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Review paper

Raltitrexed/5-fluorouracil-based combination chemotherapy regimens in anticancer therapy

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Preclinical evidence of a schedule-dependent synergism between raltitrexed and 5-fluorouracil (5-FU) has prompted clinical studies of this combination. We review the main preclinical and clinical results of raltitrexed/5-FU-based combination chemotherapy regimens in anticancer therapy. Promising results include: response rates of 25 and 23% with combinations of raltitrexed/5-FU/levofolinic acid (LFA) as first-line treatment and oxaliplatin/raltitrexed/5-FU/LFA as second-line treatment of metastatic colorectal cancer, respectively; and a 67% response rate in a phase I study of cisplatin/raltitrexed/5-FU/LFA as first-line treatment of advanced head and neck cancer, including a 100% response rate at the recommended dose. These combinations were well tolerated, with neutropenia as the main dose-limiting toxicity, allowing the drugs to be combined at the doses used in monotherapy. These results suggest a role for raltitrexed within combination regimens in colorectal cancer therapy, as well as other tumors such as head and neck cancer. A further potential application of raltitrexed in combination therapies is within multidisciplinary chemo-radiotherapy strategies, mainly in rectal cancer. Phase II studies are planned/ongoing to investigate these interesting possibilities. [© 2001 Lippincott Williams & Wilkins.]

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Raltitrexed/5-fluorouracil (5-FU) combination therapy

Rationale for combination/preclinical results

Thymidylate synthase (TS) is a rate-limiting enzyme in pyrimidine *de novo* deoxynucleotide biosynthesis and is therefore a suitable target for chemotherapeutic strategies. 5-FU exerts part of its anticancer effect through TS inhibition; one of its metabolites, 5-fluoro-2′-deoxyuridine-5-monophosphate (FdUMP), binding to the pyrimidine binding site of TS. However, a consistent part of its activity, and some of its toxicity, is attributable to the incorporation of its metabolite, 5-fluorouridine-5′-triphosphate (FUTP), into RNA. Raltitrexed is a specific TS inhibitor, which is transported into cells via a reduced folate carrier and is then extensively polyglutamated by the enzyme folylpolyglutamate synthase. The polyglutamated form binds to the folate binding site of TS and is up to 100 times more potent than the parent compound.[1] In addition, the polyglutamated form is retained intracellularly, allowing an intermittent dosing schedule.

Since raltitrexed and 5-FU have proved effective as single agents in the treatment of advanced colorectal cancer, and their modalities of action are somewhat different, the combination of the two drugs might be expected to have additive activity. In fact, preclinical results in HCT-8 cells showed that the cytotoxicity of raltitrexed/5-FU combination could be synergistic, but this was schedule dependent. If the cells were exposed to raltitrexed for 24 h prior to 4-h exposure to 5-FU, synergy was obtained, but concomitant drug exposure or the opposite sequence did not produce synergistic effects.[2] Such synergy might occur by an effect of raltitrexed on 5-FU pharmacokinetics. Indeed, Yan *et al.*[3] have shown that in rat hepatocytes raltitrexed is able to reduce the activity of dihydropyrimidine dehydrogenase (DPD), the rate-limiting step in the catabolism of 5-FU, to 12% of the control without raltitrexed.

Clinical results

Two clinical studies exploring the toxicity and the activity of combination raltitrexed/5-FU have recently

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been carried out. Schwartz *et al.*[4] ran a phase I trial of sequential administration of raltitrexed on day 1 and bolus 5-FU on day 2, every 3 weeks in predominantly pre-treated patients with metastatic colorectal cancer. A summary of the results are included in Table 1. Neutropenic fever and neutropenia (>7 days) were the dose-limiting toxicity (DLT), and the recommended dose for further study was raltitrexed 5.5 mg/m^2 and 5-FU 1200 mg/m^2. Pharmacokinetic analysis demonstrated a statistically significant increase in 5-FU exposure at raltitrexed doses of 2.5 mg/m^2 or above. Clinical activity was encouraging, with one complete response and four partial responses observed and a median survival of 13.5 months.

A second phase I study has been conducted by Mayer *et al.*[5] in chemonaive patients with metastatic colorectal cancer, in which 5-FU was administered by 24-h infusion on a weekly schedule for 5 weeks at five different dose levels (from 1200 to 2800 mg/m^2), and raltitrexed was administered with 5-FU on weeks 2 and 5 at two different dose levels (2.6 and 3 mg/m^2). Myelosuppression and diarrhea represented DLT, and the recommended dose was 2.6 and 2600 mg/m^2 for raltitrexed and 5-FU, respectively (Table 1). At dose levels 3–7, 46% of the patients achieved a partial response. Also in this study, raltitrexed showed a significant impact on 5-FU pharmacokinetics, increasing mean 5-FU C_{max} and AUC, and prolonging its terminal half-life.

The results of the two studies indicate that the raltitrexed/5-FU combination is a feasible approach. The combined treatment was well tolerated and permitted the achievement of drug doses which at least match those used in monotherapy. The activity of the regimen also seems noteworthy, and both studies suggest a pharmacokinetic interaction between the two drugs with an increase in 5-FU C_{max} and AUC induced by previous or concomitant treatment with raltitrexed.

Raltitrexed/5-FU/levofolinic acid (LFA)

Rationale for combination/preclinical results

The concept of adding LFA to 5-FU when used in combination with raltitrexed is intriguing. LFA acts as a source of reduced folates, which optimize the inhibition of TS. Although LFA interferes with the uptake of raltitrexed and the polyglutamation required to synthesize the active form of the drug, some preclinical data suggest that this interference is greatly reduced when there is a 4-h interval between raltitrexed and LFA administration, which seems to be enough for raltitrexed to undergo uptake and polyglutamation.[1]

We have recently completed *in vitro* studies[11] in which we have evaluated the cytotoxicity of raltitrexed/5-FU/LFA when used in several different schedules in two colon cancer cell lines (LoVo and HT-29), and in two head and neck cancer cell lines (KB and CAL-27). The most interesting finding in our study was the clear synergism observed in at least three cell lines when cells were exposed for 24 h to raltitrexed prior to 4-h exposure to 5-FU/LFA (Table 2). The potentiation factor of raltitrexed/5-FU by LFA clearly indicates an important role for LFA in this schedule-dependent synergistic effect (Table 2).

Clinical results

Based on the above observations, we started a phase I and pharmacokinetic study of raltitrexed followed 24 h later by 5-FU/LFA in patients with advanced head and neck or colorectal cancer.[6] Treatment was recycled every 2 weeks, in order to exploit the potential advantage of a more frequent administration of two phase-specific drugs and to possibly achieve higher dose intensity. The drugs were administered in the reverse sequence for the second cycle, so that an intrapatient assessment of 5-FU pharmacokinetics and DPD activity with and without pre-treatment with raltitrexed could be performed. The DPD sample was obtained prior to 5-FU therapy in both courses in order to minimize any influence of 5-FU on DPD activity.[12]

The results of this dose-escalation study clearly demonstrated that it is possible to combine raltitrexed and 5-FU at the doses used in monotherapy (Tables 1 and 3). Raltitrexed 3.0 mg/m^2, LFA 250 mg/m^2 and 5-FU 1050 mg/m^2 were the doses selected for further evaluation. This was the first study in which raltitrexed was given in a biweekly schedule and this allowed us to achieve a dose intensity (1.32 mg/m^2/week) at the recommended dose level which was substantially higher than that achievable with the standard schedule of once every 3 weeks. Such a high dose intensity could be achieved since toxicity was moderate and non-cumulative.

Hematological toxicities were readily manageable and no toxic deaths occurred: only four cases of grade 4 neutropenia were recorded (febrile neutropenia did not occur), and 3-day administration of granulocyte colony stimulating factor induced prompt and complete reversal of toxicity; thrombocytopenia was very rarely recorded; anemia was a frequently observed finding, even at low drug dosages, and three patients required red blood cell transfusion. Non-hematological toxicity was mild: grade 3 nephrotoxicity was observed in one patient, but was reversible after treatment interruption and adequate i.v. hydration;

Table 1. Clinical studies of raltitrexed/5-FU combination therapy in patients with advanced solid tumors

Study	Combination	Phase	Patients	First/second-line	DLT	RD	Response
Schwartz *et al.*[4]	Raltitrexed/ bolus 5-FU	I	59 patients with advanced colorectal cancer	51 pre-treated with 5-FU	Febrile neutropenia, neutropenia	Raltitrexed 5.5 mg/m^2 on day 1, 5-FU 1200 mg/m^2 on day 2, q 3 weeks	9% RR: 1/54 CR, 4/54 PR Median survival 13.5 months
Mayer *et al.*[5]	Raltitrexed/ infusional 5-FU	I	43 patients with metastatic colorectal cancer	All first-line	Neutropenia, thrombocytopenia, diarrhea	Raltitrexed 2.6 mg/m^2 on days 8 and 29, 5-FU 2600 mg/m^2 weekly $\times$5 weeks, q 6 weeks	46% RR at dose levels 3–7: 13/28 PR
Caponigro *et al.*[6]	Raltitrexed/ bolus 5-FU/ LFA	I	41 patients with 1 locally advanced/ metastatic colorectal cancer and 17 patients with head and neck cancer	42 pre-treated with 5-FU	Neutropenia, renal toxicity	Raltitrexed 3.0 mg/m^2 on day 1, 5-FU 1050 mg/m^2/LFA 250 mg/m^2 on day 2, q 2 weeks	15% RR: 3/40 CR, 3/40 PR (advanced colorectal cancer), 35% RR: 1/17 CR, 5/17 PR (head and neck cancer)
Comella *et al.*[7]	Raltitrexed/ bolus 5-FU/ LFA	II	53 patients with metastatic colorectal cancer	All first-line	NA	NA	25% RR: 2/53 CR, 11/53 PR Median survival 14.5 months
Caponigro *et al.*[8]	Cisplatin/ raltitrexed/ bolus 5-FU/ LFA	I/II	45 patients with locally advanced/ metastatic head and neck cancer	All first-line	Neutropenia, fatigue	Cisplatin 60 mg/m^2 and raltitrexed 2.5 mg/m^2 on day 1, LFA 250 mg/m^2 and 5-FU 900 mg/m^2 on day 2, q 2 weeks	67% RR: 9/45 CR, 21/45 PR 100% RR at RD: 5/15 CR, 10/15 PR
Comella *et al.*[9]	Oxaliplatin/ raltitrexed/ bolus 5-FU/ LFA	I	52 patients with advanced colorectal cancer	All second-line	Neutropenia, stomatitis, diarrhea, febrile neutropenia, cardiac toxicity	Oxaliplatin 130 mg/m^2 and raltitrexed 3.0 mg/m^2 on day 1, 5-FU 1050 mg/m^2 and LFA 250 mg/m^2 on day 2, q 2 weeks	23% RR: 1/52 CR, 11/52 PR Median time to treatment failure 24 weeks
Caponigro *et al.*[10]	Mitoxantrone/ raltitrexed/ bolus 5-FU/ LFA	I	18 patients with refractory advanced solid tumors	Second-line	Neutropenia	Mitoxantrone 7 mg/m^2 and raltitrexed 3 mg/m^2 on day 1, 5-FU 900 mg/m^2 and LFA 250 mg/m^2 on day 2, q 2 weeks	33% RR: 1/18 CR, 5/18 PR

DLT, dose-limiting toxicity; RD, recommended dose; RR, response rate; CR, complete response; PR, partial response; NA, not applicable.

Table 2. Combination index values for the combination of raltitrexed/5-FU/LFA (24-h exposure to raltitrexed followed by a 4-h exposure to 5-FU/LFA) and potentiation factor of raltitrexed/5-FU by LFA

Cell line	Tissue of origin	Combination index mean (SE)[a]	Potentiation factor of raltitrexed/5-FU by LFA[b]
CAL-27	head and neck cancer	0.778 (0.058)	1.72 (0.26)
KB	head and neck cancer	1.082 (0.083)	1.20 (0.14)
LoVo	colon cancer	0.518 (0.2)	2.01 (0.47)
HT-29	colon cancer	0.31 (0.02)	1.72 (0.53)

[a]Combination index [mean (SE) from two independent experiments] was calculated at 50% cell survival (ED_{50}) from isobologram analyses performed on CalcuSyn software. A combination index <0.80 indicates synergism, >1.20 indicates antagonism and 0.80–1.20 indicates nearly additive cytotoxic effects.
[b]Potentiation factor [mean (SE) from two independent experiments] defined as: (raltitrexed/5-FU ED_{50})/(raltitrexed/5-FU/LFA ED_{50}).

Table 3. Raltitrexed/LFA/5-FU dose-escalation results

Level	Raltitrexed/LFA/5-FU (mg/m^2)[a]	No. of patients	No. of DLTs	DLT
1	1.5/250/600	6	–	–
2	**2.0**/250/600	6	–	–
3	2.0/250/**750**	6	–	–
4	**2.5**/250/750	6	–	–
5	2.5/250/**900**	7	–	–
6	**3.0**/250/900	8	1	grade 4 neutropenia
7	3.0/250/**1050**	16	2	grade 4 neutropenia (2)
8	3.0/250/**1200**	3	2	grade 4 neutropenia (1) grade 3 renal toxicity (1)

[a]Numbers in bold denote dose escalation.

mucositis was observed in 15 patients, but reached grade 3 in only three cases, demanding parenteral nutrition in two patients. Expected toxicities, such as fatigue and hand and foot syndrome, were observed but were mild to moderate.

The activity shown by our combination in metastatic colorectal cancer is noteworthy. Three patients, one of whom had already undergone previous chemotherapy for metastatic disease, achieved a complete response to treatment, which lasted 12, 13+ and 14+ months, respectively. Three further patients (two of whom were pre-treated) achieved a partial response. Of the 17 evaluable patients with head and neck cancer, six (35%) achieved an objective response to treatment; in particular, five objective responses occurred in the eight untreated patients.

DPD activity was significantly higher in samples obtained 24 h after raltitrexed administration, compared with those obtained prior to administration of the second course of chemotherapy. These data are consistent with induction of DPD activity by raltitrexed, but are not in keeping with reported preclinical data;[3] however, the preclinical data were obtained in rat hepatocytes and might be at least partly explained by DPD *ex vivo* down-regulation.[13] Our observed DPD induction might reflect a biological feedback loop in which DPD activity is increased to accommodate an increase in uracil nucleotides resulting from inhibition of TS by raltitrexed.[14] The good clinical activity of the combination, expressed by both the high response rate and the duration of response, suggest a potential synergism between the three drugs, despite the absence of an alteration in systemic 5-FU pharmacology. This possible synergism in antitumor activity may occur at the cellular level, as the cellular pharmacology studies of Longo *et al.* suggested an enhancement of 5-FU RNA incorporation as a potential mechanism for *in vitro* synergy with raltitrexed.[2]

Although evaluation of antitumor activity was not the main end point of the phase I study and the sample size was too small to draw conclusions about the efficacy of the combination, we believed that the activity shown by our regimen was encouraging enough to justify a phase II study in chemonaive patients with metastatic colorectal cancer.[7] This last study has confirmed the feasibility and the good activity of the combination; indeed, a 25% objective response rate was obtained in 53 patients (Table 1). In

particular, two complete responses, one of which lasted over 63 weeks, and 11 partial responses were achieved, while disease control was obtained in 60% of patients. Median survival time was 63 weeks in this study, with projected 1- and 2-year survival probabilities of 54 and 26%, respectively. No toxic deaths were observed in this study and the overall treatment toxicity was manageable.

Cisplatin/raltitrexed/5-FU/LFA

Rationale for combination/preclinical results

The efficacy results of the phase I study of raltitrexed/LFA/5-FU in the subset of patients with head and neck cancer were encouraging. However, head and neck cancer is known to have a higher chemosensitivity than colorectal cancer; therefore, we decided to add cisplatin, the most active single agent in head and neck cancer,[15] to the combination.

Cisplatin acts by forming intrastrand DNA–platinum adducts/cross-links between two adjacent guanine: guanine or guanine:adenine base pairs, leading to DNA strand breaks and p53-dependent apoptosis. Preclinical evidence has indicated at least additive growth inhibitory properties of cisplatin and raltitrexed in a panel of human tumor cell lines,[16] and different mechanisms of resistance to these drugs. It is possible that excision or mismatch repair processes might be prevented by thymidine 5′-triphosphate (dTTP) depletion following raltitrexed treatment. Such evidence has supported clinical combination studies of cisplatin and raltitrexed, which are currently being performed in a number of diseases, including head and neck cancer,[17] and non-small cell lung cancer.[18]

Clinical results

We have performed a phase I/II trial of cisplatin and raltitrexed on day 1, followed by LFA and 5-FU on day 2 as first-line therapy in newly diagnosed patients with locally advanced and metastatic squamous cell carcinoma of the head and neck.[8] Treatment cycles were again repeated every 2 weeks.

The results of this dose escalation study clearly demonstrated that it is possible to combine cisplatin, raltitrexed and 5-FU at doses which are close to those used in monotherapy (Tables 1 and 4). Although DLT was observed in four of six patients at the fifth dose level, we decided to reduce the dose of raltitrexed while escalating the cisplatin dose; this proved feasible and this dose level, cisplatin 60 mg/m^2, raltitrexed 2.5 mg/m^2, LFA 250 mg/m^2 and 5-FU 900 mg/m^2, was selected for further evaluation. In particular, the mean raltitrexed dose intensity at this level is higher than that achieved with the standard every 3 week schedule. Such a high dose intensity could be achieved since toxicity was moderate. The observed activity in this study was also encouraging: a 67% overall response rate was observed in a patient population which had probably worse prognostic features than in other similar studies, since 16 of 45 patients had relapsed after surgery, 12 of 45 patients had a performance status of 2 and seven of 45 patients had metastatic disease. Furthermore, at the recommended dose, all 15 treated patients achieved an objective response (five complete responses and 10 partial responses). Activity was observed even in metastatic patients, with complete disappearance of lung metastases in one patient. These results, albeit preliminary, seem better than those achieved in our previous study with cisplatin/methotrexate, followed by LFA/5-FU;[19] a regimen which is currently our standard treatment in locally advanced and metastatic head and neck cancer. A randomized phase II study comparing cisplatin/raltitrexed/5-FU/LFA with cisplatin/methotrexate/5-FU/LFA is currently ongoing and is rapidly accruing patients.

Table 4. Cisplatin/raltitrexed/LFA/5-FU dose-escalation results

Level	Cisplatin/raltitrexed/LFA/5-FU (mg/m^2)[a]	No. of patients	No. of DLTs	DLT
1	40/2.0/250/750	6	2	grade 3 fatigue (1) grade 4 neutropenia (1)
2	40/**2.5**/250/750	6	1	grade 4 neutropenia
3	40/2.5/250/**900**	6	2	grade 4 neutropenia (2)
4	**50**/2.5/250/900	6	2	grade 4 neutropenia (2)
5	50/**3.0**/250/900	6	4	grade 4 neutropenia (4)
6	**60**/2.5/250/900	15	4	grade 4 neutropenia (4)

[a]Numbers in bold denote dose escalation.

Oxaliplatin/raltitrexed/5-FU/LFA

Rationale for combination/preclinical results

Oxaliplatin is among the most active drugs against advanced colorectal cancer, with a median response rate of 18% as first-line treatment[20,21] and of 10% as second-line treatment.[22] Preclinical observations suggest that oxaliplatin has synergistic antitumor activity with 5-FU in murine leukemia cell cultures transplanted into mice and in human colonic xenografts that are either sensitive or resistant to 5-FU.[23] Preclinical data also suggest that at least an additive interaction between oxaliplatin and raltitrexed exists, and a recently published phase I study of the combination of the two drugs[24] has shown good tolerability and promising antitumor activity in colorectal cancer and in pleural mesothelioma, a notoriously chemoresistant tumor.

Clinical results

We performed a dose-finding study of the combination of oxaliplatin, raltitrexed and modulated 5-FU in pretreated patients with advanced colorectal cancer.[9] A 2-weekly schedule of administration was again used, with oxaliplatin and raltitrexed administered on day 1, followed by LFA and 5-FU on day 2. Fifty-two patients with advanced colorectal carcinoma previously treated with one (25 cases), two or more lines of chemotherapy entered the study. Starting doses were 85, 2.5 and 750 mg/m^2 for oxaliplatin, raltitrexed and 5-FU, respectively. The results of the dose escalation are shown in Tables 1 and 5. Neutropenia qualified as DLT; severe diarrhea and stomatitis were rarely reported, while mild peripheral sensitive neurotoxicity was frequent. The recommended doses for phase II were 130 mg/m^2 for oxaliplatin, 3.0 mg/m^2 for raltitrexed and 1050 mg/m^2 for 5-FU, while LFA was used at the usual fixed dose of 250 mg/m^2. Twelve patients (23%) had a major response (one complete) with this regimen and an additional eight patients (15%) showed a minor response. Stable disease was seen in 15 patients, so that a temporary disease control was achieved in 67% of patients. Median time to treatment failure was 24 weeks in this study, which is encouraging since our study population included several patients considered refractory to 5-FU and/or irinotecan. These results indicate potential for this combination regimen as a first-line treatment of advanced colorectal cancer and a clinical trial is currently planned.

Mitoxantrone/raltitrexed/5-FU/LFA

Rationale for combination/preclinical results

Clinical combination studies have shown that raltitrexed and anthracyclines can be combined at full doses without unexpected toxicities.[25,26] Mitoxantrone is a completely synthetic DNA intercalator based on the anthracenedione structure and may be viewed as an analog of the anthracyclines. It is a manageable and active drug in the treatment of a number of neoplasms, with a safety profile that is more favorable than anthracyclines.[27] We have observed that mitoxantrone and raltitrexed have at least an additive interaction in a number of human tumor cell lines (data not shown).

Table 5. Oxaliplatin/raltitrexed/LFA/5-FU dose-escalation results

Level	Oxaliplatin/raltitrexed/LFA/5-FU (mg/m^2)[a]	No. of patients	No. of DLTs	DLT
1	85/2.5/250/750	6	1	grade 4 neutropenia
2	85/2.5/250/**900**	6	1	grade 4 neutropenia
3	85/**3.0**/250/900	7	3	grade 4 neutropenia (1) grade 4 stomatitis (1) grade 3 diarrhea (1)
4	85/3.0/250/**1050**	6	0	–
5	**105**/3.0/250/1050	6	2	grade 4 neutropenia (1) delay >2 weeks due to failure to recover from prior toxicity (1)
6	**130**/3.0/250/1050	6	2	grade 4 neutropenia (2)
7	130/3.0/250/**1200**	8	4	grade 4 neutropenia (1) grade 4 febrile neutropenia (1) grade 4 diarrhea (1) grade 4 cardiac toxicity (1)

[a]Numbers in bold denote dose escalation.

Clinical results

In our phase I study, mitoxantrone and raltitrexed were administered on day 1, followed by LFA and 5-FU on day 2, in patients with advanced solid tumors which were unresponsive or no longer amenable to standard therapies.[10] Mitoxantrone was given at the starting dose of 6 mg/m^2, raltitrexed at the fixed dose of 3 mg/m^2, LFA at the fixed dose of 250 mg/m^2 and 5-FU at the starting dose of 750 mg/m^2. Mitoxantrone and 5-FU doses were subsequently escalated alternately up to DLT. Treatment was repeated every 2 weeks. Four dose levels were tested in 18 patients. All three patients treated at the fourth dose level had grade 4 neutropenia after the first cycle; therefore, this level was defined as the maximum tolerated dose and the dose level immediately below (mitoxantrone 7 mg/m^2 and 5-FU 900 mg/m^2) was selected for further evaluation. The results of the dose escalation are shown in Tables 1 and 6. The mean raltitrexed dose intensity at the recommended dose is again higher than that achieved with the standard schedule of once every 3 weeks. Neutropenia was the main toxic effect; non-hematological side effects were mild. One complete response and five partial responses (all but one in patients with recurrent head and neck cancer) were observed, for an overall response rate of 33%. Thus, this combination is feasible, well tolerated and deserves further evaluation, particularly in patients with head and neck cancer.

Conclusions and future perspectives

Although the results achieved with raltitrexed when used alone in first-line treatment of advanced colorectal cancer did not match its high expectations, with only comparable efficacy to standard 5-FU/LV regimens,[28] raltitrexed appears suitable for use in combination in advanced colorectal cancer. Furthermore, our data show that the drug's indications might be widened to include other solid tumors, mainly head and neck cancer. In particular, combinations of raltitrexed with platinum agents merit further evaluation. A number of clinical trials support the combined use of platinum agents and TS inhibitors, with results that in most cases far exceed those expected.[24,29,30,31] Clear clinical evidence of synergism exists between the two classes of drugs, although the mechanism has not yet been entirely elucidated (possibly TS down-regulation).

In all of our studies, we have used raltitrexed in a 2-weekly schedule. This has allowed us to reach a drug dose intensity that in all studies has been higher than that achieved with the standard schedule of once every 3 weeks. The 2-weekly schedule has the additional advantage of more frequent exposure of tumor cells to phase-specific drugs such as 5-FU and raltitrexed.

A further potential application of raltitrexed in combination therapies is within multidisciplinary chemoradiotherapy strategies. In particular, in rectal cancer, like in other neoplasms, the way forward appears to be a combination of radiotherapy and chemotherapy, although an optimal schedule has not been internationally agreed upon.[32] Raltitrexed, like 5-FU, is a radiation sensitizer.[33] *In vitro* studies have shown that raltitrexed decreases the shoulder of radiation survival curves and *in vivo* tumor growth delay was observed when raltitrexed was administered intermittently with fractionated radiotherapy. The best acknowledged mechanism of action for raltitrexed and 5-FU as radiation sensitizers is their ability to slow down or to inhibit the repair of DNA strand breaks. In a clinical setting, for a drug to be used as a radiation sensitizer, it must be present when the repair of radiation damage is still taking place. This aspect limits the usefulness of bolus 5-FU, which has a serum half-life of less than 20 min; whereas, raltitrexed, although phase specific, has a much longer half-life.

Phase I studies have established the recommended dose of raltitrexed in combination with adjuvant postoperative radiotherapy,[34] in preoperative radiotherapy[35] and in combination with radiotherapy for patients with advanced inoperable/recurrent rectal cancer.[36] While phase II studies are ongoing in the

Table 6. Mitoxantrone/raltitrexed/LFA/5-FU dose-escalation results

Level	Mitoxantrone/raltitrexed/LFA/5-FU (mg/m^2)[a]	No. of patients	No. of DLTs	DLT
1	6.0/3.0/250/750	3	0	–
2	6.0/3.0/250/**900**	6	2	grade 4 neutropenia (2)
3	**7.0**/3.0/250/900	6	2	grade 4 neutropenia (2)
4	7.0/3.0/250/**1050**	3	3	grade 4 neutropenia (3)

[a]Numbers in bold denote dose escalation.

latter two settings, our group is about to start a phase I study in which our original combination of raltitrexed, 5-FU and LFA will be administered concurrently with standard radiotherapy as primary treatment in patients with stage II–III resectable rectal cancer.

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