions with the development of Colon 26 variants. Our study described the development of new tumor subtypes between two Colon 26 tumors that originated from one source but were maintained at laboratories on a different continent (Table 1). Also, we describe the development of a new tumor subtype (Colon 26-10) as a result of maintenance of tumor cells as a single-cell suspension followed by retransplantation. Both Colon 26 subtypes (Colon 26-10 and Colon 26-B) have more necrosis and fewer cachexic features than the parent tumor. In this sense, Colon 26 tumors might be regarded as a family of heterogeneous tumors. The differences in histology observed between Colon 26-A and Colon 26-B might add support to this conclusion. Van Kraanenburg-Voogd and co-workers [44] observed a change in Colon 26 histology (from an undifferentiated carcinoma to an undifferentiated carcinoma with local fibrosarcoma), changes in growth characteristics (the TD was slightly increased), and changes in sensitivity to drugs (lomustine was found to be active, whereas it proved to be inactive at SRI). In our study, Colon 26-A and Colon 26-B were growing at the same rate as they did in Van Kraanenburg-Voogd et al.'s studies [44]. The response to CDDP was reported in 1975 to be high in Colon 26, but responses were moderate in our experiments with Colon 26-A and low in those with Colon 26-B. Also, the variations in the response to LV modulation of FUra in Colon 26-A and Colon 26-B described in this study might indicate that this tumor line can change when maintained in different laboratories.

Furthermore, the level of TS catalytic activity was about twice as high in Colon 26-B, which might explain the lack of modulation by LV, since more folate cofactor would be required to form the stable ternary complex between TS and FdUMP. The reason for this resistance remains unclear but might involve mutations in Colon 26, resulting in higher TS catalytic activity, which has also been observed in tumors treated with

FUra [39]. The relationship between levels of TS catalytic activity and the response to FUra in this study is not clear. Apparently there is a threshold above which high or very high levels do not determine differences in antitumor activity. In addition, Peters et al. [5, 45] have observed marked heterogeneity of TS levels detected in colorectal tumors of untreated versus FUra treated patients and have related these observations to the antitumor activity of FUra. The observations of necrosis in Colon 26-B and, to a lower extent, in Colon 26-A might be related to the difference in sensitivity, since vascularization enables drugs to penetrate the tumor tissue. Moreover, high thymidine phosphorylase (TP) levels are seen in Colon 26-A [26]. Since TP, also known as epithelial growth factor, is associated with angiogenesis [53], Colon 26-A is likely to be well vascularized.

The observations that large-volume colon tumors show resistance to modulation of FUra by LV have not previously been described. Therefore, these observations might lead to a new approach in the treatment of very large colon tumors; rather than a dose reduction of FUra with the addition of LV, the highest possible dose of FUra might yield better therapeutic efficacy. Higher doses of FUra can be given when it is combined with uridine [24, 28], which protects against both hematological and gastrointestinal toxicity. Our observations on the differences between tumors and the effect of tumor load have implication for preclinical studies, since experiments may not be reproducible in different laboratories. However, these tumor models remain important for the development of new drug strategies and for mechanism studies. Actually, that one tumor model has different variants makes it eminently suitable for the study of different aspects of drug development, e.g., the implantation of two different tumors in one mouse [52]. Experiments should be initiated when the tumor volume reaches a range of 50–200 mm^3 and passages should be performed for a limited number of times to avoid the development of new variations. The question as to whether TS activity in untreated tumors can predict the success rate of chemotherapy cannot totally be answered by the results of this study. The finding, however, that modulation of FUra by LV diminished in tumors with high TS catalytic activity might be of prognostic value and should be evaluated clinically.

References

1. Peters GJ, Van Groeningen CJ (1991) Clinical relevance of biochemical modulation of 5-fluorouracil. Ann Oncol 2:469–480
2. Moertel CG (1994) Drug therapy – chemotherapy for colorectal cancer. N Engl J Med 330:1136–1142
3. Spiegelman S, Sawyer RC, Nayak R, Ritzi E, Stolfi RI, Martin DS (1980) Improving the antitumor activity of 5-fluorouracil by increasing its incorporation into RNA via metabolic modulation. Proc Natl Acad Sci USA 77:4866–4970
4. Santi, DV, McHenry CS, Sommer H (1974) Mechanism of interaction of thymidylate synthetase with 5-fluorodeoxyuridine. Biochemsitry 13:471–481
5. Peters GJ, Van der Wilt CL, Van Groeningen CJ, Smid K, Meijer S, Pinedo HM (1994) Thymidylate synthase inhibition after administration of fluorouracil with or without leucovorin in colon cancer patients: implications for treatment with fluorouracil. J Clin Oncol 12:2035–2042
6. Houghton JA, Adkins DA, Rahman A, Houghton PJ (1992) Interaction between 5-fluorouracil, [6RS]leucovorin combinations and recombinant human interferon-α_{2a} in cultured colon adenocarcinoma xenografts. Cancer Chemother Pharmacol 30:423–432
7. Van der Wilt CL, Peters GJ (1994) New targets for pyrimidine antimetabolites for the treatment of solid tumors. I. Thymidylate synthase. Pharm World Sci 16:84–103
8. Scanlon KJ, Newman EM, Lu Y, Priest DG (1986) Biochemical basis for cisplatin and 5-fluorouracil synergism in human ovarian carcinoma cells. Proc Natl Acad Sci USA 83:8923–8925
9. Van der Wilt CL, Van Laar JAM, Gyergyay F, Smid K, Peters GJ (1992) Biochemical modification of the toxicity and the anti-tumor effect of 5-fluorouracil and cis-platinum by WR-2721. Eur J Cancer 28A:2017–2014
10. Patt YZ, Boddie AW, Charnsangavej C, Ajani JA, Wallace S, Soski M, Claghorn L, Mavligit GM (1986) Hepatic arterial infusion with floxuridine and cisplatin: overriding importance of antitumor effect versus degree of tumor burden as determinants of survival among patients with colorectal cancer. J Clin Oncol 14:1356–1364
11. Sagaster P, Essel R, Teich G, Fritz E, Wasilewski M, Umek H, Dünser U, Mascher H, Micksche M (1994) Treatment of advanced colorectal cancer with folinic acid and 5-fluorouracil in combination with cisplatinum. Eur J Cancer 30A:1250–1254
12. Levi F (1994) Chronotherapy of cancer – biological basis and clinical application. Pathol Biol 342:3338–3341
13. De Vita VT (1983) The relationship between tumor mass and resistance to chemotherapy. Cancer 51:1209–1220
14. Jain RC (1989) Delivery of novel therapeutic agents in tumors: physiological barriers and strategies. J Natl Cancer Inst 81:570–576
15. Corbett TH, Griswold DP, Roberts BJ, Peckham JC, Schabel FM (1975) A mouse colon-tumor model for experimental therapy. Cancer Chemother Rep 5:169–185
16. Corbett TH, Griswold DP, Roberts BJ, Peckham JC, Schabel FM (1977) Evaluation of single agents and combinations of chemotherapeutic agents in mouse colon carcinomas. Cancer 40:2660–2680
17. Corbett TH, Bissery M, Mucci-Lo Russo P, Polin L (1991) 5-Fluorouracil containing combinations in murine tumor systems. Invest New Drugs 7:37–49
18. Eda H, Tanaka T, Ishitsuka H (1991) 5′-Deoxy-5-fluorouridine improves cachexia by a mechanism independent of its antiproliferative action in colon 26 adenocarcinoma-bearing mice. Cancer Chemother Pharmacol 29:1–6
19. Iigo M, Nishikata K, Hoshi A (1992) In vivo antitumor effects of fluoropyrimidines on colon adenocarcinoma 38 and enhancement by leucovorin. Jpn J Cancer Res 83:392–396
20. Kamm YJL, Rietjes IMCM, Vervoort J, Heerschap A, Rosenbusch G, Hofs HP, Wagener DJT (1994) Effect of modulators on 5-fluorouracil metabolic patterns in murine colon carcinoma determined by in vitro ^{19}F nuclear magnetic resonance spectroscopy. Cancer Res 54:4321–4326
21. Klohs WD, Steinkampf RW (1988) Possible link between the intrinsic drug resistance of colon tumors and a detoxification mechanism of intestinal cells. Cancer Res 48:3025–3030
22. Martin DS, Stolfi RL, Sawyer RC, Casper ES, Young CW (1983) Therapeutic utility of utilizing low doses of *N*-(phosphonacetyl)-L-aspartic acid in combination with 5-fluorouracil: a murine study with clinical relevance. Cancer Res 43:2317–2321

23. Nadal JC, Van Groeningen CJ, Pinedo HM, Peters GJ (1988) In vivo potentiation of 5-fluorouracil by leucovorin in murine colon carcinoma. Biomed Pharmacother 42 : 387–393
24. Nadal JC, Van Groeningen CJ, Pinedo HM, Peters GJ (1989) Schedule-dependency of in vivo modulation of 5-fluorouracil by leucovorin and uridine in murine colon carcinoma. Invest New Drugs 7 : 163–172
25. Nord LD, Martin DS (1991) Loss of murine tumor thymidine kinase activity in vivo following 5-fluorouracil (FUra) treatment by incorporation of FUra into RNA. Biochem Pharmacol 42 : 2369–2375
26. Peters GJ, Braakhuis BJM, De Bruyn EA, Laurensse EJ, Van Walsum M, Pinedo HM (1989) Enhanced therapeutic efficacy of 5′deoxy-5-fluorouridine in 5-fluorouracil resistant head and neck tumors in relation to 5-fluorouracil metabolising enzymes. Br J Cancer 59 : 327–334
27. Peters GJ, Van Dijk J, Nadal JC, Van Groeningen CJ, Lankelma J, Pinedo HM (1987) Diurnal variation in the therapeutic efficacy of 5-fluorouracil against murine colon cancer. In Vivo 1 : 113–118
28. Peters GJ, Van Dijk J, Laurensse E, Van Groeningen CJ, Lankelma J, Leyva A, Nadal J, Pindeo HM (1988) In vitro biochemical and in vivo biological studies of uridine "rescue" of 5-fluorouracil. Br J Cancer 57 : 259–265
29. Peters GJ, Nadal JC. Laurensse EJ, De Kant E, Pinedo HM (1990) Retention of in vivo antipyrimidine effects of brequinar sodium (DUP-785; NSC368390) in murine liver, bone marrow and colon cancer. Biochem Pharmacol 39 : 135–144
30. Peters GJ, Kraal I, Pinedo HM (1992) In vitro and in vivo studies on the combination of brequinar sodium (DUP-895; NSC 368390) with 5-fluorouracil; effects of uridine. Br J Cancer 65 : 229–233
31. Peters GJ, Van der Wilt CL, Gyergyay F, Van Laar JAM, Treskes M, Van der Vijgh WJF, Pinedo HM (1992) Protection by WR-2721 of the toxicity induced by the combination of cisplatin and 5-fluorouracil. Int J Radiat Oncol Biol Phys 22 : 785–789
32. Peters GJ, Lankelma J, Kok RM, Noordhuis P, Van der Wilt CJ, Van Groeningen CJ, Meijer S, Pinedo HM (1993) Long retention of high concentrations of 5-fluorouracil in human and murine tumors compared to plasma. Cancer Chemother Pharmacol 31 : 269–276
33. Spears CP, Shahinian AH, Moran RC, Heidelberger C, Corbett TH (1982) In vivo kinetics of thymidylate synthetase inhibition in FUra-sensitive and -resistant murine colon adenocarcinomas. Cancer Res 42 : 450–456
34. Sugimoto Y, Oh-Hara T, Heike Y, Watanabe M, Nakatsuru Y, Ishiwaka T, Tsuruo T (1990) Establishment of highly metastatic variants of murine colon adenocarcinoma 26 under serum-free conditions. Int J Cancer 46 : 118–124
35. Tanaka Y, Eda E, Fujimoto K, Ishiwaka T, Ishitsuka H (1990) Anticachectic activity of 5′-deoxy-5-fluorouridine in a murine tumor cachexia model, colon 26 adenocarcinoma. Cancer Res 50 : 4528–4532
36. Tanaka Y, Eda H, Tanaka T, Udagawa T, Ishikawa T, Horii I, Ishitsuka H, Kataoka T, Taguchi T (1990) Experimental cancer cachexia induced by transplantable Colon 26 adenocarcinoma in mice. Cancer Res 50 : 2290–2295
37. Van Laar JAM, Van der Wilt CL, Treskes M, Van der Vijgh WYF, Peters GJ (1992) Effect of WR-2721 on the toxicity and antitumor activity of the combination of carboplatin and 5-fluorouracil. Cancer Chemother Pharmacol 31 : 97–102
38. Van Laar JAM, Durrani F, Rustum YM (1993) Antitumor activity of the weekly iv push schedule of 5-fluoro-2′-deoxyuridine ± *N*-phosphonacetyl-L-aspartate in mice bearing advanced colon carcinoma 26. Cancer Res 53 : 1560–1564
39. Van der Wilt CL, Pinedo HM, Smid K, Peters GJ (1992) Elevation of thymidylate synthase following 5-fluorouracil treatment is prevented by the addition of leucovorin in murine colon tumors. Cancer Res 52 : 4922–4928
40. Visser GWM, Gorree GCM, Peters GJ, Herscheid JDM (1990) Tissue distribution of [^{18}F]-5-fluorouracil in mice: effects of route of administration, strain tumor and dose. Cancer Chemother Pharmacol 26 : 205–209
41. Weiss L, Ward PM (1988) Effects of metastatic cascades on metastatic patterns: studies on colon-26 carcinomas in mice. Int J Cancer 41 : 450–455
42. Winograd B, Boven E, Lobbezoo MW, Pinedo HM (1987) Tumor xenografts in the nude mice and their value as test models in anticancer drug development. In Vivo 1 : 1–14
43. Yusa K, Sugimoto Y, Yamori T, Yamamoto T, Toyoshima K, Tsuruo T (1990) Low metastatic potential of clone from murine colon 26 increased by transfection of activated c-erbB-2 gene. J Natl Cancer Inst 82 : 1633–1636
44. Van Kraanenburg-Voogd PJ, Keizer HJ, Van Putten LM (1978) Experimental chemotherapy of transplantable mouse colon tumors. Eur J Cancer 14 [Suppl]: 153–157
45. Peters GJ, Van Groeningen CJ, Laurensse E, Pinedo HM (1991) Thymidylate synthase from untreated human colorectal cancer and colonic mucosa: enzyme activity and inhibition by 5-fluoro-2′-deoxyuridine-5′-monophosphate. Eur J Cancer 27 : 263–267
46. Tomayko MM, Reynolds CP (1989) Determination of subcutaneous tumor size in athymic (nude) mice. Cancer Chemother Pharmacol 24 : 148–154
47. Boven E, Winograd B, Berger DP, Dumont MP, Braakhuis BJM, Fodstad O, Langdon S, Fiebig HH (1992) Phase II preclinical drug screening in human tumor xenografts: a first European multicenter collaborative study. Cancer Res 52 : 5940–5947
48. Pinedo HM, Peters GJ (1988) Fluorouracil: biochemistry and pharmacology. J Clin Oncol 6 : 1653–1664
49. Peters GJ, Jansen G (1996) Resistance to fluoropyrimidines. In: Schilsky RL, Milano GA, Ratain MY (eds) Principles of cancer drug pharmacology. Marcel Dekker, New York pp 543–586
50. Westerhof GR, Janssen G, Van Emmerik N, Kathman I, Rijkson G, Jackman AL, Schornagel JH (1991) Membrane transport of natural folates and antifolate compounds in murine L1210 leukemia cells: role of carrier- and receptor-mediated transport systems. Cancer Res 51 : 5507–5513
51. Moldawer LL, Georgieff M, Lundholm K (1987) Interleukin 1, tumour necrosis factor (cachectin) and the pathogenesis of cancer cachexia. Clin Physiol 7 : 263–274
52. Visser GWM, Van der Wilt CJ, Wedzinga R, Peters GJ, Herscheid JDM (1996) [^{18}F]-Radiopharmaceuticals of [^{18}F]-5-fluorouracil in a mouse bearing two colon tumors with a different 5-fluorouracil sensitivity. A study for correlation with oncological results. Nucl Med Biol 23 : 333–342
53. Moghaddam A, Zhang HT, Fan TPD, Hu DE, Lees VC, Turley H, Fox SB, Gatter KC, Harris AL, Bicknell R (1995) Thymidine phosphorylase is angiogenic and promotes tumor growth. Proc Natl Acad Sci USA 92 : 998–1002