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# High-dose 5-fluorouracil plus low dose methotrexate plus or minus low-dose PALA in advanced colorectal cancer: a randomised phase II-III trial of the EORTC Gastrointestinal Group

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**Abstract**

The aim of this study was to investigate whether *N*-(phosphonacetyl)-L-aspartic acid (PALA) can enhance the activity of low-dose methotrexate (LD-MTX) modulated infusional 5-fluorouracil (5-FU) in patients with advanced colorectal cancer. 198 patients were randomised either to (i) 5-FU 60 mg/kg as a continuous infusion over 48 h, to be given weekly four times and thereafter every 2 weeks, with methotrexate 40 mg/m$^2$ administered just before 5-FU (control regimen) or to (ii) PALA 250 mg/m$^2$ as a 15 min infusion administered 24 h before 5-FU and methotrexate which was given as described in the control regimen. The response rate was 13% in the patients randomised to the control arm and 7% in the patients randomised to the experimental arm. If stabilisation of the disease was also considered as a positive response, these figures become 54 and 46%, respectively. All these differences did not reach statistical significance. The median durations of progression-free survival (PFS) in the two treatment groups were not significantly different. The median duration of survival was 13.1 months in the control arm and 11.9 months in the experimental arm ($P=0.31$). No benefit was obtained by adding PALA to LD-MTX + infusional FU. Taking into account data from US trials, the modulating effect of PALA, although 'promising' in phase II, could not be substantiated in randomised studies.


*Keywords:* Advanced colorectal cancer; PALA; Chemotherapy; Infusional 5-FU

## 1. Introduction

Leucovorin (LV) is the most widely used biomodulator of 5-fluorouracil (5-FU) and has been added to both bolus and continuous infusion schedules [1]. Methotrexate (MTX) is probably as effective as LV, showing a doubling of response rate and 1.6 months survival advantage in a meta-analysis of comparative trials with unmodulated 5-FU [2].

Apart from LV and MTX, other agents have been shown to biochemically modulate FU metabolism, and, at least in experimental systems, efficacy [3]. One of these agents is the *N*-(phosphonacetyl)-L-aspartic acid (PALA). It inhibits aspartate transcarbamylase and thereby the *de novo* pyrimidine synthesis. This leads to an enhanced cellular anabolism of FU, including more incorporation of the FU-metabolite fluorouridine triphosphate (FUTP) into RNA over that seen with FU alone. Using mammary and

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colon carcinoma cell lines and animal models, synergistic cytotoxicity between PALA and FU has been observed [4–6]. Most clinical trials with this combination have been disappointing [7–10], but have also been criticised for giving the active drug in a too low dose and the modulating drug a too high dose [11].

Ardalan and colleagues [12] tried to mimick the synergistic *in vitro* and animal *in vivo* conditions by giving 5-FU weekly over 24 h in high doses (maximum tolerated dose (MTD) 2600 mg/m$^2$/week) 24 h after a fixed bolus administration of 250 mg/m$^2$ PALA. This dose is 15% of the MTD, but has been shown to effectively block the *de novo* pyrimidine synthesis. Pyrimidine nucleotide pools are depressed within 24 h to around 20% and remain so for at least 1 week [13,14]. In this phase I-II study, 11 of 28 patients (39%) treated with PALA-FU responded. These findings were confirmed in another phase II trial in which 37 chemotherapy-naïve colorectal cancer patients were treated with FU-PALA with doses and a schedule that were identical to those used by Ardalan. No patient had grade 4 toxicity, the response rate was 43% (95% confidence interval (CI): 27–59) and the projected median survival time was 15 months [15].

In a previous European Organization for Research and Treatment of Cancer (EORTC) trial [16], low-dose MTX (LD-MTX) was shown to induce a positive modulation of the activity of high-dose FU (HD-FU) in colorectal cancer. Such a positive effect was reached at the expense of only mild toxicity. In the present study, the possibility of an enhanced activity through PALA, as a second modulator, was investigated. Since MTX and PALA both enhance FU anabolism, though via different pathways, the HD-FU/LD-MTX/LD-PALA combination could represent a further step forward in the development of the optimum treatment regimen.

Thus, a phase III study was carried out to compare HD-FU/LD-MTX with HD-FU/LD-MTX/LD-PALA. Response rate, survival and toxicity were the main endpoints of the study. Since the HD-FU/LD-MTX/LD-PALA combination was a new one, the trial was started as a randomised phase II study that was to be continued as a phase III study if the response rate with this treatment appeared to exceed 10% and no toxicities warranting discontinuation of the trial were observed.

## 2. Patients and methods

Patients could be entered if they had histologically-verified advanced-unresectable or metastatic adenocarcinoma of the colon or rectum. In addition, the following admission criteria were applied: presence of measurable and/or evaluable disease, World Health Organization (WHO) performance status 0-2, life expectancy of at least three months, informed consent, no previous radiotherapy of the measurable disease, no previous chemotherapy with fluorodinated pyrimidines or folate antagonists within 1 year before entering the study, no other previous cytotoxic therapy within 4 weeks before entering the study, serum creatinine concentration less than or equal to 120 μmol/l, serum bilirubin concentration less than or equal to 50 μmol/l, White Blood Cell (WBC) count greater than or equal to $3\times10^9$/l or platelet count greater than or equal to $100\times10^9$/l unless there was marrow invasion by the tumour, absence of uncontrolled *angina pectoris* or myocardial infarction within three months prior to treatment start, no metastases to the central nervous system (CNS), absence of large pleural or peritoneal effusions, absence of active infection, no history of severe mental disorders, no history of other malignant disease except non-melanoma skin cancer and treated carcinoma *in situ* (CIS) of the uterine cervix, no use of salicylates or other non-steroidal anti-inflammatory agents (NSAID) which could not be discontinued.

Patients were randomised after verification of all eligibility criteria through the EORTC Data Center. At randomisation, institution and measurability of disease were used as stratification factors. The minimisation technique was used.

Eligible patients were randomised either to (i) 5-FU 60 mg/kg as a continuous infusion over 48 h, to be given weekly four times and thereafter every 2 weeks, with MTX 40 mg/m$^2$ administered just before 5-FU (control regimen) or to (ii) PALA 250 mg/m$^2$ as a 15-min infusion administered 24 h before 5-FU and MTX which had to be given as described in the control regimen.

Toxicity was recorded using WHO Toxicity Criteria. Eligible patients who started treatment were evaluated for the occurrence of toxic events. The dosing was delayed or reduced according to appropriate values in cases of toxicity. Any patient with grade 4 toxicity was taken off the protocol treatment. Adverse effects not included in the WHO Toxicity Criteria were recorded separately.

The disease was evaluated every 3 months or earlier if clinically appropriate. Palpation, X-ray, echography and computed tomography (CT)-scan were allowed, whichever was deemed appropriate.

Complete response (CR) was defined as the disappearance of all known disease on two observations not less than four weeks apart. Partial responses (PR) was defined as a reduction by at least 50% in the sum of the products of the largest perpendicular diameter of all bidimensionally measurable lesions. No change (NC) was the status recorded when a 50% decrease in tumour size or a 25% increase in the size of one or more measurable lesions could not be detected during a period of at least 12 weeks. Progressive disease (PD) was defined as a 25% or more increase in the size of at least one measurable lesion or the appearance of a new lesion. In case of PD, patients had to stop the protocol treatment.

For the phase II part of the study, 29 patients had to be included in each treatment arm. If at least one response was observed in each treatment group, the phase III comparative study had to be initiated. The probability of rejecting an active regimen (response rate >10%) from further study was less than 5% [17].

For the phase III part of the study, the 50% assumed 1-year survival in the control arm (HD-5FU/LD MTX), i.e. the median survival was based on an estimate obtained in a previous EORTC trial [16]. The aim of the phase III part of the study was to detect an absolute difference of 15% in the 1-year survival (i.e. from 50 to 65%). At least 146 patients had to be followed until death. The sample size calculation was done for a two-sided logrank test with a type I error of 5% and a power of 80% [18].

Response to treatment was compared using a Chi-square. The duration of survival and progression-free survival were estimated using the Kaplan–Meier technique [19] and compared using a two-sided logrank test [20]. For the multivariate analysis, the Cox's proportional hazards regression model [21] was used. Patient characteristics, toxicity data and drug activity are presented for all eligible patients. Overall and progression-free survival (OS and PFS, respectively) analyses were performed in compliance with an intent-to-treat policy, i.e. by ensuring that all randomised patients are analysed according to the treatment arm to which they were randomised. As a secondary step which was supposed to maximise the opportunity for the new treatment to show additional efficacy, analyses of efficacy data recorded in eligible patients were carried out.

## 3. Results

Between 1992 and 1994, 198 patients (99 in each arm) were randomised from 13 institutions in The Netherlands, Belgium and France (Fig. 1). The median number of patients per institution was 10 (range 3–44). Nine patients per arm were not eligible (9% ineligibility rate) for at least one of the following reasons: abnormal laboratory values (5 patients), treatment with a non-steroidal compound (2 patients), non-evaluable disease (3 patients), no cancer (2 patients), no proof of malignancy (1 patient), history of other malignancy (1 patient), concomitant other malignancy (1 patient), brain metastasis (1 patient), unknown primary site (1 patient), prior treatment (1 patient). 2 patients randomised to the control arm did not start the protocol treatment. The reasons were surgical treatment of an ileus (1 patient) and CNS metastases detected between randomisation and treatment start (1 patient). Characteristics of the eligible patients at study entry are given in Table 1.

Percentages of patients with side-effects during treatment are presented in Table 2.

Rates of responses in the two treatment arms are provided in Table 3. A response was observed in 13% of the patients randomised to the control arm and in 7% of the patients randomised to the experimental arm. When excluding patients for whom response could not be evaluated, the response rates were 15 and 8% in the control and experimental groups, respectively.

If stabilisation of the disease is also considered as a positive response, these figures become 54 and 46% in the control and experimental groups, respectively. All these differences did not reach statistical significance.

The median duration (Table 4) of progression-free survival in the two treatment groups were not significantly different ($P=0.44$).

Death was recorded in 87% of the patients, the median duration (95% CI) of survival was 13.1 months (9.8, 15.8) in the control arm and 11.9 months (10.1, 15.4) in the experimental arm ($P=0.31$) (Fig. 2). The 1-year survival estimates (95% CI) were 53.3% (43.5, 63.2) in

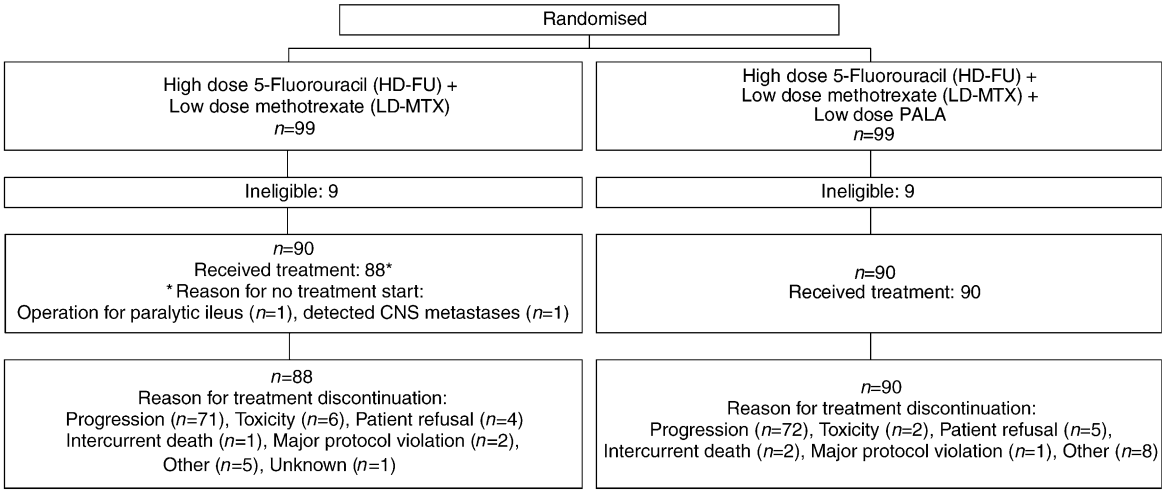


Fig. 1. Flow diagram of clinical trial EORTC 40909. CNS, Central Nervous System.

Table 1
The characteristics of the eligible patients at study entry. Patients were randomised either to high dose 5-fluorouracil/low dose methotrexate (HD-FU/LD-MTX) or to high dose 5-fluorouracil/low dose methotrexate combined with low dose PALA (HD-FU/LD-MTX + LD-PALA)

| Characteristics | HD-FU/ LD-MTX ($n=90$) | HD-FU/ LD-MTX + LD-PALA ($n=90$) |
|---|---|---|
| Age (years) median (range) | 59 (31–77) | 63 (20–79) |
| Male (%) | 54 | 59 |
| WHO Performance Status (%) | | |
| 0 | 38 | 47 |
| 1 | 60 | 46 |
| 2 | 2 | 8 |
| Weight loss > 10% (%*) | 18 | 24 |
| Time since diagnosis (months) median (range) | 5.5 (0–97) | 3.0 (0–111) |
| Primary site (%) | | |
| Colon | 32 | 22 |
| Rectosigmoid | 68 | 78 |
| Main metastatic sites (%) | | |
| Liver | 68 | 81 |
| Lung | 20 | 14 |
| Prior adjuvant chemotherapy (%) | 2 | 3 |
| Haemoglobin (g/l, median) | 129 (69–161) | 128 (66–180) |
| WBC count ($10^9$/l, median) | 8.2 (4.5–14.7) | 7.9 (3.0–16.7) |
| Serum bilirubin (μmol/l, median) | 7.0 (2.0–84.0) | 9.0 (1.0–88.0) |
| Alkaline phosphatase (IU/l, median) | 115 (46–980) | 115 (38–921) |
| LDH (IU/l, median) | 358 (144–6052) | 442 (145–4600) |

LDH, lactate dehydogenase; WHO, World Health Organization; WBC, white blood cell. *Of patients for which their status is known.

Table 2
The percentages of eligible patients with side-effects observed during the protocol treatment. Patients were treated either with high dose 5-fluorouracil/low dose methotrexate (HD-FU/LD-MTX) or with high dose 5-fluorouracil/low dose methotrexate combined with low dose PALA (HD-FU/LD-MTX + LD-PALA)

| | HD-FU/LD-MTX | | HD-FU/LD-MTX + LD-PALA | |
|---|---|---|---|---|
| | Grade 3 (%) | Grade 4 (%) | Grade 3 (%) | Grade 4 (%) |
| Haematological | | | | |
| WBC | 0 | 0 | 1 | 0 |
| Granulocytes | 4 | 0 | 2 | 0 |
| Platelets | 0 | 0 | 1 | 0 |
| Haemoglobin | 5 | 0 | 6 | 0 |
| Non-haematological | | | | |
| Stomatitis | 3 | 1 | 7 | 1 |
| Nausea/Vomiting | 13 | 0 | 7 | 0 |
| Diarrhoea | 1 | 0 | 2 | 0 |
| Renal | 1 | 0 | 0 | 0 |
| Cutaneous | 1 | 0 | 0 | 0 |
| Alopecia | 1 | 0 | 1 | 0 |
| Cardiac | 1 | 1 | 1 | 0 |
| Pulmonary | 0 | 0 | 0 | 1 |
| Fever-drug | 0 | 0 | 2 | 0 |
| Infection | 0 | 0 | 1 | 1 |
| State of consciousness | 1 | 0 | 0 | 0 |

the control arm and 49.3% (39.3, 59.3) in the experimental arm. The 2-year survival estimates (95% CI) were 28.1% (19.1, 37.1) in the control arm and 16.5% (8.9, 24.2) in the experimental arm.

Results from the analysis restricted to the eligible patients can be summarised as follows. Death was recorded in 87% of the patients, the median duration (95% CI) of survival was 13.8 months (11.0, 17.0) in the control arm and 12.3 months (10.2, 16.0) in the experimental arm ($P=0.32$) (Fig. 3). The one-year survival estimates (95% CI) were 57.6% (47.3, 67.8) in the control arm and 53.2% (42.7, 63.7) in the experimental arm. The 2-year survival estimates (95% CI) were 29.8% (20.2, 39.4) in the control arm and 17.5% (9.4, 25.6) in the experimental arm.

The Cox model for survival retained the following variables at the 5% level of significance: granulocyte count ($\leqslant 5\times10^9$/l better), platelet count ($\leqslant 350\times10^9$/l better), lung involvement (no is better), weight loss ($\leqslant 10\%$ better) and serum bilirubin (normal better). Adjusting for these five variables, the treatment effect was not statistically significant ($P=0.283$).

Table 3
Rates of clinical responses in patients either treated with high dose 5-fluorouracil/low dose methotrexate (HD-FU/LD-MTX) or treated with high dose 5-fluorouracil/low dose methotrexate combined with low dose PALA (HD-FU/LD-MTX + LD-PALA)

| Response | HD-FU/ LD-MTX ($n=90$) $n$ (%) | HD-FU/LD-MTX + LD-PALA ($n=90$) $n$ (%) |
|---|---|---|
| Complete response | 3 (3) | 1 (1) |
| Partial response | | |
| Not confirmed | 6 (7) | 4 (4) |
| Confirmed 4 weeks later | 3 (3) | 1 (1) |
| Stable disease | 37 (41) | 35 (39) |
| Progression | 28 (31) | 37 (41) |
| Early death | 2 (2) | 2 (2) |
| Not evaluable | 11 (12) | 10 (11) |

Table 4
Progression-free survival (PFS) in patients either treated with high dose 5-fluorouracil/low dose methotrexate (HD-FU/LD-MTX) or treated with high dose 5-fluorouracil/low dose methotrexate combined with low dose PALA (HD-FU/LD-MTX + LD-PALA)

| | HD-FU/ LD-MTX ($n=90$) | HD-FU/LD-MTX + LD-PALA ($n=90$) |
|---|---|---|
| Median duration (months) (95% CI) | 5.9 (4.2, 8.2) | 4.7 (3.2, 5.8) |
| PFS estimates | | |
| One year (%, 95% CI) | 12.2 (5.5, 19.0) | 13.3 (6.3, 20.4) |
| Relative risk (95% CI) | 1[a] | 1.13 (0.84–1.51) |

95% CI, 95% Confidence Interval.
[a] Reference value.

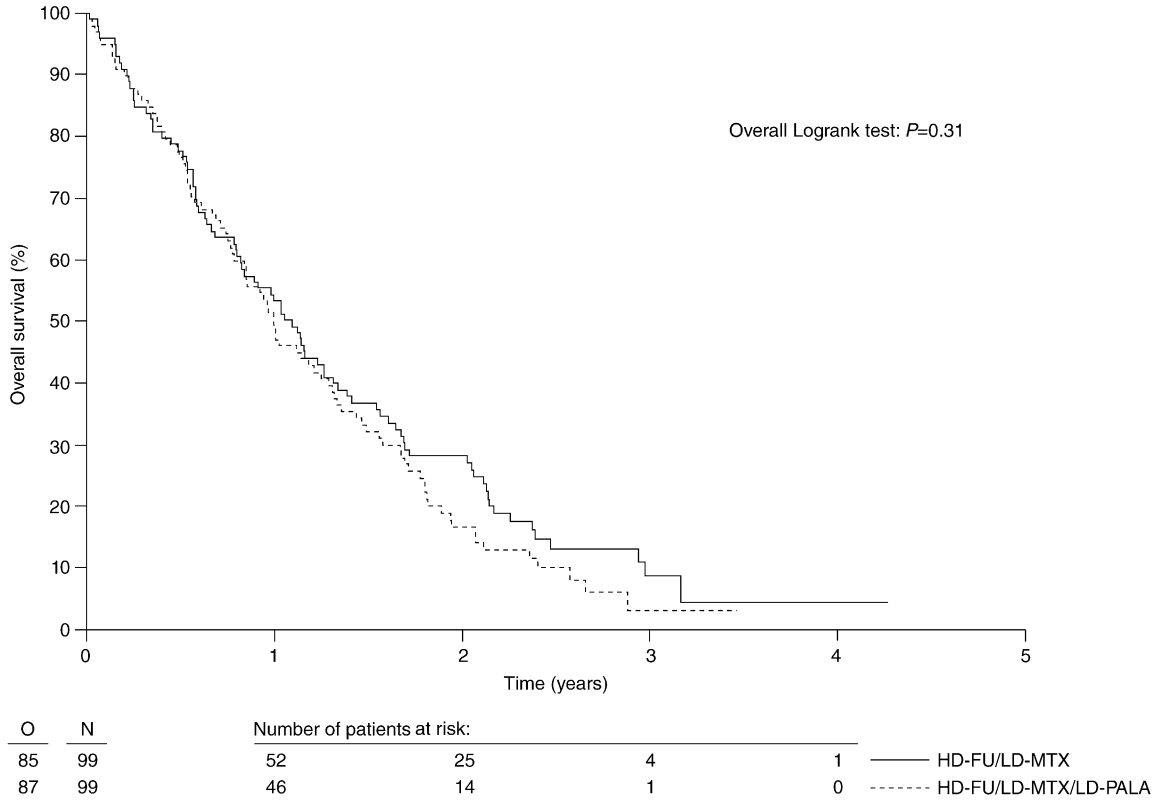


Fig. 2. Overall survival (months) in patients suffering from advanced colorectal carcinoma and treated either with high dose of 5-fluorouracil (HD-FU)/low-dose methotrexate (LD-MTX) or with HD-FU/LD-MTX plus low-dose PALA—all patients are considered. O, Observed events; N, Number of patients considered.

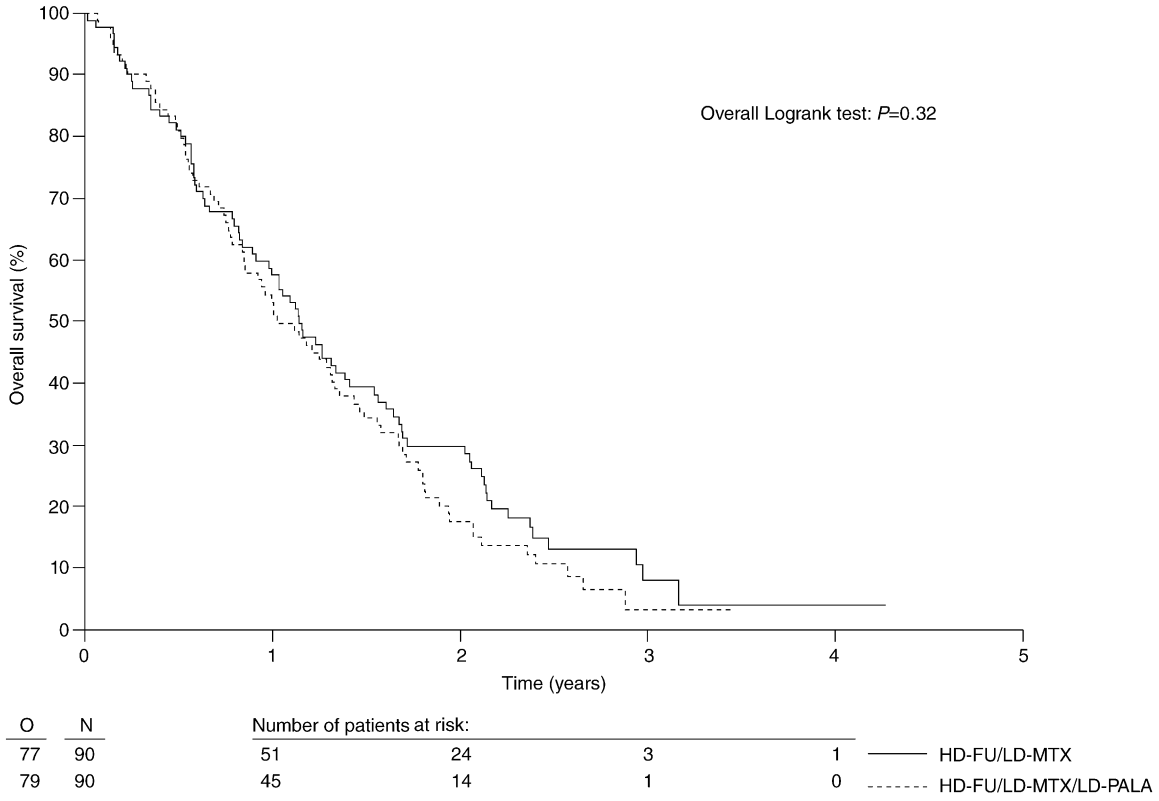


Fig. 3. Overall survival (months) in patients suffering from advanced colorectal carcinoma and treated either with high dose of 5-fluorouracil (HD-FU)/low-dose methotrexate (LD-MTX) or with HD-FU/LD-MTX plus low-dose PALA—eligible patients are considered.

## 4. Discussion

This trial was carried out when there was still interest in assessing other ways of modulating the activity of 5-FU (i.e. PALA and interferon). At that time, new drugs such as CPT-11 and oxaliplatin, that have changed current practice, were not available. Leucovorin, as well as methotrexate, were accepted modulating agents, although MTX was never widely used. At the same time as our study, two large scale studies were carried out in the US. The United States Southwest Oncology Group (US. SWOG) conducted a seven-arm randomised phase II trial assessing different schedules of 5-FU ± leucovorin and including one arm with PALA plus high-dose infusional 5-FU (the 'Ardalan' schedule) [22]. There were no significant differences among any of the seven cohorts and the only conclusion was that infusional 5-FU had a better therapeutic index than bolus regimens and that PALA was ineffective in modulating the activity of high-dose infusional 5-FU. The Eastern Cooperative Oncology Group/Cancer and Leukemia Group B (ECOG/CALGB) randomised 1120 patients in a five-arm design, including the same PALA/high-dose FU schedule. The conclusion was that PALA again failed to increase the activity of weekly 24-h infusion of 5-FU and that intermittent infusion schedules with or without LV are the safest and most effective means of delivering 5-FU [23].

The results of our trial are more or less consistent with these results from the US. We applied 48-h infusion of 5-FU in approximately the same dose weekly×4 and then every two weeks, with results in the somewhat less dose-intensive 5-FU regimen in the first 6 weeks. Meanwhile, our group adopted the weekly 24 h infusion ×6 instead of the 48 h infusion 4 weeks out of 6. Furthermore, we assessed double modulation of 5-FU by combining low-dose MTX with PALA. In fact, this trial was undertaken to test the hypothesis that the addition of PALA in a low, but biochemically active, dose to the combination of high-dose 5-FU, given as an intermittent 48 h continuous infusion, and low-dose methotrexate, given 24 h prior to 5-FU, would improve the 1-year survival rate from around 50 to 65%. Clearly, this hypothesis can now be rejected with a power of 80%. The efficacy data even suggest a lower response rate, PFS and OS with PALA, although none of these differences were statistically significant. The toxicity in both arms of our trial was moderate without any toxic death, which confirms the good tolerability of infusional 5-FU.

It appears obvious that low-dose PALA does not add any further efficacy combined with the biomodulator MTX. Data from the US also confirm this. As a result, interest in PALA as a modulating agent has disappeared and LV remains the most widely applied drug. Data from a more recent EORTC Gastro Intestinal (GI) trial suggest that LV can indeed increase the activity of 5-FU, also in high-dose infusional schedules [24].

More recent studies have clearly demonstrated the superiority of 5-FU/LV + irinotecan or oxaliplatin over 5-FU/LV treatment alone. Given the high toxicity associated with bolus 5-FU/LV regimens in combination with the new drugs [25] and the superior activity of infusional FU/LV + oxaliplatin over bolus FU/LV + CPT-11 [26], infusional 5-FU is now, even in the US, preferred over bolus schedules. Further improvements may be possible by including new 'mechanism-based' agents such as cetuximab in the therapeutic armentarium.

In conclusion, no benefit was obtained by adding PALA to low-dose MTX + infusional FU. Taking into account data from US trials, the modulating effect of PALA, although 'promising' in phase II studies, could not be substantiated in randomised studies.

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