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Modulated 5-Fluorouracil (5-FU) Regimens in Advanced Colorectal Cancer: A Critical Review of Comparative Studies

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Several modifications to the administration schedule of 5-fluorouracil (5-FU) alone or in combination with other agents have been investigated in advanced colorectal cancer. Biochemical modulation of 5-FU with leucovorin (LV) increases reponse rate compared with 5-FU alone, but without improvement of overall survival. The best treatment schedule and optimal dose of LV remain unclear, although low doses seem equally as effective as high doses, with the advantage of reduced cost. Methotrexate can increase the activity of 5-FU to a similar degree as LV and a recent meta-analysis showed a slight improvement in survival. The combination of 5-FU + interferon has been disappointing, with phase III trials showing similar activity to 5-FU + LV, but with high toxicity. Other modulators (e.g. hydroxyurea, *N*-phosphonacetyl-L-aspartate, dipyridamole) show promising but sometimes conflicting results. Standardisation of assessment criteria should be considered when comparing these data to the activity of new drugs such as 'Tomudex'™ (raltitrexed, previously known as ZD1694), CPT-11 and oxaliplatin.

Key words: 5-fluorouracil, colorectal cancer, leucovorin, treatment schedules
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INTRODUCTION

THE OPTIMAL treatment for advanced colorectal cancer has not yet been established: usually, this disease has been regarded as poorly chemosensitive and the drug of choice has been, for more than 30 years, 5-fluorouracil (5-FU). However, the reported objective response rate (OR) using bolus intravenous (i.v.) administration has been only 10–15%, with a median overall survival of 1 year [1]. Several attempts at increasing the activity of 5-FU have been made, chiefly through schedule modifications and addition of biochemical modulators.

Amongst the changes in the schedule of administration, of particular importance has been the use of longer low-dose infusions [2] and the administration of the drug according to an optimal circadian rhythm [3]. Several drugs have been employed as biochemical modulators, both in experimental and clinical studies, in order to increase the therapeutic index of 5-FU. The most interesting agents in the clinic so far have been: folinic acid or leucovorin (LV), alpha-interferon (IFN), methotrexate (MTX), *N*-phosphonacetyl-L-aspartate (PALA), hydroxyurea, dipyridamole and allopurinol.

The development of 5-FU analogues (ftorafur, doxifluridine, uracil-tegafur [UFT]) and of innovative compounds ('Tomudex'™*, irinotecan [CPT-11], oxaliplatin) has increased the number of active drugs available to the medical oncologist. However, a critical review of phase III studies evaluating the biochemical modulation of 5-FU is needed to assess the current position of potentiated 5-FU. Given the large number of published comparative trials, this article aims to provide a critical analysis of selected important papers, rather than an exhaustive review of all publications.

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* 'Tomudex' is a trademark, the property of Zeneca Limited.

LEUCOVORIN + 5-FU

An increased inhibition of thymidylate synthase (TS) is the rationale for this modulation. The administration of folic coenzymes, precursors of 5, 10-CH_2-FH_4 acid, increases intracellular levels of this cofactor, leading to enhanced formation and retention of the ternary complex with fluoro-deoxyuridine-monophosphate (FdUMP) and TS. This subsequently enhances the inibition of DNA synthesis. The first successful trial in clinical oncology was performed by Machover and associates [4] and demonstrated high activity (approximately 40% OR). This has been confirmed by other phase II studies. The regimen consisted of 200 mg/m^2 LV i.v. bolus immediately followed by 5-FU (370 mg/m^2 i.v. in 15-min infusion), both drugs being given for 5 consecutive days with a 21-day interval. The other classic regimen was designed at Roswell Park Memorial Institute (RPMI) in Buffalo [5]: patients received LV (500 mg/m^2/day in a 2-h infusion) and 5-FU (600 mg/m^2/day i.v. bolus at mid-infusion), with both drugs given weekly for 6–8 weeks. The activity was similar to that reported for the previous schedule [4]. The dose-limiting toxicity was diarrhoea rather than the mucositis seen with the Machover regimen [5]. Other studies have employed a continuous infusion or oral administration of LV, which may produce selective absorption of the active L-isomer [6].

Table 1. Randomised clinical trials of LV + 5-FU versus 5-FU

No. of patients	Schedule	% CR + PR	Survival	Reference
82	5-FU 600 mg/m^2 + LV 500 mg/m^2 weekly *versus* 5-FU 600 mg/m^2 weekly	16% versus 5% (P = 0.05)	No significant difference	[10]
318	5-FU 600 mg/m^2 + LV 500 *or* 25 mg/m^2 out of 8 weeks *versus* 5-FU 500 mg/m^2 × 5 every 4 weeks	30.3% versus 18.8% versus 12.1% (P < 0.01)	55 versus 45 versus 46 weeks (P = 0.08)	[11]
44	5-FU 600 mg/m^2 + LV 500 mg/m^2 6 out of 8 weeks *versus* 5-FU 450 mg/m^2 × 5	48% versus 11% (P = 0.0009)	No significant difference (P = 0.6)	[8]
153*	5-FU 400 mg/m^2 × 5 + LV 200 mg/m^2 × 5 every 4 weeks *versus* 5-FU 480 mg/m^2 followed by 600 mg/m^2/week	18.8% versus 17.3% (P = 0.4)	24 versus 20 weeks (P = 0.4)	[11]
181	5-FU 400 mg/m^2 × 5 + LV 200 mg/m^2 × 5 every 4 weeks *versus* 5-FU 540 mg/m^2 × 5	15% versus 16%	25 versus 21 weeks	[12]
182	5-FU 400 mg/m^2 × 5 + LV 200 mg/m^2 × 5 every 4 weeks *versus* 5-FU 400 mg/m^2 × 5 every 4 weeks	20.6% versus 10% (P = 0.046)	46 versus 44 weeks	[13]
208	5-FU 370 mg/m^2 × 5 + LV 20 *or* 200 mg/m^2 × 5 *versus* 5-FU 500 mg/m^2 × 5	43% versus 26% versus 10% (P = 0.001)	53 versus 52 versus 34 weeks (P = 0.05)	[14]
74	5-FU 370 mg/m^2 × 5 + LV 500 mg/m^2 × 5 every 4 weeks *versus* 5-FU 370 mg/m^2 × 5	44% versus 13% (P = 0.0019)	62 versus 55 weeks (P = 0.25)	[15]
124	5FU 370 mg/m^2 × 5 + LV 200 mg/m^2 × 5 *versus* 5FU 370 mg/m^2 × 5	33% versus 7% (P < 0.0005)	54 versus 41 weeks (P = 0.05)†	[16]
64	5FU 600 mg/m^2 + LV 200 mg/m^2 weekly *versus* 5FU 600 mg/m^2 weekly	26.4% versus 3.3%	No significant difference	[17]

* Randomisation 2:1. † Not confirmed in a further analysis.

From a review of 22 phase II studies of LV + 5-FU, an OR (complete [CR] or partial [PR]) was achieved in 177 of 730 evaluable patients (24%) [7]. On the basis of these promising results, at least 10 phase III trials comparing LV + 5-FU to 5-FU alone were performed and published from 1987 to 1992 in North America and Europe [8–17]. Table 1 provides an outline of these trials. They can be divided into two groups, one evaluating the RPMI weekly intensive regimen [5] and the other the daily-times-five combination designed by Machover [4]. A significant advantage in terms of OR was observed for LV + 5-FU in 8 out of 10 studies, whilst in the remaining two, the activity was similar to that of 5-FU alone. In one study only, North Central Cancer Treatment Group (NCCTG), a prolongation of survival was reported [14]. In the same trial a favourable impact on quality of life (pain relief and improvement of performance status) was also observed. Considering all trials, up to 75–80% of patients treated with this combination failed to achieve an OR and there was significant toxicity, chiefly diarrhoea and mucositis. Despite this rather modest advantage over 5-FU alone, the general opinion amongst medical oncologists after the final publication of these trials was that LV + 5-FU represented a step forward in the treatment of advanced colorectal cancer.

These studies formed the basis for the evaluation of LV + 5-FU as adjuvant therapy, with positive results reported in at least four studies [18–21] in North America and Europe. A strict meta-analysis [22] has considered all the aforementioned studies (with the exception of the NCCTG trial). In this analysis of 1381 patients, there was a significant advantage of LV + 5-FU versus 5-FU alone in terms of OR (odds ratio = 0.44), with a slight superiority of weekly compared with monthly regimens (odds ratio = 0.31 and 0.58, respectively). However, no difference was detected for overall survival (odds ratio = 0.97).

An important issue in the biochemical modulation of 5-FU with LV concerns the dose of LV needed for optimal potentiation. Whilst high doses seem to be required for the weekly schedule, in the daily-times-five regimen low doses appear to be at least equally effective in terms of response rate, symptomatic relief and overall survival. Several studies have performed a direct comparison between two dose levels within the same regimen and in the weekly and monthly schedules (Table 2) [9, 14, 23–28]. An important study [26] has compared the low-dose daily-times-five schedule developed by the Mayo Clinic with the high-dose weekly regimen designed at RPMI. The authors concluded that the low-dose modulation had similar activity to the high-dose schedule, but with less life-threatening toxicity and lower costs, and should be preferred both in clinical practice and as a reference arm in clinical trials.

Although the low-dose monthly regimen is considered the standard treatment for advanced colorectal cancer in North America and is also widely used in Europe, there is still much debate about the optimal dosage of LV [29–31]. Additionally, a lower response rate has been recently reported for this regimen compared with the high-dose L-isomer of LV [25], a higher dose combination of LV + 5-FU [27] or the new potent inhibitor of TS, 'Tomudex' [32]. A similar trend has been observed in a large seven-arm study recently published by the South West Oncology Group (SWOG) [28]. A meta-analysis, based on individual patient data of all trials comparing low-dose with high-dose LV as modulator of 5-FU in advanced colorectal cancer, has been planned by the Meta-Analysis Group In Cancer (MAGIC).

METHOTREXATE + 5-FU

MTX appears to have little activity as monotherapy in the treatment of advanced colorectal cancer [7]. However, it can potentiate the activity of 5-FU through an increase of 5-phosphoribosyl-1-pyrophosphate, which is required for the conversion of 5-FU into its active metabolites. The combination of MTX and 5-FU, generally with LV rescue, has been evaluated in phase I–III studies [8, 11, 14, 33–39]. A comparison of the various regimens is difficult because of the lack of uniformity in dosage of MTX and 5-FU, differences in the

Table 2. Randomised clinical trials comparing low-dose with high-dose LV as modulator of 5-FU in advanced colorectal cancer

Institution	Low-dose LV + 5-FU		High-dose LV + 5-FU	
GITSG [9]	LV 25 mg/m^2 (10-min infusion) 5-FU 600 mg/m^2 i.v.	weekly (6/8)	LV 500 mg/m^2 (2-h infusion) 5-FU 600 mg/m^2 i.v.	weekly (6/8)
PALL I Study Group [23]	LV 20 mg/m^2 (2-h infusion) 5-FU 500 mg/m^2 i.v.	weekly	LV 500 mg/m^2 (2-h infusion) 5-FU 500 mg/m^2 i.v.	weekly
NCCTG [14]	LV 20 mg/m^2 i.v. 5-FU 370–425 mg/m^2 i.v.	× 5 days every 4–5 weeks	LV 200 mg/m^2 i.v. 5-FU 370 mg/m^2 i.v.	× 5 days every 4–5 weeks
NCCTG [24]	LV 20 mg/m^2 i.v. 5-FU 425 mg/m^2 i.v.	× 5 days every 4–5 weeks	LV 200 mg/m^2 i.v. 5-FU 370 mg/m^2 i.v.	× 5 days every 4–5 weeks
GISCAD [25]	L-LV 10 mg/m^2 i.v. 5-FU 370 mg/m^2 (15-min infusion)	× 5 days every 4 weeks	L-LV 100 mg/m^2 i.v. 5-FU 370 mg/m^2 (15-min infusion)	× 5 days every 4 weeks
NCCTG [26]	LV 20 mg/m^2 i.v. 5-FU 425 mg/m^2 i.v.	× 5 days every 4–5 weeks	LV 500 mg/m^2 (2-h infusion) 5-FU 600 mg/m^2 i.v.	weekly (6/8)
FFCD/GERCOD/SFNMI [27]	LV 20 mg/m^2 i.v. 5-FU 425 mg/m^2 i.v.	× 5 days every 4 weeks	LV 200 mg/m^2 (2-h infusion) 5 FU 400 mg/m^2 i.v. + 600 mg/m^2 (22-h infusion)	days 1–2 every 2 weeks
SWOG [28]	LV 20 mg/m^2 i.v. 5-FU 425 mg/m^2 i.v. LV 20 mg/m^2 i.v. every week 5-FU 200 mg/m^2 continuous infusion, day 1–28, every 5 weeks	× 5 days every 4–5 weeks	LV 500 mg/m^2 (2-h infusion) 5-FU 600 mg/m^2 i.v.	weekly

GITSG, Gastrointestinal Tumour Study Group; NCCTG, North Central Cancer Treatment Group; GISCAD, Gruppo Italiano Studio Carcinomi Apparato Digerente; FFCD, Fondation Francaise de Cancerologie Digestive; SWOG, South West Oncology Group.

interval of administration between the two drugs and variations in the interval between courses. One critical point concerns the time between exposure to MTX and subsequent administration of 5-FU; in experimental models (human cell lines) the optimal interval has ranged from 18 to 24 h [7]. Several phase II studies have indicated good activity with this combination, and this promising observation has prompted phase III evaluation of this schedule (Table 3). The most important study was conducted by the Nordic Gastrointestinal Tumour Adjuvant Therapy Group [33] in which 249 patients were randomised to receive either (i) MTX (250 mg/m^2 i.v. × 2 h) + 5-FU (500 mg/m^2 i.v. push at 3 h and 23 h) + rescue with LV (15 mg × 8, beginning at 24 h), or (ii) 5-FU (600 mg/m^2 × 2 consecutive days). The therapy was repeated every 14 days for eight treatment cycles and then every 4 weeks. The combination was significantly superior to 5-FU in terms of response rate (24% versus 3%, $P = 0.0001$), median survival (8.5 versus 6 months, $P < 0.01$) and palliative effect (45% versus 23%, $P = 0.01$). A recent meta-analysis by the Advanced Colorectal Cancer Meta-Analysis Project group performed on phase III trials has compared MTX + 5-FU ± LV with 5-FU alone [34] and has demonstrated a superiority of the combination in terms of response rate (19% versus 10%) and overall survival (median 10.7 versus 9.1 months). A prolonged interval (e.g. 24 h) between administration of the two drugs permits adequate biomodulation of 5-FU with a relatively low dose of MTX, thus reducing the risk of side effects [40].

ALPHA-INTERFERON + 5-FU

After promising experimental data [41], the first clinical study was reported in 1989 by Wadler [42]. In this phase II trial, 17 previously untreated patients received a loading course of 5-FU (750 mg/m^2 by continuous i.v. infusion daily for 5 days), followed by a 1-week interval, then weekly bolus therapy at 750 mg/m^2. Alpha-2a IFN was administered subcutaneously at a dose of 9×10^6 IU three times weekly, starting on day 1. A very high response rate (76%) was reported and this observation was confirmed in a subsequent update by the same author [43]. Although the toxicity was severe (two toxic deaths were reported), these interesting results generated several confirmatory studies [44, 45], some of which obtained a similar activity [45], whilst others found a lower response rate [45]. However, the toxicity was considerable in all of these trials. In other phase II trials, a modification of the original schedule was introduced, or a double modulation of 5-FU with both IFN and LV was attempted. A response rate of approximately 20–30% was obtained from these complex data, but was coupled with a high incidence of severe toxicity [46–50]. These studies show that IFN has only a limited capability of increasing 5-FU activity and that this small advantage is not superior to that achieved, with less toxicity, by LV.

OTHER MODULATORS

PALA acts on L-aspartic acid transcarbamoylase to block *de novo* synthesis of pyrimidines. This depletes the non-fluorinated pool of uracil nucleotides, altering the competition for TS between FdUMP and dUMP, thus enhancing blockade of the enzyme. As little as 250 mg/m^2 PALA is capable of suppressing uracil synthesis for as long as 1 week. Based upon the encouraging results of a phase I study with weekly courses of PALA followed 24 h later by 5-FU (2.6 g/m^2 in 24 h infusion), O'Dwyer [51] tested this schedule in a phase II trial and obtained a 43% response rate in 37 patients. This apparently promising regimen has been recently challenged in a randomised study [27] and showed a discouraging response rate of only 15%.

Dipyridamole inhibits the efflux of 5-FU nucleosides (FUrd and FdUrd). However, no advantage in terms of antitumour activity was observed in a randomised trial comparing 5-FU + LV versus 5-FU + LV + dipyridamole [52].

ALTERNATING REGIMENS

Preclinical and clinical studies, conducted at the National Cancer Institute of Genoa, indicated that 5-FU can act in two

Table 3. Randomised trials comparing 5-FU alone with 5-FU/MTX in patients with advanced colorectal cancer

Group	5-FU alone	5-FU/MTX
EORTC [34]	5-FU 60 mg/kg over 48 h, every 7 days × 4, every 14 days × 4, and then every 21 days (n = 156)	MTX 40 mg/m^2; 5-FU 60 mg/kg over 48 h; every 7 days × 4, every 14 days × 4, and then every 21 days (n = 154)
NGTATG [33]	5-FU 600 mg/m^2 days 1 and 2, every 14 days × 8, then every 21–28 days (n = 127)	MTX 250 mg/m^2 over 2 h; 5-FU 500 mg/m^2 at 3 h and 23 h; LV at 24 h; every 14 days × 8, then every 21–28 days (n = 122)
NCCTG/Mayo Clinic [14]	5-FU 500 mg/m^2 days 1–5, every 35 days (n = 70)	MTX 40 mg/m^2, 5-FU 700 mg/m^2 at 24 h, days 1 and 8 every 28 days (n = 71) MTX 200 mg/m^2 over 4 h; 5-FU 900 mg/m^2 at 7 h; LV dose at 24 hours, every 21 days × 2, then every 28 days (n = 71)
AIO [35]	5-FU 450 mg/m^2 days 1–5, every 21 days (n = 82)	MTX 300 mg/m^2 over 4 h; 5-FU 900 mg/m^2 at 7 h; LV dose at 24 h, every 14 days × 3, then every 21 days (n = 88)
NCOG [11]	5-FU 12 mg/kg/day, days 1–5, then 15 mg/kg/day, day 1, every 7 days (n = 55)	MTX 50 mg/m^2 orally every 6 h × 5; 5-FU 500 mg/m^2 at 24 h; LV at 30 h, every 14 days (n = 103)
GOCS [36]	5-FU 1200 mg/m^2 over 2 h, every 14 days (n = 61)	MTX 200 mg/m^2; 5-FU 1200 mg/m^2 over 2 h at 20 h; LV dose at 24 h, every 14 days (n = 64)
Mar del Plata [37]	5-FU 500 mg/m^2 days 1–5, then 5-FU 500 mg/m^2 day 1, every 7 days (n = 33)	MTX 50 mg/m^2; LV 150 mg/m^2 i.v. over 6 h at 12 h; 5-FU 600 mg/m^2 at 18 h, every 14 days (n = 28)
Spain [38]	5-FU 1200 mg/m^2 day 1, every 14 days (n = 33)	MTX 500 mg/m^2 over 1 h; LV 200 mg/m^2 i.v. over 1 h at 10 h; 5-FU 600 mg/m^2, every 14 days (n = 26)
RPMI [8]	5-FU 450 mg/m^2 days 1–5 + 5-FU 200 mg/m^2 every other day × 6 doses, every 46 days (n = 23)	MTX 50 mg/m^2 over 4 h, then 5-FU 600 mg/m^2, every 7 days × 4, then every 14 days (n = 23)

Note: Treatment groups other than 5-FU or 5-FU/MTX are not shown. Mode of administration is i.v. bolus, unless otherwise specified.
EORTC, European Organization for Research and Treatment of Cancer; NGTATG, Nordic Gastrointestinal Tumour Adjuvant Therapy Group; AIO, Association of Medical Oncology of the German Cancer Society; NCOG, Northern California Oncology Group; GOCS, Grupo Oncologico Cooperativo del Sur, Argentina; RPMI, Roswell Park Memorial Institute.
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different ways according to the schedule of administration and the specific modulator employed [53, 54]. In particular, 5-FU bolus, modulated by MTX, can be successfully alternated with 5-FU administered by continuous infusion and modulated by weekly low-dose LV. In a phase II trial the response rate exceeded 40%, with an apparent increase in overall survival [55]. This exciting hypothesis is now being explored in a phase III study (Genoa, GISCAD Italian Group for the Study of Digestive Tract Cancer, collaborating centres), comparing this innovative strategy to MTX + 5-FU.

CONCLUSIONS

The biochemical modulation of 5-FU is one of the most interesting strategies developed in recent years for the treatment of colorectal cancer. Experimental data provided a rationale and prompted its evaluation in the clinical setting. Several phase II trials with various agents demonstrated a promising activity, even though toxicity was significant in many studies. In phase III trials, however, evaluation of this treatment in comparison to standard chemotherapy (5-FU alone) led to less positive results. In general, the advantage of modulated 5-FU over 5-FU as monotherapy is limited (usually only in terms of response rate and not overall survival) and this appears to be of little value in the treatment of advanced colorectal cancer. However, this small improvement is important for the outcome of this strategy in the adjuvant setting, as demonstrated by several studies, chiefly in combination with LV.

For optimal biochemical modulation, several issues (e.g. patient selection, optimum dose and schedule, minimisation and management of toxicity) need to be clarified. An obvious first step would be to use a less empirical choice of schedule. Closer interaction between laboratory and clinic could be seen as mandatory. Moreover, in interpreting the clinical results, it must be realised that several factors can influence the outcome of treatment. Such factors include patient selection, definition and application of response criteria, variable assessment of progression or response, whether an independent review of response was included, fluctuations in drug-dose intensity, and differences in administration and statistical interpretation of the results. This is also important to bear in mind when biochemical modulation is compared with new drugs.

At present, no standard chemotherapeutic regimen can be recommended for advanced colorectal cancer. The choices seem to lie between giving 5-FU alone in appropriate dosage schedules, biochemical modulation of 5-FU or with the promising new drugs. An in-depth knowledge of the comparative studies mentioned in this article and of other emerging data may help the medical oncologist in clinical decisions for individual patients and in the design of new trials.

1. Moertel CG. Chemotherapy for colorectal cancer. *New Engl J Med* 1994, **330**, 1136–1142.
2. Lokich JJ, Ahlgren JD, Gullo JJ, *et al.* A prospective randomized comparison of continuous infusion 5-fluorouracil with a conventional bolus schedule in metastatic colorectal carcinoma. A MAOP study. *J Clin Oncol* 1989, **7**, 425–432.
3. Anderson N, Lokich JJ. Controversial issues in 5-fluorouracil infusion use. *Cancer* 1992, **70**, 998–1002.
4. Machover D, Schwarzenberg L, Goldschmidt E, *et al.* Treatment of advanced colorectal and gastric adenocarcinomas with 5FU combined with high-dose folinic acid: a pilot study. *Cancer Treat Rep* 1982, **66**, 1803–1807.

5. Madajewicz P. Phase I/II trial of high-dose calcium leucovorin and 5-fluorouracil in advanced colorectal cancer. *Cancer Res* 1984, **44**, 4667–4669.
6. Brenckman WD, Laufman LR, Adamiewicz BB, *et al.* Is fluorouracil as effective as equitoxic doses of 5FU plus high dose oral leucovorin in colorectal carcinoma? *Proceedings ASCO* 1990, **9**, 421 (Abstract).
7. Luporini G, Labianca R, Pancera G. Treatment of metastatic colorectal cancer: improvement of 5-fluorouracil activity with modulating agents. *Forum* 1991, **1**, 246–256.
8. Petrelli N, Herrera L, Rustum Y, *et al.* A prospective randomized trial of 5-fluorouracil versus 5-fluorouracil and leucovorin in previously untreated patients with advanced colorectal carcinoma. *J Clin Oncol* 1987, **5**, 1559–1565.
9. Petrelli N, Douglass HD, Herrera L, *et al.* The modulation of fluorouracil with leucovorin in metastatic colorectal carcinoma. A perspective randomized phase III trial. *J Clin Oncol* 1989, **7**, 1419–1426.
10. Nobile MT, Vidili MG, Sobrero A, *et al.* 5-fluorouracil alone or combined with high-dose folinic acid in advanced colorectal cancer patients: a randomized trial. *Proceedings ASCO* 1988, **7**, 97 (Abstract).
11. Valone FH, Friedman MA, Wittlinger PS, *et al.* Treatment of patients with advanced colorectal carcinomas with fluorouracil alone, high-dose leucovorin plus fluorouracil or sequential methotrexate, fluorouracil, leucovorin: a randomized trial of the Northern California Oncology Group. *J Clin Oncol* 1989, **7**, 1427–1436.
12. Di Costanzo F, Bartolucci R, Sofra M, *et al.* 5-fluorouracil alone versus high dose folinic acid and 5FU in advanced colorectal cancer. A randomized trial of the Italian Oncology Group for Clinical Research. *Proceedings ASCO* 1989, **8**, 410 (Abstract).
13. Labianca R, Pancera G, Aitini E, *et al.* Folinic acid + 5-fluorouracil (5FU) versus equidose 5FU in advanced colorectal cancer. Phase III study of GISCAD (Italian Group for the Study of Digestive Tract Cancer). *Ann Oncol* 1991, **2**, 673–679.
14. Poon MA, O'Connell MJ, Moertel CG, *et al.* Biochemical modulation of fluorouracil. Evidence of significant improvement of survival and quality of life in patients with advanced colorectal carcinoma. *J Clin Oncol* 1989, **7**, 1407–1417.
15. Doroshow JH, Multhauf P, Leong L, *et al.* Prospective randomized comparison of fluorouracil versus fluorouracil and high-dose continuous infusion leucovorin calcium for the treatment of advanced measurable colorectal cancer in patients previously unexposed to chemotherapy. *J Clin Oncol* 1990, **8**, 491–501.
16. Erlichman C, Fine S, Wong A, *et al.* A randomized trial of fluorouracil and folinic acid in patients with metastatic colorectal carcinoma. *J Clin Oncol* 1988, **6**, 469–475.
17. Cricca A, Martoni A, Guaraldi M, *et al.* Randomized clinical trial of 5FU + folinic acid versus 5FU in advanced gastrointestinal cancers. *Proceedings ESMO* 1988, **13**, 427 (Abstract).
18. O'Connell MJ, Maillard J, MacDonald J, *et al.* An intergroup trial of intensive course 5FU and low-dose leucovorin as surgical adjuvant therapy for high risk colon cancer. *Proceedings ASCO* 1993, **12**, 552 (Abstract).
19. Wolmark N, Rockette H, Fisher B, *et al.* The benefit of leucovorin-modulated fluorouracil as post operative adjuvant therapy for primary colon cancer: results from the National Surgical Adjuvant Breast and Bowel Project protocol C-03. *J Clin Oncol* 1993, **11**, 1879–1887.
20. Francini G, Petrioli R, Lorenzini L, *et al.* Folinic acid and 5-fluorouracil as adjuvant chemotherapy in colon cancer. *Gastroenterology* 1994, **106**, 899–906.
21. IMPACT investigators (Labianca R, Marsoni S, Pancera G, *et al.*). Efficacy of adjuvant fluorouracil and folinic acid in colon cancer. *Lancet* 1995, **345**, 939–944.
22. Advanced Colorectal Cancer Meta-Analysis Project. Modulation of fluorouracil by leucovorin in patients with advanced colorectal cancer: evidence in terms of response rate. *J Clin Oncol* 1992, **10**, 896–903.
23. Jaeger E, Klein O, Bernhard H, *et al.* Weekly high-dose folinic acid (FA)/5-fluorouracil (FU) versus low-dose FA/FU in advanced colorectal cancer. Results of randomized multicenter trial. *Proceedings ASCO* 1994, **13**, 556 (Abstract).
24. Poon MA, O'Connell MJ, Wieand HS, *et al.* Biochemical modulation of fluorouracil with leucovorin: confirmatory evidence of improved therapeutic efficacy in advanced colorectal cancer. *J Clin Oncol* 1991, **9**, 1967–1972.
25. Valsecchi R, Labianca R, Cascinu S, *et al.* High-dose versus low-dose L-leucovorin as a modulator of 5 days' 5-fluorouracil in advanced colorectal cancer: a GISCAD phase III study. *Proceedings ASCO* 1995, **14**, 457 (Abstract).
26. Buroker TR, O'Connell MJ, Wieand HS, *et al.* Randomized comparison of two schedules of fluorouracil and leucovorin in the treatment of advanced colorectal cancer. *J Clin Oncol* 1994, **12**, 14–20.
27. de Gramont A, Bosset JF, Milan C, *et al.* A prospective randomized trial comparing 5FU bolus with low-dose folinic acid (FUFOLld) and 5FU bolus plus continuous infusion with high-dose folinic acid (LV5FU2) for advanced colorectal cancer. *Proceedings ASCO* 1995, **14**, 455 (Abstract).
28. Leichman CG, Fleming TR, Muggia FM, *et al.* Phase II study of fluorouracil and its modulation in advanced colorectal cancer: a Southwest Oncology Group study. *J Clin Oncol* 1995, **13**, 1303–1311.
29. Borner MM, Sartor O. More is not always better: a case for low-dose leucovorin. *J Clin Oncol* 1993, **11**, 382–383.
30. Petrelli N, Rustum Y. Fluorouracil and leucovorin: there is a choice. *J Clin Oncol* 1993, **11**, 1434.
31. Brook J. Fluorouracil and low-dose leucovorin versus fluorouracil and high-dose leucovorin. What is the real cost? What is the answer? *J Clin Oncol* 1995, **13**, 1830–1831.
32. Cunningham D, Zalcberg J, Rath U, *et al.* 'Tomudex' (ZD1694): results of a randomised trial in advanced colorectal cancer demonstrate efficacy and reduced mucositis and leucopenia. The 'Tomudex' Colorectal Cancer Study Group. *Eur J Cancer* 1995, **31A**, (12), 1945–1954.
33. Nordic Gastrointestinal Tumour Adjuvant Therapy Group. Superiority of sequential methotrexate, fluorouracil and leucovorin to fluorouracil alone in advanced symptomatic colorectal carcinoma: a randomized trial. *J Clin Oncol* 1989, **7**, 1437–1446.
34. Advanced Colorectal Cancer Meta-analysis Project. Meta-analysis of randomized trials testing the biochemical modulation of fluorouracil by methotrexate in metastatic colorectal cancer. *J Clin Oncol* 1994, **12**, 960–969.
35. Blijham GH, Stellestags J, Sahmoud T, *et al.* The modulation of high-dose 5-fluorouracil with low-dose methotrexate in metastatic colorectal cancer. A phase III study of the EORTC GI Cancer Cooperative Group. *Proc Am Soc Clin Oncol* 1993, **12**, 586 (Abstract).
36. Herrmann R, Knuth A, Kleeberg U, *et al.* Sequential methotrexate and 5-fluorouracil (FU) vs FU alone in metastatic colorectal cancer. *Ann Oncol* 1992, **3**, 539–543.
37. Machiavelli M, Leone BA, Romero A, *et al.* Advanced colorectal carcinoma: a prospective randomized trial of sequential methotrexate, 5-fluorouracil, and leucovorin versus 5-fluorouracil alone. *Am J Clin Oncol* 1991, **14**, 211–217.
38. Delfino C, Caccia G, Maniago O. 5-fluorouracil alone versus methotrexate + leucovorin + FU in patients with metastatic colorectal cancer. *Proc Am Soc Clin Oncol* 1992, **11**, 521 (Abstract).
39. Abad A, Garcia P, Gravalos C, *et al.* Phase III trial with methotrexate, 5-FU and high-dose leucovorin vs. 5-FU, leucovorin vs. 5-FU in advanced and metastatic colorectal cancer. *Proc Am Soc Clin Oncol* 1992, **11**, 459 (Abstract).
40. Marsh J, Bertino J, Katz KH, *et al.* The influence of drug interval on the effect of methotrexate and 5-fluorouracil in the treatment of advanced colorectal cancer. *J Clin Oncol* 1991, **9**, 371–380.
41. Elias L. Interferon effects upon the adenocarcinoma 38 and HL-60 cell lines: antiproliferative responses and synergistic interaction with halogenated pyrimidine antimetabolites. *Cancer Res* 1988, **48**, 4868–4873.
42. Wadler S, Schwartz EL, Goldman M, *et al.* Fluorouracil and recombinant alpha-2a interferon: an active regimen against advanced colorectal carcinoma. *J Clin Oncol* 1989, **7**, 1769–1775.
43. Wadler S, Wiernik PM. Clinical update on the role of fluorouracil and recurrent interferon alpha-2a in the treatment of colorectal cancer. *Semin Oncol* 1990, **18** (suppl), 16–23.
44. Kemeny N, Younes A, Seiter K, *et al.* Interferon alpha-2a and 5-flurouracil for advanced colorectal carcinoma. Assessment of activity and toxicity. *Cancer* 1990, **66**, 2470–2475.
45. Pazdur R, Ajani JA, Patt YZ. Phase II study of fluorouracil and recombinant interferon alpha-2a in previously untreated advanced colorectal carcinoma. *J Clin Oncol* 1990, **8**, 2027–2031.
46. Labianca R, Giaccon G, Barni S, *et al.* Double modulation of

5-fluorouracil in advanced colorectal cancer with low-dose interferon alpha-2b and folinic acid. The GISCAD experience. *Eur J Cancer* 1994, **30A**, 1611–1616.
47. York M, Greco FA, Figlin RA, *et al.* A randomized phase III trial comparing 5FU with or without interferon alpha-2a for advanced colorectal cancer. *Proceedings ASCO* 1993, **12**, 590 (Abstract).
48. Hill M, Norman A, Cunningham D, *et al.* Royal Marsden phase III trial of fluorouracil with or without interferon alpha-2b in advanced colorectal cancer. *J Clin Oncol* 1995, **13**, 1297–1302.
49. Kocha W. 5-fluorouracil plus interferon alpha-2a (Roferon-A) versus 5-fluorouracil plus leucovorin in metastatic colorectal cancer. Results of a multicentre, multinational phase III study. *Proceedings ASCO* 1993, **12**, 562 (Abstract).
50. Kohne CH, Wilke H, Hecker H, *et al.* Interferon alpha does not improve the antineoplastic efficacy of high-dose infusional 5-fluorouracil plus folinic acid in advanced colorectal cancer. *Ann Oncol* 1995, **6**, 461–466.
51. O'Dwyer PJ, Paul AR, Walczac J, *et al.* Phase II study of biochemical modulation of fluorouracil by low-dose PALA in patients with colorectal cancer. *J Clin Oncol* 1990, **8**, 1497–1503.
52. Kohne CH, Hiddemann W, Schuller J, *et al.* Failure of orally administered dipyridamole to enhance the antineoplastic activity of fluorouracil in combination with leucovorin in patients with advanced colorectal cancer: a prospective randomized trial. *J Clin Oncol* 1995, **13**, 1201–1208.
53. Aschele C, Sobrero A, Faderan MA, *et al.* Novel mechanism of resistance to 5-fluorouracil in human colon cancer (HCT-8) sublines following exposure to two different clinically relevant dose schedules. *Cancer Res* 1992, **52**, 1855–1864.
54. Mori A, Bertoglio S, Guglielmi A, *et al.* Activity of continuous infusion 5-fluorouracil in patients with advanced colorectal cancer clinically resistant to bolus 5-fluorouracil. *Cancer Chemother Pharmacol* 1993, **33**, 179–180.
55. Sobrero A, Aschele C, Guglielmi A, *et al.* Schedule selective biochemical modulation of 5-fluorouracil: a phase II study in advanced colorectal cancer. *Clin Cancer Res* 1995, **1**, 955–960.