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Review paper

Chemotherapy of colorectal cancer

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Advanced colorectal cancer belongs to the most chemotherapy-resistant human malignancies. The cytotoxic agent with the most consistent antitumor activity has been 5-fluorouracil (5-FU). With this drug, response rates vary between 15% (with conventional weekly or 5-day bolus injection) and 30% (with continuous infusions of 24 h to 12 weeks); survival advantages of the latter approach have not been demonstrated. Combinations of 5-FU with other cytotoxic agents with some activity have been found unsuccessful. Because of its complex metabolism the efficacy-toxicity ratio of 5-FU can be positively influenced by biochemical modulation with agents leading to decreased availability of competing substrates, increased availability of co-substrates or more efficient interactions with target substances. Superior response rates (20–40%) have been observed upon the addition of leucovorin, methotrexate and *N*-(phosphonacetyl)-L-aspartic acid; survival was modestly prolonged (2–3 months) in two leucovorin and one methotrexate study. Interferon-alpha, inactive as a single agent, appears to synergize with 5-FU at the cost of considerable toxicity; results from randomized trials of this combination are awaited. 5-FU has also been the mainstay of adjuvant treatments; in poor-prognosis rectal cancer it appears to improve survival if added to radiotherapy, whereas in combination with levamisol the survival of node-positive colonic carcinoma patients can be prolonged.

Key words: Chemotherapy, colorectal cancer, 5-FU.

Introduction

In the Western world colorectal cancer is the second most frequent malignancy after lung cancer. About half of the patients afflicted with this disease will die from it as a consequence of locoregional or distant spread.[1] Thus, colorectal cancer constitutes a formidable challenge to those engaged in the systemic treatment of human malignancy. Despite enormous preclinical and clinical research efforts, however, this disease has virtually escaped all attempts to obtain clinically meaningful chemotherapeutic results. In fact, if the results of 180 phase II studies with 13 new drugs performed between 1971 and 1984 are analysed in relation to type of disease, colorectal cancer appears to be the most resistant of all malignancies; this is even more striking since many of these patients were treated with new drugs without being pretreated extensively or at all.[2] It is tempting to speculate about the background of this primary or inherent resistance. Recent data suggest a role for the multidrug resistance gene, although other mechanisms certainly are also involved.[3] Further research in this complex area is of great importance in order to be able to pass this main obstacle on the way to more successful chemotherapy of colorectal cancer.

Treatment of 5-fluorouracil (5-FU)

Ever since its synthesis by Heidelberger in 1957 the antimetabolite 5-FU has been the most important cytotoxic drug for the treatment of inoperable or disseminated colorectal cancer. Numerous studies have been devoted to the question of how to administer 5-FU in the most effective way.[4–11] Two schedules have emerged as being most often applied: weekly bolus administration (sometimes after loading doses) and bolus injections on five consecutive days, to be repeated every 4–5 weeks. In phase III studies with dose escalation to maximal tolerated doses, dose intensities of 575 mg/m^2/week for the weekly[12] and 550 mg/m^2/week for the 5-day schedules[13] have been described. Response rates in these trials were 17 and 12% with median survival times of 345 and 322 days, respectively. It appears a fair conclusion that these figures are the best that can be obtained with the conventional modes of 5-FU administration.

Hryniuk and co-workers found a clear dose-intensity/response relationship for 5-FU in colorectal cancer, which is one of the reasons to look for different schedules of administration with the aim

Table 1. Bolus 5-FU treatment in colorectal cancer: efficacy and toxicity at maximal dose intensity in phase III trials

Study	Schedule	Dose intensity (mg/m²/wk)		Response rate (%)	Toxicity (severe)
		Planned	Actual		
Petrelli *et al.*[13]	500 mg/m² for 5 days every 4 wks; (de)escalation with 25 mg/m²	625	615	12	Leucopenia: 27% Diarrhea: 9% Mucositis: 15%
Valone *et al.*[12]	450 mg/m² for 5 days, then 580 mg/m² weekly; (de)escalation with 10%	670	620	17	Leucopenia: 21% Diarrhea: 14% Mucositis: 6%

to obtain better efficacy–toxicity relationships and higher dose intensities.[14] Moreover, for a S-phase specific agent such as 5-FU it is theoretically attractive to prolong the duration of exposure; this concept of superior cytotoxicity from 5-FU with longer times of exposure has been substantiated *in vitro*.[15] On the basis of this rationale a number of continuous infusion schedules have been developed of long (12 weeks) or short (24–48 h) duration[16–20] (Table 2). With these treatments, dose intensities in the order of 2000–2500 mg/m² per week can be obtained and response rates in the order of 20–30% have been observed. In one randomized study, the response rate of long-term infusional therapy (300 mg/m²/day) was significantly superior to that obtained with bolus therapy (500 mg/m² for 5 days every 5 weeks): 30 vs 7%.[17] Survival benefit, however, has not been demonstrated.

With bolus treatment of 5-FU, toxicity is mainly myelosuppression with, to a lesser extent, gastrointestinal symptoms and skin changes. This toxicity pattern is different with continuous infusion schedules. With protracted infusion, myelosuppression and gastrointestinal toxicity are mild and skin toxicity becomes the most prominent side effect. This so-called 'hand–foot syndrome' consists of palmar and plantar redness, peeling, hyperpigmentation and sometimes pain.[18] The continuous infusion schedules of short duration are remarkably non-toxic; with these high doses occasionally neurotoxicity (somnolence, ataxia) and cardiotoxicity (pain, EKG changes) have been observed.[21,22] Clearly with continuous infusions high dose-intensities of 5-FU can be obtained without increase in toxicity. Response rates, although superior to those obtained with bolus injections, are certainly not consistent with the steep dose–response curve as described by Hrynink.[14] This may at least in part be related to the increase in clearance rate that occurs with continuous infusions and may be the result of increased pulmonary extraction.[23] At the present time continuous infusions of 5-FU must still be considered experimental and are particularly attractive as targets for the effects of the addition of modulating agents.

Agents with a steep dose–response curve and high first-pass extraction rate are attractive for local administration. Since metastatic colorectal cancer is often clinically limited to the liver, intrahepatic administration of fluorinated pyrimidines has been the subject of considerable interest. Most of these studies have been performed with the 5-FU derivative fluorodeoxyuridine (FUdR), since more than 90% of this drug is extracted from the blood

Table 2. Continuous infusion 5-FU treatment in colorectal cancer: dose intensity and efficacy

Study	Schedule	Dose intensity (mg/m²/wk)	Response rate (%)
Kemeny *et al.*[a 43]	1000 mg/m² over 5 days every 4 wks	1220	3
Shah *et al.*[16]	2300 mg/m² over 2 days every wk	2300	30
Ardalan *et al.*[19]	2600 mg/m² over 1 day every wk	2600	
Diaz-Rubio *et al.*[18]	3500 mg/m² over 2 days every wk	3500	43
Blijham *et al.*[20]	2300 mg/m² over 2 days every wk for 6 wks, then every 2 wks	1750	19
Lokich *et al.*[a 17]	300 mg/m²/day daily for 12 weeks	2100	30

[a] Randomized studies.

by the liver during its first pass.[24] Intrahepatic administration can be achieved by the intraportal and the intra-arterial route; since established liver metastases derive their blood supply mainly from the hepatic artery the latter route is the preferred one except in the adjuvant setting. Two randomized trials comparing intra-arterial and systemic FUdR treatment have confirmed the higher response rate with the former route (10 and 20% vs 37 and 50%).[25,26] It has been much more difficult to demonstrate a survival advantage, partly because of the cross-over design of these trials but mainly since survival (around 15 months in both arms in these trials) is probably not or only marginally prolonged. A phase II study combining intra-arterial and systemic treatment did not yield better results with a 44% response rate and a median duration of response of 30 weeks.[27] Local toxicity of intrahepatic therapy along the arterial route is considerable and includes catheter problems and chemical hepatitis and biliary sclerosis. The benefit of intrahepatic chemotherapy (intra-arterial as well as intraportal) for patients with clinically evident liver metastases remains unproven.

Combination chemotherapy

Apart from 5-FU very few cytotoxic agents are active in disseminated colorectal cancer. In a review published in 1982[28] only nitrosureas and mitomycin were reported as having some activity. Combinations of 5-FU with semustine, vincristin, dacarbazine and streptozocine have been investigated in several phase III trials.[29–31] Although in some instances response rates were slightly higher with the combined treatment, survival was certainly not improved and toxicity considerably more severe. More recently developed cytostatic agents with activity in several other tumors such as epirubicin, mitoxantrone, vindesine, etoposide and iphosphamide were found equally inactive and have therefore not been tested in combination with 5-FU.

Although as a single agent cisplatin has only a very low level of activity in colorectal cancer, it has nevertheless been investigated in combination with 5-FU because of the synergistic interaction between these two drugs in animal systems and esophageal and head and neck cancer.[32,33] In several phase II studies encouraging results were obtained with response rates varying from 0 to 70%.[34–38] Subsequently a number of randomized trials were initiated showing no improvement over 5-FU treatment or, in one study, a superior response rate without prolongation of survival (10 months for cisplatin plus 5-FU and 12 months for 5-FU alone)[39–43] (Table 3). The European Organisation for Research and Treatment of Cancer (EORTC) conducted a phase III trial comparing 5-FU with 5-FU in combination with cisplatin and allopurinol.[44] This latter drug has been reported to rescue normal cells from 5-FU toxicity.[45] Again, response rates and survival were not significantly different.

In summary, combination chemotherapy has not been found to be superior to treatment with 5-FU alone. This is due to the lack of truely active drugs with mechanisms of action different from those of the fluorinated pyrimidines. It remains important to enter patients with colorectal cancer in phase II trials of new agents, in particular if these agents are exploring different and new modes of action.

Biochemical modulation of 5-FU

5-FU is a fluorinated pyrimidine base that by itself is cytotoxically inactive and has to be anabolized to one of its active nucleotides in order to delay growth or kill cells. The biochemistry of 5-FU has been excellently reviewed and is summarized in Figure 1.[23,46] From this figure the three proposed mechanisms of action of 5-FU become apparent: inhibition of the enzyme thymidylate synthase (TS) by FdUMP, incorporation of FdUTP into DNA and incorporation of FUTP into RNA. Because of the complexity of its metabolism and the multiple mechanisms of action it comes as no surprise that multiple factors have been found to be associated

Table 3. 5-FU and cisplatin in colorectal cancer: response rates in randomized trials

Study	5-FU schedule	Response rate (%)	
		5-FU	5-FU + cisplatin
Diaz-Rubio *et al.*[39]	Bolus × 5	24	17
Poon *et al.*[61]	Bolus × 5	10	15
Labianca *et al.*[40]	Weekly	14	19
Loehrer *et al.*[41]	Weekly	19	22
Lokich *et al.*[42]	Continuous infusion for 12 weeks	31	35
Kemeny *et al.*[43]	Continuous infusion for 5 days	3	25[a]

[a] $p = 0.001$; survival 12 (5-FU) vs 10 (5-FU + cisplatin) months.

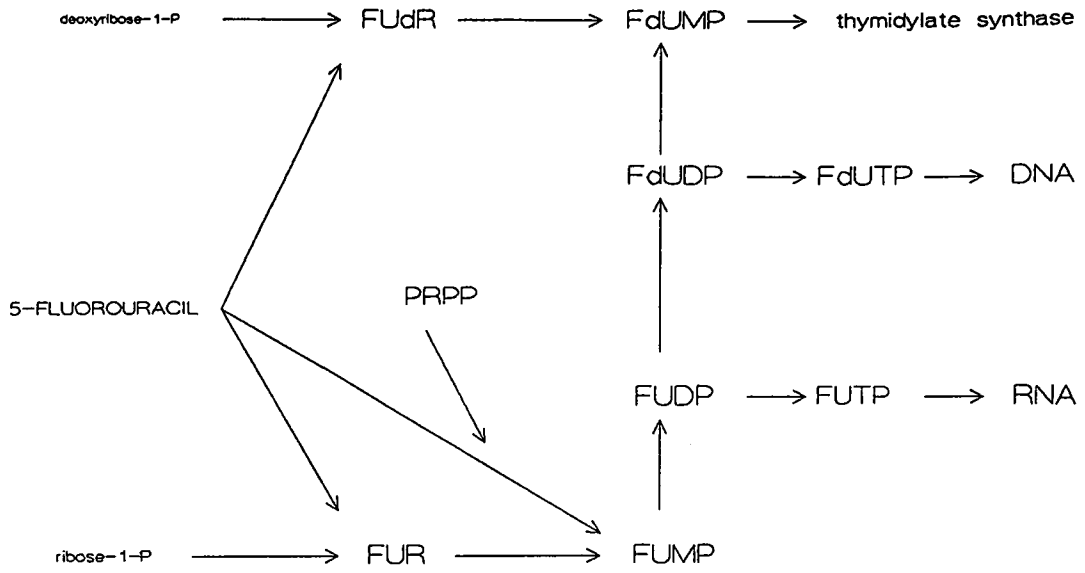

Figure 1. Anabolic pathways of 5-FU. The following substances are indicated: FUdR, 5-fluorodeoxyribose; FdUMP, 5-fluorodeoxyuridinemonophosphate; FUR, 5-fluororibose; FUMP, 5-fluorouridinemonophosphate; FuDP, 5-fluorouridinediphosphate; FUTP, 5-fluorouridinetriphosphate; FdUDP, 5-fluorouridinediphosphate; FdUTP, 5-fluorodeoxyuridinetriphosphate.

with the sensitivity (or lack of sensitivity, i.e. resistance) of cells to 5-FU. These determinants of sensitivity are summarized in Table 4. Biochemical modulation of 5-FU activity may be accomplished by interfering with each of these determinants; the aim is to improve the therapeutic index by rescuing normal cells or increase cytotoxic activity in tumor cells. This concept has been most extensively and successfully tested in the treatment of human colorectal cancer.[23,47]

A great number of 5-FU-modulator combinations have been designed and tested in cell cultures or tumor-bearing animals. Most of these have not yet been adequately tested in humans or were found not to be successful. To this category of modulators belong competing nucleosides such as uridine, thymidine and cytidine, the nucleoside-transport inhibitor dipyridamol and the OPRT-inhibitor allopurinol.[23,48–51] Clinically interesting data have been obtained with three modulators: leucovorin, methotrexate and *N*-(phosphonacetyl)-L-aspartic acid (PALA). These results will be reviewed in more detail.

Table 4. Determinants of sensitivity for 5-FU

Speed of 5-FU transport in/out cell.
Presence of co-substrates (e.g. PRPP).
Activity of anabolic enzymes (e.g. OPRT).
Presence of competing nucleosides (e.g. uridine) and nucleotides (e.g. dTTP).
Kinetics of catabolism of 5-FU, FUMP and FdUMP.
Alterations in thymidylate synthase (e.g. gene amplification).

PRPP, phosphoribosylpyrophosphate; OPRT, orotate phosphoribosyl-transferase.

Combination of 5-FU and leucovorin

The duration and degree of thymidylate synthase inhibition by 5-FU can be enhanced by the addition of reduced folates as a consequence of the formation of a stable ternary complex between FdUMP, TS and the cofactor 5,10-methylene tetrahydrofolate.[52] The predicted enhanced cytotoxicity of a combination of 5-FU and reduced folates, e.g. leucovorin, was confirmed *in vitro* and in human colon carcinoma xenografts.[53,54] Promising response rates were subsequently obtained in a number of phase II studies in patients with metastatic colorectal cancer.[55–58]

Recently, detailed results have become available of six phase III studies comparing 5-FU with 5-FU plus leucovorin (Table 5); abstracts have been excluded from this tabulation.[12,13,59–62] It appears that in five of these studies significantly higher response rates in the order of 30% (26–48%) have been obtained. In the one negative study[12] the 5-FU treatment was significantly more toxic than the 5-FU–leucovorin combination, suggesting that also

Table 5. 5-FU–leucovorin (LV) combinations: efficacy in phase III trials

Study	5-FU schedule	5-FU–LV schedule	Response rates (%)		Survival (wks)	
			5-FU	5-FU–LV	5-FU	5-FU–LV
Petrelli *et al.*[59]	450 mg/m^2 for 5 days then 200 mg/m^2 qod × 6	600 mg/m^2 5-FU 500 mg/m^2 LV 6 of 8 wks	11	48[a]	52	52
Ehrlichman *et al.*[60]	370 mg/m^2 for 5 days every 4 wks	370 mg/m^2 × 5 5-FU 200 mg/m^2 × 5 LV every 4 wks	7	33[a]	41	54[a]
Poon *et al.*[61]	500 mg/m^2 for 5 days every 5 wks	370 mg/m^2 × 5 5-FU 200 mg/m^2 × 5 LV every 4–5 wks	10	26[a]	34	52[a]
		or 425 mg/m^2 × 5 5-FU 20 mg/m^2 × 5 LV every 4–5 wks		43[a]		51[a]
Petrelli *et al.*[13]	500 mg/m^2 for 5 days every 4 wks (escalation)	600 mg/m^2 5-FU 500 mg/m^2 LV 6 of 8 wks	12	30[a]	46	55
		or 600 mg/m^2 5-FU 25 mg/m^2 LV 6 of 8 wks		19		45
Valone *et al.*[12]	600 mg/m^2 weekly (escalation)	400 mg/m^2 × 5 5-FU 200 mg/m^2 × 5 LV every 4 wks	17	19	20	24
Doroshow *et al.*[62]	370 mg/m^2 for 5 days every 4 wks	370 mg/m^2 × 5 5-FU 500 mg/m^2 × 5 LV every 4 wks	13	44[a]	55	62

[a] $p \leq 0.05$.

in this trial a superior benefit:toxicity ratio was observed. In contrast a significant survival advantage was only seen in two trials[60,61] with a prolongation of the median survival time of 3–4 months and without an increase in long-term survival. The other studies mostly showed some prolongation of survival without this being significant at the $p < 0.05$ level. Overall, these results suggest a doubling of response rate and a moderate improvement in survival upon the addition of leucovorin to 5-FU.[63] The maximally tolerated doses of 5-FU are lower if combined with leucovorin, showing that the leucovorin not only potentiates efficacy but also toxicity. Myelosuppression, mucositis and diarrhea are the most important side effects and may be severe and life-threatening (Table 6). Diarrhea in particular has been recognized as a rather frequent and in some patients potentially lethal toxicity; it may be especially prominent with schedules of weekly administration.[59] As far as the limited data justify conclusions in this respect, efficacy of 5-FU–leucovorin combinations does not appear to be dependent on the schedule (weekly or 5-day courses), mode of administration (bolus or continuous infusion) or dose of leucovorin, as long as leucovorin is given shortly before and/or simultaneously with 5-FU. The dose question is particularly relevant since in *in vitro* systems divergent results in this respect have been obtained. For some cell lines leucovorin levels of 1 μmol/l (obtainable with 20 mg/m^2 leucovorin in humans) are sufficient to potentiate 5-FU cytotoxicity, whereas in other cell lines 10–20 μmol/l levels should be achieved (obtainable with ≥200 mg/m^2 leucovorin in humans).[64,65]

Several questions remain to be investigated. The optimal dose, schedule and route of administration of leucovorin need to be further analysed. With oral administration of leucovorin, the biologically active 1-stereoisomer is preferentially absorbed and

Table 6. 5-FU–leucovorin (LV) combinations in colorectal cancer: toxicity profile

Study	Leukopenia (<2000/mm^3)	Mucositis (severe; grade 3/4)	Diarrhea (severe; grade 3/4)	Toxic deaths
Poon *et al.*[61]				
low dose LV	21%	26%	14%	0/73
high dose LV	19%	30%	9%	1/69 (leukopenia)
Petrelli *et al.*[13]				
low dose LV	4%	0%	13%	4/115
high dose LV	8%	4%	25%	7/115 (diarrhea)
Valone *et al.*[12]	12%	16%	14%	4/107 (3 leukopenia)
Doroshow *et al.*[62]	9%	13%	6%	0/36

quickly metabolized to 5-CH3THF (5-methyltetrahydrofolate). With oral doses of 100 mg every 4–6 h, leucovorin plasma levels exceed 1 μmol/l throughout the duration of administration.[66] If this leucovorin treatment was given concomitantly with a 5-day continuous infusion of 5-FU, 800 mg/m^2/day of 5-FU appeared to be the maximally tolerated dose with mucositis being dose-limiting. In contrast we were able to administer 90 mg leucovorin every 6 h to a 48-h continuous infusion of 2300 mg/m^2/day 5-FU without dose-limiting toxicity; 10/30 patients with colorectal cancer experienced a partial response.[67] Further studies combining dose-intense 5-FU and alternative leucovorin schedules are to be undertaken.

Combination of 5-FU and methotrexate

Methotrexate inhibits purine synthesis resulting in an intracellular accumulation of PRPP and thereby in an increased ribosylphosphorylation of 5-FU to FUTP and incorporation into RNA.[68] The predicted enhanced cytotoxicity of the 5-FU–methotrexate combination was confirmed *in vitro*.[69] A number of subsequently performed phase II studies were summarized by Kemeny *et al.*; results suggested an increased response rate (around 30%) provided methotrexate preceded 5-FU by at least 4 h and was given in a dose of at least 40 mg/m^2.[70–73]

Subsequent phase III trials yielded equivocal results and are summarized in Table 7. In three of six trials significantly higher response rates were observed, whereas survival was significantly prolonged in one study.[74] The low performance of the control group in this latter study, though treated with a rather intense 5-FU dose of 1200 mg/m^2 every 2 weeks, may be due to patient selection (only symptomatic patients were included) and stringent response criteria (minimum duration 4 months). Since the 5-FU schedule in the experimental arm was similar but less intense (1000 mg/m^2 every 2 weeks) and toxicity was similar (except increased conjunctivitis), the results of this trial indicate that clinical synergism had indeed occurred. Valone *et al.*[12] found an unchanged response rate with the 5-FU–methotrexate combination despite considerably lower 5-FU doses and reduced toxicity, which also suggests the occurrence of an improved benefit:toxicity ratio. Poon and co-workers,[77] in an expansion of their original trial,[61] found the combination of 5-FU with intermediate dose methotrexate to be inferior to 5-FU–leucovorin combinations.

In almost all trials mentioned leucovorin was given to rescue normal cells from methotrexate toxicity. In view of the reported modulating activity of low-dose leucovorin a contribution to the results obtained cannot be totally excluded.[61,78] However, in all instances low-dose leucovorin was started at least 1 h after bolus administration of 5-FU and therefore is unlikely to synergize. Taken together methotrexate indeed appears to modulate 5-FU cytotoxicity in patients with colorectal cancer. The response rates are in the order of 25% and with most regimens tested survival is not appreciably prolonged.

Much attention has been given to the dose of methotrexate and the interval between methotrexate and 5-FU in designing trials of the combination of these two drugs. With most of the studies reported in Table 7, doses of methotrexate are in the so-called intermediate range (200–250 mg/m^2) and should provide the *in vitro* necessary concentrations of 1–10 μmol/l.[69] In a recent randomized study, 5-FU following methotrexate after 24 h was significantly superior in response rate (15 vs 29%) and survival (11 vs 15 months) as compared with a 1-h interval.[79] Doses of 5-FU have generally been low with the

Table 7. 5-FU and methotrexate (MTX) in colorectal cancer: efficacy in randomized trials

Study	Schedule 5-FU–MTX	Response rate (%)		Survival (wks)	
		5-FU	5-FU–MTX	5-FU	5-FU–MTX
Petrelli *et al.*[59]	$T = 0$: 50 mg/m^2 MTX $T = 1$: 600 mg/m^2 5-FU q wk × 4, then q 2 wks	11	5	52	48
Valone *et al.*[12]	$T = 0$: 50 mg/m^2 MTX p.o.q. 6 h × 5 $T = 24$: 500 mg/m^2 5-FU $T = 30$: LV p.o. started q 2 wks	17	20	49	51
Poon *et al.*[61]	$T = 0$: 200 mg/m^2 MTX $T = 7$: 1000 mg/m^2 5-FU $T = 24$: LV p.o. started q 3–4 wks or	10	12	34	32
	$T = 0$: 40 mg/m^2 MTX $T = 24$: 700 mg/m^2 5-FU days 1 + 8 q 4 wks		26[a]		38
Glimelius *et al.*[74]	$T = 0$: 250 mg/m^2 MTX $T = 3$: 500 mg/m^2 5-FU $T = 23$: 500 mg/m^2 5-FU $T = 24$: LV p.o. started q 2 wks × 8, then 3/4 wk	3	24[a]	26	36[a]
Herrman *et al.*[75]	$T = 0$: 200 mg/m^2 MTX $T = 7$: 1000 mg/m^2 5-FU $T = 24$: LV p.o. started q 3–4 wks	15	28	57	48
Machiavelli *et al.*[76]	$T = 0$: 200 mg/m^2 MTX $T = 20$: 1200 mg/m^2 5-FU $T = 24$: LV p.o. started q 2 wks	12	28[a]	37	49

[a] $p = 0.05$.

exception of the Nordic Study.[74] The question remains whether a more pronounced modulating effect can be achieved by adding methotrexate to a more dose-intense 5-FU schedule. The EORTC Gastrointestinal Tract Cancer Cooperative Group (GICCG) is currently testing this hypothesis.[20]

Combination of 5-FU and PALA

PALA inhibits the enzyme aspartate transcarbamylase and thereby *de novo* pyrimidine synthesis.[23] This leads to an enhanced cellular anabolism of 5-FU, including more incorporation of the 5-FU-metabolite FUTP into RNA over that seen with 5-FU alone. Using mammary and colon carcinoma cell lines and animal models, synergistic cytotoxicity between PALA and 5-FU has been observed.[80–83] Most clinical trials with this combination have been disappointing,[84–88] but have also been criticized for giving the active drug in a too low dose and the modulating drug in a too high dose.[89]

Ardalan *et al.*[19] tried to mimick the synergistic *in vitro* and animal *in vivo* conditions by giving 5-FU weekly over 24 h in high doses (2600 mg/m^2/week, the maximum tolerated dose) 24 h after a fixed bolus administration of 250 mg/m^2 PALA. This dose is 15% of the maximum tolerated dose of PALA but has been shown to effectively block *de novo* pyrimidine synthesis. Pyrimidine nucleotide pools are depressed within 24 h to around 20% and remain so for at least 1 week.[90,91] In this phase I–II study, 11 of 28 patients (39%) treated with PALA–5-FU responded. The design of this study (mixed patient categories, dose-finding as far as 5-FU is concerned) does not allow conclusions regarding the effectiveness of this particular scheduling of PALA–5-FU, but suggests that further randomized trials of low-dose PALA followed by high-dose 5-FU are needed.

O'Dwyer *et al.* recently confirmed these findings.[92] They treated 37 chemotherapy-naive colorectal cancer patients with 5-FU–PALA in doses and schedule identical to those used by Ardalan *et al.*[19] No patient had grade 4 toxicity, the response rate was 43% (95% confidence interval between 27 and 59%) and the projected median survival time

was 15 months. Taken together, these data suggest that the combination of high-dose 5-FU in continuous infusion of short duration and low-dose PALA may be very active and needs further study. The Mid-Atlantic Oncology Program recently started such a study, in which low-dose PALA is added to long-term 5-FU infusions; pilot data were recently published.[93]

Cytokines

Of the clinically available cytokines most experience has been obtained with recombinant interferon-alpha (IFNa). By itself this agent has minimal or no activity against metastatic colorectal carcinoma.[94,95] In cell culture experiments the 50% inhibitory dose of 5-FU markedly decreased following IFNa treatment.[96] This shows that apart from being a biological response modifier, IFNa is able to synergize with 5-FU in a metabolic way; mechanisms are probably related to the inhibition of thymidine kinase and increased accumulation of FdUMP.[97,98] In fact, the IFNa–5-FU interaction may be another, though rather unexpected example of biochemical modulation.

Wadler *et al.*[99] performed the first clinical phase II study with a combination of 5-FU (750 mg/m^2/day continuous infusion for 5 days followed by weekly bolus injection of 750 mg/m^2) and recombinant IFNa-2a (9×10^6 units subcutaneously three times weekly). Thirteen of 17 previously untreated patients responded (95% confidence interval 56 to 96%). Almost all responses were observed in visceral metastases in liver and/or lungs. Toxicity was considerable and consisted of myelosuppression, mucositis, diarrhea, neurotoxicity and fever. Of a total of 30 patients, the weekly 5-FU dose had to be reduced by 25% in 16 and the IFNa dose by 50% in 12; one fatal and one life-threatening toxicity was encountered, both consisting of a combination of diarrhea, leukopenia and fever. None of 12 patients previously treated with 5-FU and methotrexate or leucovorin responded.

Since this initial report, two papers have appeared in which an identical 5-FU–IFNa combination was applied.[100,101] Investigators from the M.D. Anderson Cancer Center treated patients without prior chemotherapy and found a response rate of 35% (one complete response) with a median duration of response of 7.5 months. Kemeny *et al.*,[100] also using the Wadler regimen in chemotherapy-naive patients, found comparable results with a 26% response rate (none complete) with a median duration of 7.5 months (Table 8). In both studies toxicity was severe and consisted of granulocytopenia, mucositis, diarrhea and neurotoxicity. Taken together, these 5-FU–IFNa response rates approximate those reported for 5-FU–leucovorin combinations and continuous 5-FU infusion; toxicity, however, is considerably more severe. An improvement in therapeutic ratio can seriously be doubted. These results do constitute a basis, however, for further development of alternative schedules of 5-FU modulation that include interferon.

Table 8. 5-FU plus interferon-alpha in colorectal cancer: efficacy and toxicity

Study	*n*	Responders	Toxicity (severe; grade 3/4)
Pazdur *et al.*[101]	51	18	Granulocytopenia: 43% Mucositis: 37% Diarrhea: 16% Seizures: 2 patients Toxic death: 1
Kemeny *et al.*[100]	35	9	Leukopenia: 8% Mucositis: 11% Diarrhea: 13% Neurotoxicity: 7% Toxic deaths: 0

Patients with disseminated colorectal cancer have been included in a number of phase I–II studies of interleukin-2 with or without adoptive cellular therapy. Results have been disappointing.[102] In some experimental models combination therapy with IFNa and interleukin-2 appeared to be synergistic.[103,104] Recently combinations of IFNa and interleukin-2 with acceptable toxicity have been designed, which can be given in an out-patient setting.[105,106] In one of these studies six patients with colorectal cancer were treated; none responded and the five with liver metastases tolerated the combined treatment poorly.[106]

Adjuvant chemotherapy

Buyse *et al.*[1] reviewed all randomized controlled trials of adjuvant chemotherapy in comparison to no treatment published up to December 1986 in the English language. Seventeen trials were identified with 6791 patients. From the meta-analysis of those trials using 5-FU (the majority) or 5-FU-containing chemotherapy given for at least 1 year it appeared that chemotherapy resulted in a small but significant benefit with an increase in 5-year overall survival of 3.4% (95% confidence interval between 1.2 and

8%). It was suggested that this benefit occurs predominantly in patients with rectal cancer.

Since this meta-analysis results from three major adjuvant studies in colon cancer have become available.[107–109] The NSABP study Ca-1[107] demonstrated a small but significant improvement in disease-free survival in colon cancer with the combination of methyl-CCNU, vincristine and 5-FU (MOF). Overall survival, however, was not prolonged and the results have to be weighted against the side effects of methyl-CCNU and a number of negative trials with 5-FU–methyl-CCNU combinations.[110]

Levamisole is an antihelminthic drug with immunomodulating activity.[111] It has no antitumor activity in patients with metastatic colorectal cancer.[112] In the adjuvant setting the EORTC and the Western Cancer Study Group found levamisole to be no better than placebo,[113,114] although the opposite was predicted from animal models.[115] The North Central Cancer Treatment Group (NCCTG) conducted a randomized trial comparing levamisole (150 mg/day for 3 days every 2 weeks) without or with 5-FU (450 mg/m^2/day for 5 days, then weekly) for 1 year with no treatment.[108] In this trial only 5% of patients had rectal cancer. After correction for imbalances in prognostic determinants, 5-FU plus levamisole was found to reduce recurrence rate (with around 15% at 5 years) and prolonged survival (with around 10% at 5 years) significantly. This advantage could only be demonstrated in patients with positive lymph nodes; in stage B patients a similar trend was observed. To confirm these results a larger intergroup study was started that was similarly designed and only enrolled colon cancer patients.[109] In a preliminary analysis 3.5 year survival rates were estimated to be 71 vs 55% for the levamisole–5-FU and control group, respectively. Again, results were only significant for patients in stage III, but significance may emerge for stage II patients as well with longer follow-up. Positive results from adjuvant 5-FU–levamisole treatment were also reported in a much smaller and differently designed British trial.[116]

The role of 5-FU–levamisole treatment in standard practice and clinical research has become a matter of debate, as is the significance of levamisole in the results obtained.[117] Further trials will certainly address these and other questions such as the efficacy in rectal and stage II cancer and the role of 5-FU modulators such as leucovorin.

In rectal cancer, results from two new studies reinforce the benefit from adjuvant chemotherapy, in particular in combination with radiotherapy[118,119] (Table 9). In the NSABP study R-01 chemotherapy with methyl-CCNU, vincristine and 5-FU is significantly better than no treatment in Dukes' B and C rectal cancers for disease-free and overall survival; this benefit is relatively small however and restricted to males.[118] The NCCTG compared a combination of post-operative radiotherapy and chemotherapy (5-FU plus methyl-CCNU) to radiotherapy alone in rectal cancer that was deeply invasive or metastatic to regional lymph nodes.[119] After a median follow-up of 7 years the estimated 5-year recurrence rate was 63% in the radiotherapy and 42% in the combined-modality group; 62% of radiotherapy and 47% of radiotherapy plus chemotherapy patients had died ($p = 0.04$). As in colon cancer the role of methyl-CCNU in obtaining these results is controversial; preliminary data suggest that methyl-CCNU does not contribute to the adjuvant chemotherapy effect in rectal cancer.[120] In view of these results it appears likely that both radiotherapy and 5-FU are important determinants of a positive adjuvant effect and should be offered to patients with poor-prognosis rectal cancer.

On the basis of early results obtained by Taylor *et al.*[122] several groups have started to investigate early post-operative intrahepatic therapy with 5-FU through the portal vein as adjuvant therapy in colorectal cancer. Results have been rather conflicting and have been reviewed.[123,124] In the two largest studies no reduction in the incidence of liver

Table 9. Adjuvant chemotherapy in poor-prognosis rectal cancer

Study	*n*	Five-year survival rate (%)			
		Surgery	Radiotherapy	Chemotherapy	Radiotherapy + chemotherapy
GITSG 7175[121]	202	43	52	56	59[a]
NSABP R-01[118]	555	43	41	53[a]	—
NCCTG 794751[119]	204	—	47	—	58[a]

[a] $p < 0.05$.

metastases as first site of failure was observed despite some modest prolongation of disease-free and overall survival.[125,126] The results of other ongoing trials including one by the EORTC are awaited.

Conclusion

For over 30 years 5-FU has been the drug of choice for the treatment of patients with inoperable or metastatic colon or rectal cancer. The performance of this drug is poor with less than one of five patients showing objective responses. In the past 5 years response rates have improved with the use of continuous infusions, intrahepatic administration and with the addition of biochemical modulators such as leucovorin, methotrexate, PALA and interferon-alpha. Survival benefit of these newer treatments so far has only been shown for 5-FU–leucovorin, 5-FU–levamisole (in adjuvant setting) and less convincingly 5-FU–methotrexate combinations; in all cases this benefit is modest at best. However, in view of the number of patients involved even modest improvements may positively influence the life of many individuals. Finally, any improvement in the treatment of a tumor as resistant as colorectal cancer is reason for optimism, as it suggests that our research efforts are going in the right direction.

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