BMC Cancer

Research article

Schedule-selective biochemical modulation of 5-fluorouracil in advanced colorectal cancer – a phase II study

Shannon K Tomlinson [1], Susan A Melin [2], Vetta Higgs [2], Douglas R White [2], Paul Savage [2], Douglas Case [3] and A William Blackstock *[1]

Address: [1]Dept of Radiation Oncology, Wake Forest University School of Medicine, Winston-Salem, NC 27157, USA, [2]Div. of Medical Oncology, Wake Forest University School of Medicine, Winston-Salem, NC 27157, USA and [3]Dept of Radiation Biostatics, Wake Forest University School of Medicine, Winston-Salem, NC 27157, USA

E-mail: Shannon K Tomlinson - stomlins@wfubmc.edu; Susan A Melin - melin@wfubmc.edu; Vetta Higgs - higgs@wfubmc.edu; Douglas R White - dwhite@wfubmc.edu; Paul Savage - psavage@wfubmc.edu; Douglas Case - dcase@wfubmc.edu; A William Blackstock* - ablackst@wfubmc.edu

*Corresponding author

Published: 2 May 2002

BMC Cancer 2002, **2**:9

Received: 21 November 2001
Accepted: 2 May 2002

This article is available from: http://www.biomedcentral.com/1471-2407/2/9

Abstract

Background: 5-fluorouracil remains the standard therapy for patients with advanced/metastatic colorectal cancer. Pre-clinical studies have demonstrated the biological modulation of 5-fluorouracil by methotrexate and leucovorin. This phase II study was initiated to determine the activity and toxicity of sequential methotrexate – leucovorin and 5-fluorouracil chemotherapy in patients with advanced colorectal cancer.

Methods: Ninety-seven patients with metastatic colorectal cancer were enrolled onto the study. Methotrexate – 30 mg/m^2 was administered every 6 hours for 6 doses followed by a 2 hour infusion of LV – 500 mg/m^2. Midway through the leucovorin infusion, patients received 5-fluorouracil – 600 mg/m^2. This constituted a cycle of therapy and was repeated every 2 weeks until progression.

Results: The median age was 64 yrs (34–84) and the Eastern Cooperative Group Oncology performance score was 0 in 37%, 1 in 55% and 2 in 8% of patients. Partial and complete responses were seen in 31% of patients with a median duration of response of 6.4 months. The overall median survival was 13.0 months. The estimated 1-year survival was 53.7%. Grade III and IV toxic effects were modest and included mucositis, nausea and vomiting.

Conclusions: This phase II study supports previously reported data demonstrating the modest clinical benefit of 5-FU modulation utilizing methotrexate and leucovorin in patients with metastatic colorectal cancer. Ongoing studies evaluating 5-fluorouracil modulation with more novel agents (Irinotecan and/or oxaliplatin) are in progress and may prove encouraging.

Background

Although a number of recently introduced chemotherapeutic agents have demonstrated significant anti-tumor activity in advanced colorectal cancer, [1–9] standard therapy for patients with metastatic disease remains 5-fluorouracil (5-FU) – based chemotherapy.[10] A vast series of pre-clinical and clinical studies have suggested 5-FU is a more active anti-tumor agent when modulated by

a host of compounds including leucovorin, [11–13] methotrexate,[14] folinic acid, [15,16] N-phosphonacetyl-L-aspartic acid (PALA) [17–22] and recombinant interferon alfa-2a (IFNα-2a). [23,24] Protracted venous infusion (PVI) 5-FU regimens have also resulted in increased response rates in some studies, but only results in a modest benefit in median survival. [25,26] Oral fluoropyrimidines and oral regimens using prodrugs of 5-FU or inhibitors of dihydropyrimidine dehydrogenase (DPD) which pharmacologically simulate the intravenous continuous infusion administration of 5-FU are currently under clinical evaluation. Although these studies are important and may demonstrate equivalency, it is not clear these oral compounds will result in a significant improvement compared to the intravenous infusion of 5-FU.[27]

Potentiation of the anti-tumor activity of 5-FU by methotrexate (MTX) and leucovorin (LV) requires careful scheduling to achieve the most favorable interactions between these 3 drugs. In vitro and in vivo studies have both suggested a synergistic as well as antagonistic effect when 5-FU, MTX and LV are used in combination. [28–32] In this report, two modulators of 5-FU were used in a sequential manner, anticipating an enhancement of the efficacy of 5-FU before the development of 5-FU resistance and subsequent disease progression. The order of administration of the three agents was chosen to optimally exploit the reported differing mechanisms of biochemical modulation. The objectives of this phase II study were to determine the activity and toxicity of MTX/LV/5-FU in patients with advanced colorectal cancer.

Patients and Methods

Between August of 1987 and August of 1988, 97 patients with advanced colorectal cancer were enrolled onto this study. Informed consent was obtained in writing from all patients according to Institutional Review Board guidelines. Eligibility criteria included histologic proof of metastatic colorectal carcinoma and the presence of at least one bidimensionally measurable lesion on computed tomography scan. All patients were required to have an Eastern Cooperative Oncology (ECOG) Group performance score of 0 – 2 and have a life expectancy greater than 2 months. Patients also were required to meet the following criteria: adequate bone marrow, renal, and hepatic function as evidenced by a white blood cell count = 4,000 cells/ul, platelet count = 100,000 cells/ul, creatinine = 2.0 mg/dl or creatinine clearance of 60 ml/min and a serum bilirubin = 1.5 mg/dl and no serious intercurrent medical or mental illness. Patients could not have received prior chemotherapy (excluding adjuvant chemotherapy).

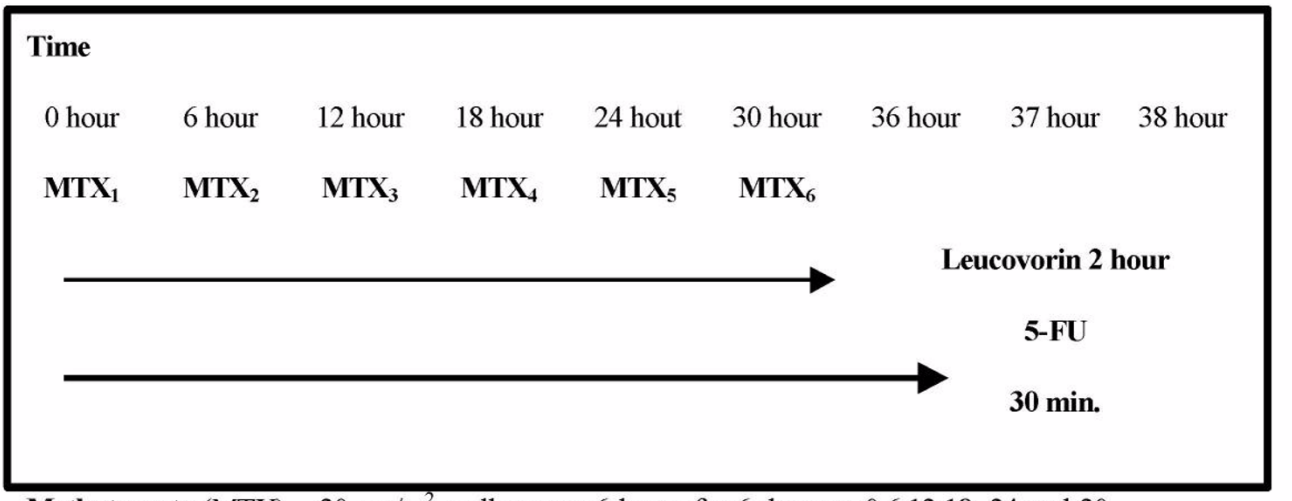
Treatment Plan

Consistent with the traditional design of phase II studies, all patients received therapy as outlined in the treatment schema in figure 1. Methotrexate was administered at a dose of 30 mg/m^2 orally every 6 hours on an empty stomach for 6 doses. At hour 36 a 2 hour intravenous infusion of leucovorin was administered at a dose of 500 mg/m^2. Midway through the leucovorin infusion, 5-FU at a dose of 600 mg/m^2 was given as a 30 minute intravenous bolus infusion. This represented a course of therapy and was repeated every two weeks. Dose modifications for hematologic toxicity were made on the basis of counts on the day of treatment. For a white blood count = 3,500 cells/ul or a platelet count = 100,000 platelets/ul – patients received full doses of all three drugs as described above. For a white blood count between 3,000 and 3,500 cells/ul and a platelet count = 100,000 platelets/ul, a 50% dose reduction of all three drugs was performed. For white blood counts less than 3,000 cells/ul and a platelet count < 100,000 platelets/ul – all therapy was held until the counts had fully recovered (white blood count = 3,500 cells/ul and a platelet count = 100,000). Patients experiencing a white blood count = 2,000 and/or a platelet count = 75,000 any time during therapy subsequently received 50% of the calculated dose even if the counts fully recovered.

Weekly complete blood counts, including platelet counts and white blood count differentials were conducted to determine the level of myelosuppresion. Before every other cycle of treatment (q 4 weeks), a chest x-ray and complete chemistry panel including electrolytes and serum creatinine were obtained. All lesions were measured bidimensionally and assessed for changes by either an imaging study (computed tomography, chest x-ray, ultrasound) or by clinical examination to determine response to therapy. Treatment was continued until disease progression became evident or side effects became intolerable.

Statistical Methods

This study was planned to recruit 50 evaluable patients, allowing us to estimate objective tumor response to within +/- 15% with 95% confidence. Additional patients were subsequently accrued to increase the precision of our estimates of response and survival. The trial was conducted with one interim analysis after nine evaluable patients. If no patient had experienced an objective tumor response, the study would have been terminated. The two-stage design used in this study was first proposed by Ed Gehan in 1961 (Gehan E.A. The determination of the number of patients required in a preliminary and a follow-up trial of a new chemotherapeutic agent. J. Chron. Dis. 13:346–353, 1961). The probability of observing no responses was less than 5% if the true response was 30% or greater. Progression-free survival was defined as the time from study entry to the first observation of disease progression or death as



Methotrexate (MTX) – 30 mg/m^2 orally every 6 hours for 6 doses at 0,6,12,18, 24 and 30 hours.
Leucovorin – (LV) 500 mg/m^2 by 2 hour infusion in 250 cc D5/W from 36-38 hours.
5-fluorouracil – (5-FU) 600 mg/m^2 at hour 37 by rapid intravenous bolus midway during the leucovorin infusion.

Figure 1
Treatment Schema

a result of any cancer. Survival was defined as the time of study entry until death as a result of any cause. These time-to-event parameters were summarized using Kaplan-Meier product-limit estimates. Log rank tests were used to assess which factors were univariately predictive of the time to progression and survival, and the Cox proportional hazards regression model was used to assess which factors were jointly predictive of these outcomes. The statistical analysis was performed using the SAS package, version 6.12 (SAS Institute, Cary, NC).

Results

From August of 1987 through September of 1988, 97 patients were accrued to this study with two patients subsequently found to be ineligible. Data was not collected for the first patient due to a protocol violation and a second patient was found to have an ovarian primary cancer after enrollment. Patient characteristics for the 95 eligible patients are listed in table 1. The median age was 64. The median ECOG performance score was 1. All patients had a histologic diagnosis of adenocarcinoma. The major sites of metastatic disease were liver, lung, soft tissue and lymph node. None of the patients remain on study; 71 patients progressed on study, 16 patients were removed from study either at the discretion of the treating physician or patient refusal to continue treatment, 3 patients were removed from study due to toxicity, 2 patients expired while receiving therapy. Three patients were removed for other reasons; one patient failed to adhere to the treatment schedule, one relocated, and a third patient never received therapy due to rapid medical decline.

Treatment and Response

A total of 1257 cycles of treatment were delivered during the trial (median 9 cycles; range 0 to 41 cycles). Table 2 reflects the average percent of ideal dose given at each selected cycle of treatment. Even after 25 cycles of therapy, at least 75% of the ideal doses for all drugs were being administered. Ninety-three of the 95 eligible patients enrolled onto the study were assessable for toxicity. Mucositis, nausea/vomiting and diarrhea were the most commonly observed toxicities and are listed in table 3. The hematologic toxicities are also shown in table 3 and in general were infrequently observed. Overall the regimen was well tolerated.

Table 1: Patient Characteristics

Total Patients	95	100%
Median Age Years (range)	64 (34–84)	
Sex		
Female	49	52%
Male	46	48%
Race		
African American	21	22%
Caucasian	74	78%
Primary Disease		
Colon	82	86%
Rectal	13	14%
Performance Status		
0	35	37%
1	52	55%
2	8	8%

Table 2: Percent of Ideal Dose (mean ± SD)

Course	# of Patients	% Methotrexate	% Leucovorin	% 5-FU
1	94	98.9 ± 7.2	99.9 ± 0.7	98.8 ± 10.3
5	76	90.1 ± 18.9	91.8 ± 17.4	86.3 ± 24.4
10	48	88.6 ± 19.9	87.8 ± 20.2	84.7 ± 44.2
15	34	90.3 ± 19.8	93.5 ± 16.4	89.0 ± 19.7
20	25	83.0 ± 24.2	89.7 ± 19.3	84.3 ± 22.9
25	15	76.7 ± 28.2	80.7 ± 26.7	77.2 ± 28.7

Of the 90 patients evaluable for response, ten patients (11%) demonstrated a complete response of their disease (median response duration 14.3 months). Remarkably, 3 of the 10 patients remain alive, 2 without evidence of disease with 51, 73 and 138 months of follow-up, respectively. An additional 18 patients (20%) attained partial responses; 16 have died of progressive disease while 2 patients are alive and without disease progression. Table 4 reflects the overall best response for the entire patient cohort.

The overall estimated median time to progression was 5.6 months. The estimated progression free survival at 12 months was 25.5% (standard error 4.5%). The overall estimated median survival time was 13.0 months. The estimated survival rate at 12 months was 53.7% (standard error 5.1%). Table 5 reflects overall survival for various subgroups of patients. The prognostic factors found to be significant on univariate analysis were ECOG performance score, gender and number of metastatic sites. Female patients, patients with fewer metastatic sites and patients with an ECOG performance 0 had a statistically significant improved survival. Survival by metastatic site is shown in Table 6. Those patients without lung metastasis and those without intra-abdominal metastasis had longer survival times. In the multivariate analysis, presence of lymph nodes, lower LDH levels female sex, better performance status and fewer metastatic sites were statistically predictive of longer survival (Data not shown).

Table 3: Toxicity

Hematologic Toxicity	# of Patients	Grade II (%)	Grade III (%)	Grade IV (%)
Toxicity				
Neutropenia	93	10%	1%	0%
Thrombocytopenia	93	8%	0%	0%
Anemia	93	18%	4%	0%
Non Hematologic Toxicity				
Diarrhea	93	17%	8%	0%
Mucositis	93	30%	8%	1%
Nausea/Vomiting	93	9%	11%	1%

Table 4: Best Objective Response

Response	# of Patients (%) (n = 90 evaluable)	95% C. I.
Complete	10 (11%)	5.5 – 19.5
Partial	18 (20%)	12.3 – 29.8
Complete/Partial	28 (31%)	21.8 – 41.7
Stable Disease	33 (37%)	26.8 – 47.5
Progressive Disease	29 (32%)	22.8 – 42.9

Discussion

The rationale, at least in part, for this phase II study comes from pre-clinical data suggesting MTX and LV potentiate the anti-tumor activity of 5-FU. The cytotoxic effects of 5-FU are mediated through inhibition of DNA and/or RNA synthesis.[33] DNA synthesis inhibition is mediated by the binding of the active 5-FU metabolite 5-fluoro-2'deoxyuridine – 5-monophosphate (F-dUMP) to the enzyme

Table 5: Subgroup analysis of survival

Characteristic	# of Patients	# Deaths	Median Survival (months)	Log rank *p*-value
Overall	95	90	13.0	
Age				
= 60	40	40	12.4	.1170
> 60	55	50	13.9	
Sex				
Female	49	44	15.8	.0113
Male	46	46	12.1	
Race				
African American	21	20	14.8	.5929
Caucasian	74	70	12.6	
Performance Status				
0	35	32	17.9	.0001
1	50	50	11.4	
2	8	8	4.8	
LDH				
= 230	44	41	15.4	.1255
> 230	47	45	10.8	
Alkaline Phosphatase				
= 130	48	46	14.3	.1732
> 130	46	43	12.3	

Table 6: Outcome in relation to sites of metastatic disease

Site of Metastasis	# of Patients	Median Survival (mo.)	*p* – value
Lung			
No	64	13.1	.0474
Yes	31	11.9	
Liver			
No	24	14.7	.5114
Yes	71	12.7	
[1] Intra-abdominal			
No	59	17.1	.0043*
Yes	36	9.3	
Lymph Nodes			
No	85	13.2	.3319
Yes	10	12.5	
# of Metastasis			
1	49	17.1	.0009*
2	35	10.9	
3	11	6.5	

[1]Intra-abdominal metastasis refers to disease involving the colon, rectum, pancreas, abdominal wall, bladder, prostate, pelvis or adrenals.

thymidylate synthetase (TS). This binding is enhanced by high concentrations of reduced folate cofactors.[34] Although this ternary complex is covalent, it dissociates with a half-life of 2–3 hours in the absence of excess 5, 10-methylene tetrahydrofolate. Thus high levels of 5, 10-methylene tetrahydrofolate derived from LV not only allows optimum ternary complex formation, but also prevents subsequent breakdown of the complex.[35] Additional in vitro studies suggest cells may need prolonged exposure to reduced folates in order to obtain maximum inhibition of TS by FdUMP.[36] A second observed means of potentiating the cytotoxic effects of 5-FU is through the pre-treatment of cells with MTX. MTX-induced enhancement of 5-phosphoribosyl-1-pyrophosphate (PRPP) pools with subsequent increased synthesis of 5-FU nucleotides and incorporation of 5-fluoriuridine – 5'-triphosphate (FUTP) into RNA has been proposed as the basis for this biochemical modulation. [37–39] Subsequent in vivo studies using fluorine 19 magnetic resonance spectroscopy confirmed a three-fold increase in 5-fluoronucleotide (FNuct), the active 5-FU metabolite, following the pre-treatment of animals with MTX [32]. These observed changes in 5-FU catabolism resulted in a greater anti-tumor activity than MTX given alone or MTX given after 5-FU. Consistent with these pre-clinical studies, the design of this phase II trial involved a fluorouracil infusion that was initiated one hour into the 2 hour high dose infusion of LV and 36 hours after the initial methotrexate dosing.

Our phase II study provides long-term efficacy and safety data for sequential MTX-LV-5-FU chemotherapy administered on a 14-day cycle. The 31% response rate and median survival duration (median 13.0 months) observed in this trial are slightly improved when compared to the results reported for intravenous 5-FU therapy. Overall, treatment with sequential MTX-LV-5-FU was very well-tolerated. Grade III diarrhea was seen in only 8% of patients, grade III and IV mucositis in 8% and 1% of patients respectively and grade III and IV nausea/vomiting in 11% and 1% of patients respectively. The most common hematologic toxicity observed was grade II and III anemia in 18% and 4% of patients respectively.

Other similar studies have shown comparable results in terms of the efficacy and safety of MTX-LV-5-FU for patients with advanced colorectal cancer [44–48]. In a meta analysis of eight randomized trials of 5-FU/MTX versus 5-FU alone, the complete and partial response rates for the 5-FU patient cohort was 10% compared with 19% for patients receiving MTX/5-FU.[14] In addition, the median survival was improved for patients receiving MTX/5-FU, 10.7 months versus 9.1 months for patients receiving 5-FU alone (p = .024). Marsh et al. in a randomized trial, reported a statistical improvement in response, time to progression (9.9 months versus 5.9 months) and median survival (15.3 months versus 11.4 months) when the interval between the MTX and 5-FU infusion was increased from 1 hour to 24 hours.

Conclusions

We suggest our results and the pre-clinical and clinical data discussed indicate the sequence of administration of 5-FU with MTX and LV is important. Future studies combining 5-FU with other cytotoxic / biologic compounds should consider the mechanism of the interaction and incorporate that information into the design of the trial.

Abbreviations

5-FU: 5-fluorouracil

PALA: N-phosphonacetyl-L-aspartic acid

IFNα-2a: Interferon alfa-2a

PVI: Protracted venous infusion

DPD: dihydropyrimidine dehydrogenase

MTX: Methotrexate

LV: Leucovorin

ECOG: Eastern Cooperative Oncology

F-dUMP: 5-fluoro-2'deoxyuridine – 5-monophosphate

TS: thymidylate synthetase

PRPP: 5-phosphoribosyl-1-pyrophosphate

FUTP: 5-fluoriuridine – 5'-triphosphate

FNuct: 5-fluoronucleotide

Authors' Contributions

DRW and PS were involved in the original design and accrual to this phase II clinical trial. The manuscript was prepared and written by SKT and AWB. The manuscript was then read and revised by members of the Gastrointestinal Research Group at Wake Forest University (SAM and VH). LDC wrote and performed all statistical analysis and preparation of the data presented in this manuscript.

Acknowledgements

The authors wish to thank Carrie G. Blackstock, Ph.D., UNC – Chapel Hill Department of English, for her editorial support.

References

1. Andre T, Bensmaine MA, Louvet C, *et al*: **Multicenter phase II study of bimonthly high-dose leucovorin, fluorouracil infusion, and oxaliplatin for metastatic colorectal cancer resistant to the same leucovorin and fluorouracil regimen.** *J Clin Oncol* 1999, **17:**3560-8
2. Becouarn Y, Ychou M, Ducreux M, *et al*: **Phase II trial of oxaliplatin as first-line chemotherapy in metastatic colorectal cancer patients. Digestive Group of French Federation of Cancer Centers.** *J Clin Oncol* 1998, **16:**2739-44
3. Becouarn Y, Rougier P: **Clinical efficacy of oxaliplatin monotherapy: phase II trials in advanced colorectal cancer.** *Semin Oncol* 1998, **25:**23-31
4. Diaz-Rubio E, Sastre J, Zaniboni A, *et al*: **Oxaliplatin as single agent in previously untreated colorectal carcinoma patients: a phase II multicentric study.** *Ann Oncol* 1998, **9:**105-8
5. Machover D, Diaz-Rubio E, de Gramont A, *et al*: **Two consecutive phase II studies of oxaliplatin (L-OHP) for treatment of patients with advanced colorectal carcinoma who were resistant to previous treatment with fluoropyrimidines.** *Ann Oncol* 1996, **7:**95-8
6. Rothenberg ML, Cox JV, DeVore RF, *et al*: **A multicenter, phase II trial of weekly irinotecan (CPT-11) in patients with previously treated colorectal carcinoma.** *Cancer* 1999, **85:**786-95
7. Cunningham D, Glimelius B: **A phase III study of irinotecan (CPT-11) versus best supportive care in patients with metastatic colorectal cancer who have failed 5-fluorouracil therapy. V301 Study Group.** *Semin Oncol* 1999, **26:**6-12
8. Saltz LB: **Irinotecan in the first-line treatment of colorectal cancer.** *Oncology (Huntingt)* 1998, **12:**54-8
9. Conti JA, Kemeny NE, Saltz LB, *et al*: **Irinotecan is an active agent in untreated patients with metastatic colorectal cancer.** *J Clin Oncol* 1996, **14:**709-15
10. Moertel CG: **Chemotherapy for colorectal cancer [see comments].** *N Engl J Med* 1994, **330:**1136-42
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, and leucovorin: a randomized trial of the Northern California Oncology Group.** *J Clin Oncol* 1989, **7:**1427-36
12. Petrelli N, Douglass HO Jr, Herrera L, *et al*: **The modulation of fluorouracil with leucovorin in metastatic colorectal carcinoma: a prospective randomized phase III trial. Gastrointesti-**

nal Tumor Study Group [published erratum appears in J Clin Oncol 1990 Jan; 8(1):185]. *J Clin Oncol* 1989, **7:**1419-26
13. Jager E, Heike M, Bernhard H, *et al*: **Weekly high-dose leucovorin versus low-dose leucovorin combined with fluorouracil in advanced colorectal cancer: results of a randomized multicenter trial. Study Group for Palliative Treatment of Metastatic Colorectal Cancer Study Protocol I.** *J Clin Oncol* 1996, **14:**2274-9
14. **Meta-analysis of randomized trials testing the biochemical modulation of fluorouracil by methotrexate in metastatic colorectal cancer. Advanced Colorectal Cancer Meta-Analysis Project.** *J Clin Oncol* 1994, **12:**960-9
15. Erlichman C: **5-Fluorouracil (FUra) and folinic acid (FA) therapy in patients with colorectal cancer.** *Adv Exp Med Biol* 1988, **244:**185-92
16. 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* 1988, **6:**469-75
17. O'Dwyer PJ: **Biochemical modulation of 5-fluorouracil by PALA.** *Cancer Invest* 1990, **8:**261-2
18. Martin DS: **Biochemical modulation of 5-fluorouracil by PALA: mechanism of action.** *Adv Exp Med Biol* 1993, **339:**153-63
19. Kemeny N, Conti JA, Seiter K, *et al*: **Biochemical modulation of bolus fluorouracil by PALA in patients with advanced colorectal cancer.** *J Clin Oncol* 1992, **10:**747-52
20. Rosvold E, Schilder R, Walczak J, *et al*: **Phase II trial of PALA in combination with 5-fluorouracil in advanced pancreatic cancer.** *Cancer Chemother Pharmacol* 1992, **29:**305-8
21. O'Dwyer PJ, Paul AR, Walczak J, Weiner LM, Litwin S, Comis RL: **Phase II study of biochemical modulation of fluorouracil by low-dose PALA in patients with colorectal cancer.** *J Clin Oncol* 1990, **8:**1497-503
22. Kemeny NE, Schneider A, Martin DS: **Phase I trial of PALA, methotrexate, fluorouracil, leucovorin, and uridine rescue in patients with advanced cancer. The use of uridine to decrease fluorouracil toxicity.** *Cancer Invest* 1990, **8:**263-4
23. Pazdur R, Ajani JA, Patt YZ, *et al*: **Phase II study of fluorouracil and recombinant interferon alfa-2a in previously untreated advanced colorectal carcinoma.** *J Clin Oncol* 1990, **8:**2027-31
24. Kim J, Zhi J, Satoh H, *et al*: **Pharmacokinetics of recombinant human interferon-alpha 2a combined with 5-fluorouracil in patients with advanced colorectal carcinoma.** *Anticancer Drugs* 1998, **9:**689-96
25. Hansen RM, Ryan L, Anderson T, *et al*: **Phase III study of bolus versus infusion fluorouracil with or without cisplatin in advanced colorectal cancer.** *J Natl Cancer Inst* 1996, **88:**668-74
26. Aranda E, Diaz-Rubio E, Cervantes A, *et al*: **Randomized trial comparing monthly low-dose leucovorin and fluorouracil bolus with weekly high-dose 48-hour continuous-infusion fluorouracil for advanced colorectal cancer: a Spanish Cooperative Group for Gastrointestinal Tumor Therapy (TTD) study.** *Ann Oncol* 1998, **9:**727-31
27. Pazdur R: **Phase II study of UFT plus leucovorin in colorectal cancer.** *Oncology* 1997, **54:**19-23
28. Romanini A, Li WW, Colofiore JR, Bertino JR: **Leucovorin enhances cytotoxicity of trimetrexate/fluorouracil, but not methotrexate/fluorouracil, in CCRF-CEM cells.** *J Natl Cancer Inst* 1992, **84:**1033-8
29. Kamm VJ, Rietjens IM, Vervoort J, *et al*: **Effect of modulators on 5-fluorouracil metabolite patterns in murine colon carcinoma determined by in vitro 19F nuclear magnetic resonance spectroscopy.** *Cancer Res* 1994, **54:**4321-6
30. Van der Wilt CL, Braakhuis BJ, Pinedo HM, De Jong M, Smid K, Peters GJ: **Addition of leucovorin in modulation of 5-fluorouracil with methotrexate: potentiating or reversing effect?** *Int J Cancer* 1995, **61:**672-8
31. Ardalan B, Luis R, Jaime M, Franceschi D: **Biomodulation of Fluorouracil in colorectal cancer [see comments].** *Cancer Invest* 1998, **16:**237-51
32. McSheehy PM, Prior MJ, Griffiths JR: **Enhanced 5-fluorouracil cytotoxicity and elevated 5-fluoronucleotides in the rat Walker carcinosarcoma following methotrexate pre-treatment: a 19F-MRS study in vivo.** *Br J Cancer* 1992, **65:**369-75
33. Chabner BA: **Pyrimidine antagonists.** *In: Pharmacologic Principles of Cancer Treatment: W.B. Saunders, (Edited by: Chabner BA)* 1982
34. Santi DV, McHenry CS, Sommer H: **Mechanism of interaction of thymidylate synthetase with 5-fluorodeoxyuridylate.** *Biochemistry* 1974, **13:**471-81
35. Mini E, Moroson BA, Bertino JR: **Cytotoxicity of floxuridine and 5-fluorouracil in human T-lymphoblast leukemia cells: enhancement by leucovorin.** *Cancer Treat Rep* 1987, **71:**381-9
36. Yin MB, Zakrzewski SF, Hakala MT: **Relationship of cellular folate cofactor pools to the activity of 5-fluorouracil.** *Mol Pharmacol* 1983, **23:**190-7
37. Cadman E, Heimer R, Davis L: **Enhanced 5-fluorouracil nucleotide formation after methotrexate administration: explanation for drug synergism.** *Science* 1979, **205:**1135-7
38. Benz C, Schoenberg M, Choti M, Cadman E: **Schedule-dependent cytotoxicity of methotrexate and 5-fluorouracil in human colon and breast tumor cell lines.** *J Clin Invest* 1980, **66:**1162-5
39. Fernandes DJ, Bertino JR: **5-fluorouracil-methotrexate synergy: enhancement of 5-fluorodeoxyridylate binding to thymidylate synthase by dihydropteroylpolyglutamates.** *Proc Natl Acad Sci U S A* 1980, **77:**5663-7
40. Mullany S, Svingen PA, Kaufmann SH, Erlichman C: **Effect of adding the topoisomerase I poison 7-ethyl-10-hydroxycamptothecin (SN-38) to 5-fluorouracil and folinic acid in HCT-8 cells: elevated dTTP pools and enhanced cytotoxicity.** *Cancer Chemother Pharmacol* 1998, **42:**391-9
41. Guichard S, Hennebelle I, Bugat R, Canal P: **Cellular interactions of 5-fluorouracil and the camptothecin analogue CPT-11 (irinotecan) in a human colorectal carcinoma cell line.** *Biochem Pharmacol* 1998, **55:**667-76
42. Rostagno P, Formento P, Ciccolini J, *et al*: **Mechanisms of the synergy between oxaliplatin (LOHP) and 5 fluorouracil (FU)-folinic acid (FA).** *Proc. Amer. Assoc. Can. Res.* 2000
43. Hsu C, Yeh KH, Yeh PY, Chen ML, Lin ZR, Cheng AL: **Cytotoxicity of oxaliplatin in gastric cancer cells – Studies on single-agent activity and combination effect with 5-fluorouracil.** *Proc. Amer. Assoc. Can. Res.* 2000
44. Stabuc B, Markovic A, Plesnicar A, Cizej TE: **Double modulation of 5-fluorouracil by leucovorin and low-dose methotrexate in advanced colorectal cancer [In Process Citation].** *Neoplasma* 2000, **47:**248-52
45. Comella P, Lorusso V, Casaretti R, *et al*: **Concurrent modulation of 5-fluorouracil with methotrexate and L-leucovorin: an effective and moderately toxic regimen for the treatment of advanced colorectal carcinoma. A multicenter phase II study of the Southern Italy Cooperative Oncology Group.** *Tumori* 1999, **85:**465-72
46. Polyzos A, Tsavaris N, Giannopoulos A, *et al*: **Biochemical modulation of fluorouracil: comparison of methotrexate, folinic acid, and fluorouracil versus folinic acid and fluorouracil in advanced colorectal cancer: a randomized trial.** *Cancer Chemother Pharmacol* 1996, **38:**292-7
47. Romero AO, Perez JE, Cuevas MA, *et al*: **Double modulation of 5-fluorouracil by methotrexate and high-dose L-leucovorin in advanced colorectal cancer.** *Am J Clin Oncol* 1998, **21:**94-8
48. Marsh JC, Bertino JR, Katz KH, *et al*: **The influence of drug interval on the effect of methotrexate and fluorouracil in the treatment of advanced colorectal cancer [see comments].** *J Clin Oncol* 1991, **9:**371-80

Pre-publication history

The pre-publication history for this paper can be accessed here:

http://www.biomedcentral.com/1471-2407/2/9/prepub