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5-FLUOROURACIL (5FU) WITH OR WITHOUT FOLINIC ACID (LV) IN HUMAN COLORECTAL CANCER? MULTIVARIATE META-ANALYSIS OF THE LITERATURE

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This meta-analysis is based on 106 evaluations of response from 77 clinical studies about 5-fluorouracil (5FU) treatment with or without leucovorin (LV) in metastatic colorectal carcinoma. Overall, in naive patients, LV is associated with a median response rate of 31% as compared with a 12% figure with 5FU alone.

Using a forward stepwise multilinear regression analysis, it is shown that as much as 44% of the variance in the reported response rates in naive patients can be accounted for by treatment-related variables ($P < 0.001$). The significant parameters are LV adjunction (partial R = 0.636), cumulative total 5FU dose (R = 0.344), and 5FU weekly schedule (R = 0.246). In pretreated patients, the latter parameter is the only significant one (R = 0.443). Unexpectedly, LV administration behaves like an all-or-nothing governor, without any obvious dose–effect relationship.

Protracted 5FU infusion over weeks allows a mean cumulative drug delivery, 3 times higher than bolus regimens (21.3 vs 7.02 g m^{-2}, $P < 0.001$) and may represent the best clinical approach to influence the 5FU-related variables. Accordingly, it is suggested that 5FU protracted infusion, titrated to the highest tolerable doses and potentiated with low doses of leucovorin, could represent the most efficacious way for using 5FU in colorectal disseminated cancer. This hypothesis and its eventual impact on survival should be tested in randomized trials.

Key words: **5-Fluorouracil, Folinic acid, Meta-analysis, Human, Colon cancer.**

INTRODUCTION

Colorectal cancer is a major health problem, and little progress in chemotherapy has been achieved since the introduction of 5-fluorouracil decades ago. A renewed interest has recently arisen, as 5FU cytotoxicity could be enhanced by the adjunction of folinic acid. Special issues of renowned cancer journals have been dedicated to the topics.[1,2] The rationale for using LV is to enhance thymidylate synthase inhibition by the formation of a stable ternary complex between the enzyme, FdUMP and reduced folate co-factor.[3] However, it is not the sole mechanism of action of 5FU. Fluorouracil may be incorporated into RNA or DNA and interfere with their functions. Other determinates of cell sensitivity to 5FU than dTMP synthase activity have been described.[3] So far, on theoretical grounds, the potentiating role of LV looks somehow restricted. Indeed, present knowledge is controversial. *In vitro*, evidence exists for LV enhanced 5FU cytotoxicity on human colon cell lines, but with questionable clinical relevance[4] or without marked importance of LV concentration, length of exposure or preincubation time.[5] In a human clinical assay, low and high doses of folinic acid had been found equally effective.[6] In a murine model, therapy with 5FU at the maximal tolerated dose was not improved by LV adjunction.[7] The latter authors have emphasized that almost all the completed or ongoing phase III trials used either a 5FU control arm in which the 5FU was administered in a different schedule from the 5FU/LV arm or one in which the 5FU was not at the maximally tolerated dose, so far weakening the assertion of a clinically relevant modulating effect of leucovorin. Indeed, both schedule and dose intensity of 5-fluorouracil are prominent parameters influencing cancer response[8,9] and should be differentiated from the LV effect. Furthermore, it is worth mentioning that the commercially available form of LV is a mixture of two stereomers and the question has arisen as whether the inactive *d*-isomer might inhibit the membrane transport of the *l*-isomer and compete for intracellular polyglutamation.[10] Not the least, some investigators have reported an exceedingly

An interim report of the results of this study was presented at the poster session of the 15th ESMO Congress in Copenhagen, 2–5 December 1990.

increased toxicity of the LV/5FU association and have recommended not to use it further.[11] Up to now, no definitive data exist supporting an unrestricted use of LV with 5FU, and the value of their co-administration still needs to be defined.

In the present study, a multivariate regression analysis was used to interpret the apparently controversial literature. Hierarchical models were created to encompass multiple variables, especially LV and 5FU doses and schedules, and isolate the most significant ones.

METHODS

Data search

This study was initiated after the receipt of a précis prepared by Lederle Literature Service, Wayne, U.S.A., summarizing clinical studies about folinic acid and 5FU in cancer treatment. Forty-three abstracts about colon cancer fitted this meta-analysis. Twenty-one could be cross-checked with the original full-length publications.[6,12–30] The authors of the data only retrieved from the aforementioned précis are given in Appendix 1. The second data core-sample came from the 1989 and 1990 Proceedings of the American Society of Clinical Oncology. Fourteen abstracts[31–43] were suited for the present analysis. One was used in its definitive published form.[44] Finally, the MEDLINE/EBSCO CD-ROM™ 1978–1990 database was consulted, yielding twenty new pertinent studies.[11,45–63]

Eligibility

The studies were taken into account if they gave an objective response rate, in a defined number ($n \geq 8$) of patients evaluated for response, with explicit time- and dose-defined regimens of 5FU alone or 5FU + LV, in metastatic colorectal cancer.

Study design

The leading hypothesis of this meta-analysis was that the different authors dealt with samples of patients from the same original colon cancer population, and that the differences in the reported response rates could be accounted for by explanatory variables, some of which related to the applied treatment.

Parameters

The outcome variable was the objective response rate (partial + complete) expressed in percent (RR%). Square root or logistic transformations were also used. The other considered variables were the number of evaluated patients, the type of study (comparative randomized study or open phase II one), the type of patients (naive or pretreated subjects), the 5FU loading dose during the first week (g m^{-2}), the total cumulative dose of 5FU over the first 12 weeks (g m^{-2}), the schedule of 5FU administration (weekly doses or cycles with longer intervals), the manner of delivering 5FU (continuous IV perfusion for 96 or more hours against any other mode). The last parameters were related to LV, i.e. the co-administration of LV (digital categorization according to administration before 5FU, or after 5FU or in an imprecise schedule, or none), the LV dose given with the loading dose of 5FU (mg m^{-2}), the ratio of LV to 5FU dose (mg/mg) and the LV unit dose (mg m^{-2}). The rules used to standardize the parameters are given in Appendix 2.

Statistical analysis

The response rates in subgroups were compared by non-parametric bilateral Mann–Whitney or Kruskal–Wallis tests as appropriate. The cumulative frequency curves of the observed response rates were compared using Kolmogorov–Smirnov test statistics. The dependency of the response rate upon explanatory variables was evaluated by forward stepwise multivariate regression analysis. The calculations were performed with the NWA Statpak ver 3.1 software (Northwest Analytical Inc., Portland, Oregon).

RESULTS

According to the type of study, the naive status of the patients and the described 5FU or 5FU/LV regimens, the data were split down, yielding 106 different evaluations of response from the 77 collected studies. The descriptive values of the most salient parameters are shown in Table 1. Eighty-one evaluations (76.4%) concerned naive patients. A randomization process was used in 52 studies (49%), almost only in naive patients ($n = 50$). Leucovorin, in any schedule, was given in 69 investigations (65.1%), 47 in naive patients (58% in this group) and 22 in pretreated ones (88% in this latter subset).

The most important variables influencing the response rate (RR%) were delineated by multivari-

Table 1. Descriptive data of the surveyed studies about colon cancer treatment with 5FU alone or with folinic acid, classified as dealing with naive patients or not (the ± values represent the mean ± S.D. of the variables between the different studies)

	Naive patients	Pretreated patients
Number of studies	81	25
Response rate (RR%)	24.1 ± 15.0	20.4 ± 16.1
range	0–60	0–50
Randomized studies	50	2
Number of patients	45 ± 28	21 ± 8
range	8–125	10–36
5FU loading dose (1st week, g m^{-2})	1.90 ± 0.93	1.53 ± 0.80
range	0.4–5.0	0.4–2.8
5FU 12w-cumulative dose (g m^{-2})	8.49 ± 5.12	8.80 ± 6.89
range	4.44–31.2	1.6–31.2
Weekly 5FU schedules	26	12
5FU continuous infusions (CIV)	10	2
Protracted CIV	4	2
LV administration before 5FU	46	18
after or unknown	1	4
none	34	3
LV loading dose (mg m^{-2})	380 ± 491	567 ± 647
range	0–2750	0–3000
LV/5FU dose ratio	0.24 ± 0.30	0.42 ± 0.39
range	0–1.49	0–1.62
LV unit dose (mg m^{-2})	118 ± 145	210 ± 197
range	0–500	0–500

ate regression analysis as to be LV administration (any dose), 5FU cumulative dose intensity, absence of previous treatment, and weekly administration of 5FU (R = 0.594, $P < 0.001$, Table 2). The logistic expression of RR% was far less fruitful (R = 0.236), isolating only LV delivery as a significant parameter. RR% transformation as a square root did not change the selection of the most significant explanatory parameters nor the overall definition of the statistical model (not shown). When the studies dealing only with naive patients were considered separately, the stepwise regression analysis pointed out LV administration, total cumulative 5FU dose and 5FU weekly schedule as the explanatory variables determining RR% (Table 3, R = 0.667, $P < 0.001$). The logistic response rate was only dependent upon the first and the latter parameters (R = 0.508). In naive patients with LV given before 5FU, the only significant parameter for response was 5FU dose intensity (R = 0.335). When no LV was given, the determinant variables (R = 0.674) were 5FU weekly schedule (partial R = 0.513) and 5FU cumulative dose (partial R = 0.436). In these studies dealing with naive patients, the median reported response rate was 31% with LV before 5FU (mean value ± S.D. = 31.1 ± 14.4%) and 12% without LV (mean value 14.8 ± 10.1). The relative importance of the 5FU total cumulative doses and LV admini-

Table 2. Forward stepwise multivariate analysis of the response rate in all selected studies (n = 106)

Dependent variable = RR%

Step	Explicative variable	F to enter	Partial R
1	x_1 = LV use	20.79	0.527
2	x_2 = 5FU cumulative dose	12.21	0.293
3	x_3 = Naive status	7.26	0.294
4	x_4 = Weekly 5FU schedule	7.58	0.264

equation: $RR\% = -3.44\ (\pm 4.28) + 8.64\ (\pm 1.39)\ x_1 + 0.74\ (\pm 0.24)\ x_2 + 9.16\ (\pm 2.96)\ x_3 + 7.31\ (\pm 2.65)\ x_4$
(R = 0.594, $P < 0.001$)

Table 3. Forward stepwise multivariate analysis of the response rate in studies dealing only with naive patients (n = 81)

Dependent variable = RR%			
Step	Explicative variable	F to enter	Partial R
1	x_1 = LV use	34.28	0.636
2	x_2 = 5FU cumulative dose	14.05	0.344
3	x_3 = Weekly 5FU schedule	4.97	0.246

equation: RR% = 3.79 (± 3.28) + 9.74 (± 1.35) x_1 + 0.86 (± 0.27) x_2 + 6.22 (± 2.79) x_3
(R = 0.667, $P < 0.001$)

stration on the response rate is illustrated in Fig. 1. As suggested in the forward multivariate regression analyses, LV was a hit-or-miss governor, only acting as a categorical variable. The absence of any dose–effect relationship is illustrated in Fig. 2. In pre-treated patients (25 response assessments), the response rate was related only to 5FU weekly schedule (R = 0.443), and no variable could be brought up in the logistic model. LV was not recognized as an explanatory parameter, but it should be emphasized that no leucovorin was used only in very few studies (12%). The mean response rate values reported in the subgroups without LV, with LV before 5FU, or another schedule, were

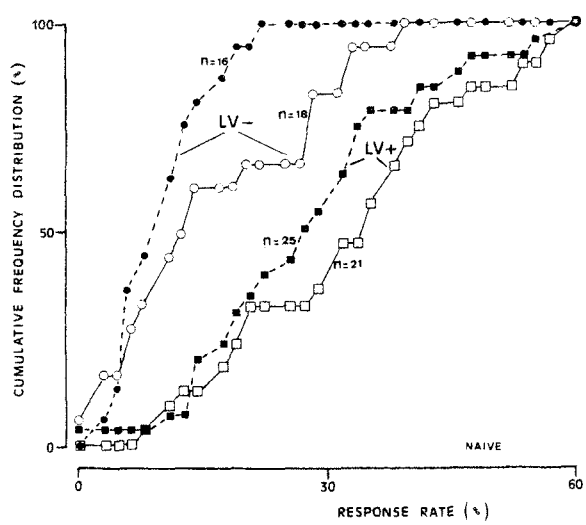

Fig. 1. Displacement of the response rate distribution curves by leucovorin and high 5FU cumulative doses. Circles: series without LV; Squares: series with LV. Full symbols: low 5FU doses (≤ the median value). Open symbols: high 5FU doses (above the median value). The LV− curves are significantly different from the LV+ ones (Kolmogorov–Smirnov test: ●-■, d_{max} = 0.697, $P < 0.01$; ○-■, d_{max} = 0.423, $P < 0.05$; ●-□, d_{max} = 0.627, $P < 0.01$; ○-□, d_{max} = 0.482, $P < 0.05$).

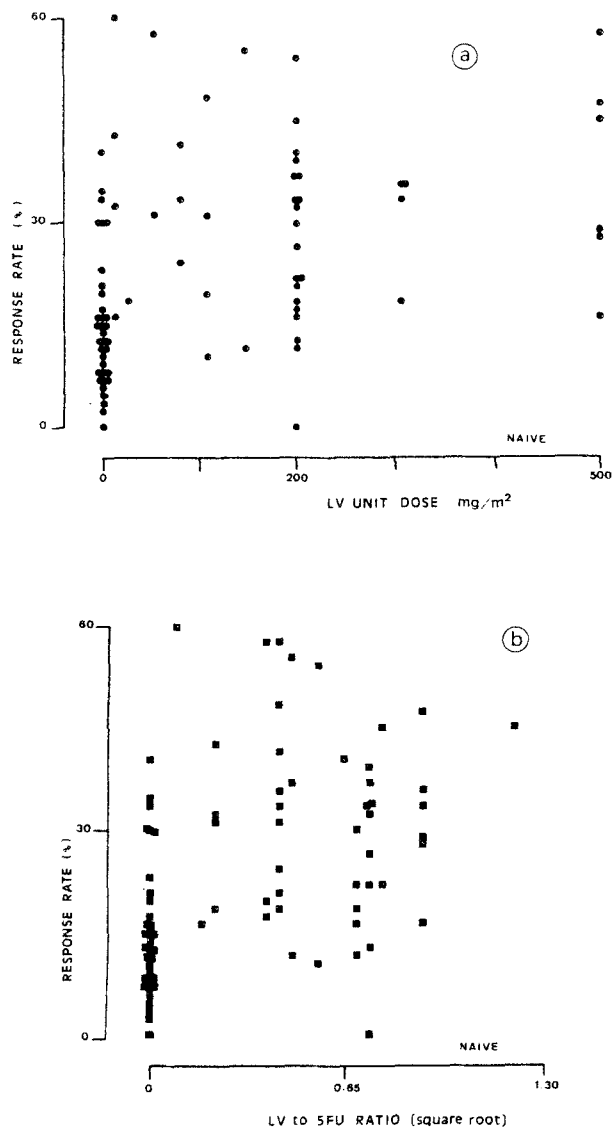

Fig. 2. Absence of relationship between increasing LV doses and response to 5FU chemotherapy in naive patients (scattergram). (a) In abscissae, the LV dose is given as the raw unit dose (mg m^{-2}); (b) the LV dose is given as its ratio to the co-administered 5FU dose (square root transformation).

respectively 26.9 ± 10.5, 21.5 ± 17.5 and 10.2 ± 8.1 ($P = 0.30$). In the series with LV before 5FU, there was a slight trend for better response rates with increasing LV unit doses ($R = 0.460$, $0.05 < P < 0.10$). The 5FU weekly schedule was the most prominent determinant variable, since in that case, the median cumulative response rate was 29% as compared to only 6% (Fig. 3).

The mode of administration of 5FU as a bolus or a continuous infusion, was not found of major importance. However, the different schedules influenced deeply (Table 4) the delivered cumulative 5FU dose, a strong predictor of response as previously discussed. Moreover, according to the parametrization rules, a protracted continuous 5FU infusion was classified as a weekly schedule, the other significant parameter influencing the response rate. Thereby, the *per se* effect of the different modes could have been obscured.

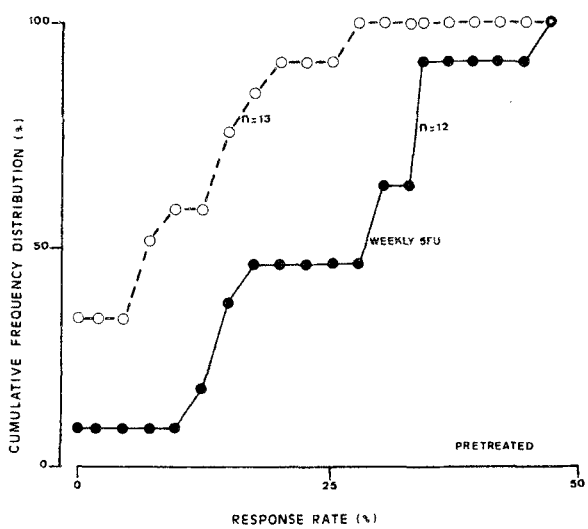

Fig. 3. Achievement of higher response rates in pretreated patients by weekly administration of 5FU (full symbols) as compared to not-weekly schedules (open symbols). (Kolmogorov–Smirnov statistics: $d_{max} = 0.546$, $P < 0.05$).

DISCUSSION

The purpose of meta-analysis is to integrate the findings of several inconclusive or apparently contradictory independent studies so that the accumulated evidence can provide guidance to clinicians in managing their patients or planning new strategies to be tested in randomized trials.[65]

In this study, aiming to reconcile discrepant data on LV benefit in colon cancer treatment with 5FU, each reported response rate was considered for itself and assumed to be a fair estimation of the response in a sample of patients issued from the same colon cancer population. It was hypothesized that the differences among the results could have arisen from differences in the study designs or treatment protocols. To avoid the so-called Simpson's paradox, potentially leading to conclusions of meta-analysis contradicting the conclusions of the contributive studies,[65] no attempt was made to integrate the reported response rates in a single figure. Rather, it was opted to compare the frequency distribution functions of the outcome parameter, as drawn out from the surveyed studies, and to find explanations accounting for the discrepancies in the quoted response rates. To minimize the effect of under-reporting negative studies,[66] the results of phase III ones were pooled with those of phase I/II studies, mixing peer-reviewed published data and abstracts from congresses and précis.

A multivariate regression approach was used for the present meta-analysis of colon cancer chemotherapy with 5FU with or without leucovorin. It was established that up to 35–44% of the variance of the target parameter, the objective response rate, could be determined by a few explanatory variables.

Table 4. Influence of the mode of 5FU administration upon the delivered doses (g m^{-2})

	Bolus regimens		Infusion regimens	
	Discontinuous cycles	Weekly doses	Discontinuous cycles	Protracted treatment
Number of studies	62	32	6	6
5FU loading dose	1.98 ± 0.56	1.10 ± 0.77	3.72 ± 1.30	1.98 ± 0.29
range*	0.4–3.3	0.4–3.5	1.9–5.0	1.4–2.1
5FU total dose	7.02 ± 2.37	8.44 ± 7.02	12.5 ± 2.77	21.3 ± 4.6
range**	1.6–20.0	4.8–31.2	7.5–15.0	12.6–25.2

*Kruskal–Wallis test: $P = 0.0014$.
**$P < 0.0001$.

Patient naiveness to treatment was one of the delineated parameters influencing response (partial R = 0.294, Table 2). The seeming absence of any difference in the response rate between naive and pretreated patients in Table 1, is due to the few studies without LV in the latter case. Indeed, the 20.4 mean percentage should better be compared with the 31.1 figure in naive patients treated with LV and 5FU. So far, prior treatment must be regarded as increasing resistance to 5FU/LV regimens.

The other determining variables delineated in the multivariate models were directly related to the therapeutic regimens. With leucovorin concomitant use, higher response rates to 5FU were more frequently reported for all patients (Table 2) or, more specifically, in naive subjects (Table 3 and Fig. 1). However, the LV effect appeared as an all-or-nothing effect, without any obvious dose–response relationship (Fig. 2). Such a finding is in contradistinction with currently held opinions but in good keeping with some *in vitro* studies.[5] In a randomized clinical study,[6] though low and high LV doses were found equally effective as far as survival or health improvement were concerned, the low LV doses resulted in a higher response rate. As depicted in Fig. 2, the right shift of the response rate distribution curve following LV adjunction, is accompanied by flattening and dispersion. This suggests an increased heterogeneity in response and can hardly be reconciled with the idea that LV promotes 5FU cytotoxicity in an unrestricted way. Obviously, this study does not provide sound evidence for using high LV doses. Doses as low as 20 mg m^{-2} may be quite sufficient. Such a therapeutic approach, the efficacy of which has however to be further confirmed in properly designed clinical studies, could be cost-saving and less toxic, the major drawbacks of LV treatment with 5FU.

In pretreated patients, a possible LV dose–effect exists ($R = 0.431$, $0.05 < P < 0.10$). The point should be addressed in randomized trials, bitterly lacking in this survey of the literature.

The mode of 5FU administration appeared also to be a predictor of response, independent from LV use. Both dose and schedule play important roles. It seems important to deliver the highest possible cumulative doses of 5FU and optimally, in a weekly schedule, despite the obvious interdependency of these variables (Table 4). Since the report of Ansfield *et al.*[67] in 1977, intravenous loading doses of 5FU had been favoured. But, consistently with the findings of this meta-analysis, the higher response rate corresponded also with the most intensive 5FU treatment arm. On the other side, it has long been advocated that loading followed by titrated weekly doses could be the most convenient and efficacious way for giving 5FU.[9] The protracted continuous infusion of 5FU might be the method of choice, allowing the delivery of very high cumulative doses over weeks (Table 4), with minimal toxicity.[43,58,68] The high response rate obtained with such a schedule and low LV doses, as reported by Leichman *et al.*,[43] is worth being mentioned as another piece of evidence strengthening the conclusions of this meta-analysis. In pretreated patients, 5FU weekly administration was the single crucial variable (R = 0.443). However, as already mentioned, the genuine LV effect could have been missed, since LV was used in almost all studies.

The number of evaluated patients in the different studies, the open or the randomized design of the studies, did not emerge as critical parameters, providing indirect evidence for the fair comparability of the reviewed studies. This points also to the present standards of medical reporting higher than those practised in early studies.[69] Other parameters related to the disease and the host, such as the burden and localization of metastases, sex, performance status and age of the subjects,[9] are likely to have influenced the response rate, but could not be investigated since commonly lacking in the reviewed papers.

Though LV may double the expected remission rate, it should not be inferred that a substantial gain in survival would ensue. Indeed, from the seven available controlled trials of 5FU- LV chemotherapy in advanced colorectal cancer, five show an improved response rate, and only two do demonstrate a significant prolongation of patient survival.[70] It has long been known that combination chemotherapy could enhance tumor response, in the order of 40–50% as compared with 20% for 5FU alone, but had failed to alter the overall survival.[9] Such a disappointing result seems all the more inescapable with LV, since the remission rates climb hardly to 31% as compared to the 12–15% obtained with 5FU alone. In other tumors, significant improvements in survival and cure had only followed response rates over 80%.

It is my opinion that leucovorin should not be used lavishly, but could add the finishing touches to a finely tuned-up schedule of 5-fluorouracil. It is proposed that protracted 5FU infusion and low doses of leucovorin should be used. However, this assumption awaits confirmation in properly designed clinical trials.

REFERENCES

1. Development of Folates and Folic Acid Antagonists in Cancer Chemotherapy. *NCI Monographs* **5** (1987).

2. Advances in folate chemotherapy: Status of 5-FU/leucovorin in advanced malignancies. *Cancer* **63**, supplement 6 (1989).
3. Grem J L: Fluorinated pyrimidines, in Chabner B A, Collins J M (eds): *Cancer Chemotherapy, Principles & Practice*, pp. 180–224. Philadelphia, Lippincott (1990).
4. Park J G, Collins J M, Gazdar A F, Allegra C J, Steinberg S M, Greene R F, Kramer B S: Enhancement of fluorinated pyrimidine-induced cytotoxicity by leucovorin in human colorectal carcinoma cell-lines. *J Natl Cancer Inst* **80**, 1560 (1988).
5. Scheithauer W, Temsch E M: A study of various strategies to enhance the cytotoxic activity of 5-fluorouracil/leucovorin in human colorectal cancer cell lines. *Anticancer Res* **9**, 1793 (1989).
6. Poon M A, O'Connell M J, Moertel C G, Wienand H S, Cullinan S A, Everson L K, Krook J E, Maillard J A, Laurie J A, Tschetter L K, Wiesenfeld M: Biochemical modulation of fluorouracil: evidence of significant improvement of survival and quality of life in patients with advanced colorectal carcinoma. *J Clin Oncol* **7**, 1407 (1989).
7. Martin D S, Stolfi R L, Colofiore J R: Failure of high-dose leucovorin to improve therapy with the maximally tolerated dose of 5-fluorouracil: a murine study with clinical relevance? *J Natl Cancer Inst* **80**, 496 (1988).
8. Hryniuk W M, Figueredo A, Goodyear M: Applications of dose intensity to problems in chemotherapy of breast and colorectal cancer. *Sem Oncol* **14**, 3 (1987).
9. Greenspan E M, Ratner L H: Gastrointestinal cancer chemotherapy, in Greenspan E M (ed): *Clinical Interpretation and Practice of Cancer Chemotherapy*, pp. 195–241. New York, Raven Press (1982).
10. Bleyer W A: New vistas for leucovorin in cancer chemotherapy. *Cancer* **63**, 995 (1989).
11. Asbury R F, Boros L, Brower M, Woll J, Chang A, Bennett J: 5-Fluorouracil and high-dose folic acid treatment for metastatic colon cancer. *Am J Clin Oncol* **10**, 47 (1987).
12. Barone C, Astone A, Garufi C, Grieco A, Cavallero A, Netri G, Rossi S, Cassano A, Ricevuto E, Noviello M R: High-dose folinic acid (HDFA) combined with 5-fluorouracil (5-FU) in first line chemotherapy of advanced large bowel cancer. *Eur J Cancer Clin Oncol* **23**, 1303 (1987).
13. Bertrand M, Doroshow J H, Multhauf P, Blayney D W, Carr B I, Cecchi G, Goldberg D, Leong L, Margolin K, Metter G, Staples R: High-dose continuous infusion folinic acid and bolus 5-fluorouracil in patients with advanced colorectal cancer: a phase II study. *J Clin Oncol* **4**, 1058 (1986).
14. Budd G T, Fleming T R, Bukowsi R M, McCracken J D, Rivkin S E, O'Bryan R M, Balcerzak S P, Macdonald J S: 5-Fluorouracil and folinic acid in the treatment of metastatic colorectal cancer: a randomized comparison. A Southwest Oncology Group study. *J Clin Oncol* **5**, 272 (1987).
15. Cunningham J, Bukowski R M, Budd G T, Weick J K, Purvis J: 5-Fluorouracil and folinic acid: a phase I-II trial in gastrointestinal malignancy. *Invest New Drugs* **2**, 391 (1984).
16. Di Costanzo F, Bartolucci R, Padalino D, Brugia M, Buzzi F: High-dose folinic acid and 5-fluorouracil in advanced colorectal cancer. *Cancer Invest* **6**, 133 (1988).
17. Doroshow J H, Multhauf P, Leong L, Margolin K, Litchfield T, Akman S, Carr B, Bertrand M, Goldberg D, Blayney D, Odujinrin O, DeLap R, Shuster J, Newman E: Prospective randomized comparison of fluorouracil versus fluorouracil and high-dose continuous infusion of leucovorin calcium for the treatment of advanced measurable colorectal cancer in patients previously unexposed to chemotherapy. *J Clin Oncol* **8**, 491 (1990).
18. Erlichman C, Fine S, Wong A, Elhakim T: A randomized trial of fluorouracil and folinic acid in patients with metastatic colorectal carcinoma. *J Clin Oncol* **6**, 469 (1988).
19. Hines J D, Zakem M H, Adelstein D J, Rustum Y M: Treatment of advanced-stage colorectal adenocarcinoma with fluorouracil and high-dose leucovorin calcium: a pilot study. *J Clin Oncol* **6**, 142 (1988).
20. Hines J D, Adelstein D J, Spiess J L, Giroski P, Carter S G: Efficacy of high-dose oral leucovorin and 5-fluorouracil in advanced colorectal carcinoma. Plasma and tissue pharmacokinetics. *Cancer* **63**, 1022 (1989).
21. Laufman L R, Brenckman W D, Stydnicki K A, Morgan E D, Collier M, Knick V B, Duch D S, Mullin R, Ferone R: Clinical experience with leucovorin and 5-fluorouracil. *Cancer* **63**, 1031 (1989).
22. Machover D, Goldschmidt E, Chollet P, Metzger G, Zittoun J, Marquet J, Vandenbulcke J M, Misset J L, Schwarzenberg L, Fourtillan J B, Gaget H, Mathé G: Treatment of advanced colorectal and gastric adenocarcinoma with 5-fluorouracil and high-dose folinic acid. *J Clin Oncol* **4**, 685 (1986).
23. Madajewicz S, Petrelli N, Rustum Y M, Campbell J, Herrera L, Mittelman A, Perry A, Creaven P J: Phase I-II trial of high-dose calcium leucovorin and 5-fluorouracil in advanced colorectal cancer. *Cancer Res* **44**, 4667 (1984).
24. Petrelli N, Herrera L, Rustum Y, Burke P, Creaven P, Stulc J, Emrich L J, Mittelman A: A prospective randomized trial of 5-fluorouracil versus 5-fluorouracil and high-dose leucovorin versus 5-fluorouracil and methotrexate in previously untreated patients with advanced colorectal carcinoma. *J Clin Oncol* **5**, 1559 (1987).
25. Petrelli N, Douglass H O, Herrera L, Russell D, Stablein D M, Bruckner H W, Mayer R J, Schinella R, Green M D, Muggia F M, Megibow A, Greenwald E S, Bukowski R M, Harris J, Levin B, Gaynor E, Loutfi A, Kalser M H, Barkin J S, Benedetto P, Wooley P V, Nauta R, Weaver D W, Leichman L P, for the Gastrointestinal Tumor Study Group: The modulation of fluorouracil with leucovorin in metastatic colorectal carcinoma: a prospective randomized phase III trial. *J Clin Oncol* **7**, 1419 (1989).
26. Rougier P, Droz J P, Ducreux M, Kac J, Spielman M, Crespon B, Ley G, Zimmermann P, Theodore C, Chavy A, Rouëssé J, Amiel J L: Treatment of advanced colonic and rectal adenocarcinoma: combination of 5-fluorouracil and high-dose folinic acid. *Bull Cancer* **70**, 434 (1983).

27. Scholnik A P, Arnold D J, Walker W S, Schwenke P, Suhrland L G, Balcueva E P, Dimitrov N V: High-dose folinic acid and 5-fluorouracil in the treatment of advanced colon cancer. *Am J Clin Oncol* **11**, 558 (1988).
28. Valone F H, Friedman M A, Wittlinger P S, Drakes T, Eisenberg P D, Malec M, Hannigan J F, Brown B W: Treatment of patients with advanced colorectal carcinomas with fluorouracil alone, high-dose leucovorin plus fluorouracil, or sequential methotrexate, fluorouracil, and leucovorin: a randomized trial of the Northern California Oncology Group. *J Clin Oncol* **7**, 1427 (1989).
29. van Groeningen C J, Peters G J, Pinedo H M: Lack of effectiveness of combined 5-fluorouracil and leucovorin in patients with 5-fluorouracil-resistant advanced colorectal cancer. *Eur J Cancer Clin Oncol* **25**, 45 (1989).
30. Wilke H, Schmoll H J, Schober C, Stahl M, Preusser P, Freund M, Poliwoda H: Folinic acid (FA) plus 5-fluorouracil (FU) in progressive advanced colorectal cancer. *Biomed Pharmacother* **42**, 373 (1988).
31. O'Connell M, Maillard J, Martin J, Fitzgibbons R, Nagorney D, Wieand H, Tschetter L, Krook J: A controlled trial of regional intra-arterial Fudr versus systemic 5FU for the treatment of metastatic colorectal cancer confined to the liver. *Proc Am Soc Clin Oncol* **8**, 98 (1989).
32. Israel K, Kemeny N, Vinciguerra V, Rosenbluth R, Bosselli B, Cochran C, Niedzwiecki D, Chapman D, Sheehan K: Randomized study of continuous infusion 5-fluorouracil (FU) vs FU plus cisplatin (DDP) in patients with metastatic colorectal cancer. *Proc Am Soc Clin Oncol* **8**, 100 (1989).
33. Di Costanzo F, Bartolucci R, Sofra M, Calabresi F, Malacarne P, Belsanti V, Boni C, Bacchi M: 5-Fluorouracil (5-FU) alone vs high dose folinic acid (FA) and 5-FU in advanced colorectal cancer (CA): a randomized trial of the Italian Oncology Group for Clinical Research (GOIRC). *Proc Am Soc Clin Oncol* **8**, 106 (1989).
34. Labianca R, Pancera G, Beretta G, Aitini E. Barni S, Beretta A, Beretta G D, Cesana B, Comella G, Cozzaglio L, Cristoni M, Deponte A, Frontini L, Geccherle A, Gottardi O, Martignoni G, Smerieri F, Zadro A, Zabinoni A, Luporini G, for the the FONCAD-Medical Treatment Group: A randomized study of intravenous fluorouracil ± folinic acid (f) in advanced metastatic colorectal carcinoma (AMC). *Proc Am Soc Clin Oncol* **8**, 118 (1989).
35. Bruckner H, Glass L L, Chesser M R, McKenna A, Holland J F: Leucovorin's (LV) dose-dependent effect on high-dose (HD) every two week 5-fluorouracil's (5FU) neurotoxicity (NT) and efficacy against colorectal cancer. *Proc Am Soc Clin Oncol* **8**, 120 (1989).
36. Macchiavelli M, Leone B, Romero A, Rabinovich M, Vallejo C, Bianco A, Pérez J, Rodriguez R, Cuevas M, Alvarez L, Chacon R, Hannois A: Advanced colorectal carcinoma (CR): a prospective randomized trial of sequential methotrexate (MTX)–5-fluorouracil (5FU) vs 5FU alone. *Proc Am Soc Clin Oncol* **9**, 102 (1990).
37. Weinerman B, Shah A, Fields A, Kerr I, Cripps C, Shepherd F, Wierzbicki R, Temple W, Maroun J, Bogues W, Pater J: A randomized trial of continuous systemic infusion (SI) vs bolus therapy (B) with 5 fluorouracil (5FU) in metastatic measurable colorectal cancer (MCC). *Proc Am Soc Clin Oncol* **9**, 103 (1990).
38. Chua L, Ardalan B, Sridhar K S, Hussein A, Richman S, Robinson D, Livingston A: A phase II study of short term protracted infusion with high dose 5-fluorouracil (5-FU) with leucovorin (LV) in colorectal carcinoma. *Proc Am Soc Clin Oncol* **9**, 104 (1990).
39. O'Connell M, Poon M, Wieand H, Moertel C, Krook J, Gerstner J: Biochemical modulation of 5-fluorouracil (5FU) with leucovorin (LV): confirmatory evidence of improved therapeutic efficacy in the treatment of advanced colorectal cancer. *Proc Am Soc Clin Oncol* **9**, 106 (1990).
40. Brenckman W D, Laufman L R, Adamkiewicz B B, Collier M A, Sullivan B A, and the BW Colorectal Cancer Cooperative Group. Is fluorouracil (FU) plus placebo (P) as effective as equitoxic doses of FU plus high dose oral leucovorin (LV) in colorectal cancer? *Proc Am Soc Clin Oncol* **9**, 109 (1990).
41. Bonaventura A, Ackland S, Stewart J: Intermediate dose leucovorin and 5-fluorouracil (LV/5FU) in metastic colorectal cancer. *Proc Am Soc Clin Oncol* **9**, 111 (1990).
42. Köhne-Wömpner C H, Wilke H, Weiss J, Hiddemann W, Gropp C, Schmitz-Hübner U, Lohrmann H P, Bodenstein H, Preusser P, Urbanitz D, Hotz J, Preiß J, Knuth A, Schwark J, Bade J, Kohler B, Schmoll H J: 5-FU, folinic acid (FA) ± dipyridamol (D) in advanced and progressive colorectal cancer (CC) — A randomized multi-center phase II trial. *Proc Am Soc Clin Oncol* **9**, 123 (1990).
43. Leichman C, Leichman L, Spears C P, Jeffers S, Chavez J, Groshen S, Waugh W, Rosen P: Phase II study of protracted infusion 5-fluorouracil (5FU) with weekly leucovorin (LV) in disseminated, measurable adenocarcinoma of the large bowel. *Proc Am Soc Clin Oncol* **9**, 129 (1990).
44. Lokich J J, Ahlgren J D, Cantrell J, Heim W J, Wampler G L, Gullo J J, Fryer J G, Alt D E: A prospective randomized comparison of protracted infusional 5-fluorouracil with or without bolus cisplatin in metastatic colorectal carcinoma. A Mid-Atlantic Oncology Program study. *Cancer* **67**, 14 (1991).
45. Alberto P, Mermillod B, Germano G, Kaplan S, Weber W, Joss R, Spati B, Martz G, Cavalli F: A randomized comparison of doxifluridine and fluorouracil in colorectal carcinoma. *Eur J Cancer Clin Oncol* **24**, 559 (1988).
46. Andersen E, Pedersen H: Oral ftorafur versus intravenous 5-fluorouracil. A comparative study in patients with colorectal cancer. *Acta Oncol* **26**, 433 (1987).
47. Bjerkeset T, Fjösne H E: Comparison of oral ftorafur and intravenous 5-fluorouracil in patients with advanced cancer of the stomach, colon or rectum. *Oncology* **43**, 212 (1986).
48. Buroker T R, Moertel C G, Fleming T R, Everson L K, Cullinan S A, Krook J E, Maillard J A,

Marschke R F, Klaassen D J, Laurie J A, Moon M D: A controlled evaluation of recent approaches to biochemical modulation or enhancement of 5-fluorouracil therapy in colorectal carcinoma. *J Clin Oncol* **3**, 1624 (1985).

49. Cortesi E, Aschelter A M, Giocchini N, Pelligrini A, Frati L, Ficorella C, Mazzei N, Marchetti P: Efficacy and toxicity of 5-fluorouracil and folates in advanced colon cancer. *J Chemother* **2**, suppl 1, 47 (1990).
50. De Gramont A, Krulik M, Cady J, Lagadec B, Maisani J E, Loiseau J P, Grange J D, Gonzalez-Canali G, Demuynck B, Louvet C, Seroka J, Dray C, Debray J, for the GERCOD: High-dose folinic acid and 5-fluorouracil bolus and continuous infusion in advanced colorectal cancer. *Eur J Cancer Clin Oncol* **24**, 1499 (1988).
51. Douglass H O, Lavin P T, Woll J, Conroy J F, Carbone P: Chemotherapy of advanced measurable colon and rectal carcinoma with oral 5-fluorouracil, alone or in combination with cyclophosphamide or thioguanine, with intravenous 5-fluorouracil or beta-2'-deoxythioguanosine or with oral 3(4-methyl-cyclohexyl)-1(2-chlorethyl)-1-nitrosourea. A phase II-III study of the Eastern Cooperative Oncology Group. *Cancer* **42**, 2538 (1978).
52. Engstrom P F, MacIntyre J M, Mittelman A, Klaassen D J: Chemotherapy of advanced colorectal carcinoma: fluorouracil alone vs two drug combinations using fluorouracil, hydroxyurea, semustine, dacarbazine, razoxane, and mitomycin. A phase III trial of the Eastern Cooperative Oncology Group. *Am J Clin Oncol* **7**, 313 (1984).
53. Hansen R, Quebbeman E, Ausman R, Frick J, Ritch P, Schulte W, Haas C, Beatty P, Anderson T: Continuous systemic 5-fluorouracil infusion in advanced colorectal cancer: results in 91 patients. *J Surg Oncol* **40**, 177 (1989).
54. Kemeny N, Israel K, Niedzwiecki D, Chapman D, Botet J, Minsky B, Vinciguerra V, Rosenbluth R, Bosselli B, Cochran C: Randomized study of continuous infusion fluorouracil versus fluorouracil plus cisplatin in patients with metastatic colorectal cancer. *J Clin Oncol* **8**, 313 (1990).
55. Lerol A, Filippi M H, Belpomme D, Olivier A, René E: Association fluoro-uracile-acide folinique pour le traitement des cancers digestifs métastasés. *Entretiens de Bichat* **7**, 34–36 (1988).
56. Labianca R, Pancera G, Cesana B, Clerici M, Montinari F, Luporini G: Cisplatin + 5-fluorouracil versus 5-fluorouracil alone in advanced colorectal cancer: a randomized study. *Eur J Cancer Clin Oncol* **24**, 1579 (1988).
57. Loehrer P J, Turner S, Kubilis P, Hui S, Correa J, Ansari R, Stephens D, Woodburn R, Meyer S: A prospective randomized trial of fluorouracil versus fluorouracil plus cisplatin in the treatment of metastatic colorectal cancer: a Hoosier Oncology Group trial. *J Clin Oncol* **6**, 642 (1988).
58. Lokich J J, Ahlgren J D, Gullo J J, Philips J A, Fryer J G: A prospective randomized comparison of continuous infusion fluorouracil with a conventional bolus schedule in metastatic colorectal carcinoma: A Mid-Atlantic Oncology Program study. *J Clin Oncol* **7**, 425 (1989).
59. Nordic Gastrointestinal Tumor Adjuvant Therapy Group: Superiority of sequential methotrexate, fluorouracil, and leucovorin to fluorouracil alone in advanced symptomatic colorectal carcinoma: a randomized trial. *J Clin Oncol* **7**, 1437 (1989).
60. Presant C A, Denes A E, Liu C, Bartolucci A A: Prospective randomized reappraisal of 5-fluorouracil in metastatic colorectal carcinoma. A comparative trial with 6-thioguanine. *Cancer* **53**, 2610 (1984).
61. Quebbeman E, Ausman R, Hansen R, Becker T, Caballero G, Ritch P, Jenkins D, Blake D, Tangen L, Schulte W: Long-term ambulatory treatment of metastatic colorectal adenocarcinoma by continuous intravenous infusion of 5-fluorouracil. *J Surg Oncol* **30**, 60 (1985).
62. Shah A, MacDonald W, Goldie K, Gudauskas G, Brisebois B: 5-FU infusion in advanced colorectal cancer: a comparison of three dose schedules. *Cancer Treat Rep* **69**, 739 (1985).
63. Windschitl H, Scott M, Schutt A, McCormack G, Everson L, Cullinan S, Gerstner J, Krook J, Laurie J, Shreck R, Marschke R, Del Pfeifle: Randomized phase II studies in advanced colorectal carcinoma: a North Central Cancer Treatment Group study. *Cancer Treat Rep* **67**, 1001 (1983).
64. McGuire B W, Sia L L, Haynes J D, Kisicki J C, Gutierrez M L, Stokstad E L R: Absorption kinetics of orally administered leucovorin calcium. *NCI Monographs* **5**, 47 (1987).
65. Halvorsen K D: Combining results from independent investigations. Meta-analysis in medical research, in Bailar J C III, Mosteller F (eds): *Medical Uses of Statistics*, pp. 392–416. Waltham, NEJM Books (1986).
66. Simes R J: Publication bias: the case for an international registry of clinical trials. *J Clin Oncol* **4**, 1529 (1986).
67. Ansfield F, Klotz J, Nealon T, Ramirez G, Minton J, Hill G, Wilson W, Davis H, Cornell G: A phase III study comparing the clinical utility of four regimens of 5-fluorouracil: a preliminary report. *Cancer* **39**, 34 (1977).
68. Lokich J J: Colorectal cancer, in Lokich J J (ed): *Cancer Chemotherapy by Infusion*, pp. 291–303. Chicago, Precept Press (1987).
69. Moertel C G: Chemotherapy of gastrointestinal cancer. *N Engl J Med* **299**, 1049 (1978).
70. Mayer R J, O'Connell M J, Tepper J E, Wolmark N: Status of adjuvant therapy for colorectal cancer. *J Natl Cancer Inst* **81**, 1359 (1989).

APPENDIX 1

Authors only referenced in Lederle's précis: Bruckner *et al.* (1982), Byrne *et al.* (1984), Lopez *et al.* (1984), Kaplan and Rivkin (1984), Price (1984), Mortimer and Higano (1985), Greene *et al.* (1986), Loffler and Hausamen (1986), Lottici *et al.* (1986), Lucas *et al.* (1986), Raju *et al.* (1986), Zakem *et al.* (1986), Marini *et al.* (1987), Sanzo *et al.* (1987), Beretta *et al.* (1988), Hiddemann *et al.* (1988), Kurschel *et al.* (1988), La Ciuria *et al.* (1988), Migeod *et al.* (1988), Nobile *et al.* (1988), Schalhorn *et al.* (1988), Seeger *et al.* (1988).

APPENDIX 2

For oral LV, a 0.31 conversion factor was used for intravenous-dose-like standardization, as established by McGuire *et al.*[65] Other interpretative rules were used: when drug doses were given per kg weight, a correcting factor of 37 was used for transformation in dose m^{-2}; when absolute doses were given, doses m^{-2} were calculated by dividing by 1.73; when an interval or a dose range was given, the median value was used for calculation; when it was possible, the actual total cumulative 5FU dose was utilized, either as given by the authors or as calculated from explicit rules for drug escalation or desescalation. In regression analysis, the continuous variables were used as raw values or as mathematical transformees, either square roots for variables including zero or natural logarithms (ln), to get the tightest fit of the model and the optimal coefficient of determination. The logistic response rate was computed as ln(RR%) minus ln(100 − RR%). By definition, RR% = 0% or 100% were equated respectively to 0.1% and 99.9% for calculation.