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TOLERABILITY OF
RALTITREXED AND 5-FLUOROURACIL CHEMOTHERAPY
IN COLORECTAL CANCER PATIENTS

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ACADEMIC DISSERTATION

To be publicly discussed, by the permission of the Medical Faculty of the University of Helsinki,
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Pohjanmaalainen Pia pyysi pumppuhoitajaa parikseen puuhastelemaan.
Poliklinikan professori patisti pistämään päämäärät paperille.
Pumppuhoito parantava, palliativinen.
Puuhaan pilkkannuimme, porttiin, pumppuun perehdyimme.
Potilaalle puudutuksessa portti pistetään.
Potilas petiin pötkölleen, palpoi portti, pistä porttiin, pumppu pussiin.
Pakkasella peitä pumppu puserolla.
Pumppun poisto perehdyttiin. Pois passivinen potilas päivähoito-osastolta.
Projektin pultteissa potilas pisti Pialle p.tä pussissa postiin.
Pakastimesta plasmaputkia pengottiin.
Pulmania piisasi pohdittavaksi:
Pahoinvointi pilia päivän, Primeran -pilleristä pelastus.
PPE pirullinen pulma, pumpun parinkymmenen prosentin pienennykseillä, paussilla pulmaa pakoon.
Paljalle päälle peitoksi peruukki.
Päivänpaisteesta pois palamasta.
Popsittavaa pataan periseman: parsaa, paprikkaa, papua, perunaa,
pastaa, persiljaa, pinaattia, puuroa, puolukkaa,
purtavaaorkkalaa, perinteistä perusruokaa päivittäin.
Palapanikkeeks pilsneriä, piimää, pullovettä.
Pihviä plus punaviniä piristykseksi pyhänä.
Potenssinkin pilleriä.
Patoumat puhumalla, paijaamalla pois.
Preventiona polyyvit pois poltettava.
Perinnöllisydestäkin puhua pitäisi.

Pumppuhoitaja Anne H enttinen
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1. **LIST OF ORIGINAL PUBLICATIONS**


2. ABBREVIATIONS

CARS = compensatory anti-inflammatory response syndrome
CRP = c-reactive protein
DFS = disease free survival
DLT = dose limiting toxicity
DPD = dihydropyrimidine dehydrogenase
dUMP = deoxy uracil monophosphate
EGFR = epidermal growth factor receptor
ESR = erythrocyte sedimentation rate
5-FU = 5-fluororacil
FdUMP = fluorodeoxy uracil monophosphate
FdUrd = 5-fluoro-2-deoxyuridine; floxuridine
GI = gastrointestinal
Gr = grade
HAI = hepatic arterial infusion
HR = hazard ratio
IFN = interferon
IL = interleukin
IL-1RA = interleukin 1 receptor antagonist
LV = leucovorin
meTHF = methyl tetrahydrofolate
MTD = maximal tolerated dose
NO = nitric oxide
OR = odds ratio
OS = overall survival
PALA = N-phosphonoacetyl-L-aspartate
pCR = pathologic complete response
PD = progressive disease
PFS = progression free survival
PPE = palmoplantar erythrodysesthesia
PR = partial response
PVI = protracted venous infusion
RR = response rate
SCFA  = short chain fatty acids
SD    = stable disease
SIRS  = systemic inflammatory response syndrome
STNFR = soluble tumour necrosis factor receptor
TGFβ  = transforming growth factor-beta
TMP   = thymidine 5’- monophosphate
TNFα  = tumour necrosis factor alpha
TS    = thymidylate synthetase
ThTP  = thymidine 5’-triphosphate
TTP   = time to progression
TTF   = time to treatment failure
ULN   = upper limit of normal
VEGF  = vascular endothelial growth factor
WHO PS = World Health Organization performance status
3. ABSTRACT

Treatment of colorectal cancer has improved considerably over the past 50 years. In Finland 19% of the 449 colorectal patients survived in 1953 and predictions for 2003 are 2452 new cases of whom 55% might survive for 5 years following diagnosis. These encouraging trends can be attributed to better diagnostics, surgical techniques, oncologic therapy and follow-up. In the future, screening is also likely to influence these trends. Oncological knowledge in colorectal cancer has exploded in half a decade. Treatment in metastatic cancer has always included developing best supportive care, and modern chemotherapy is likely to prolong survival by more than a year with improved quality of life. Curative resections of metastases are performed and may be possible in up to 15% of cases due to chemotherapy. The adjuvant therapies with chemotherapy, radiotherapy, immunotherapy and their combinations render an absolute survival benefit of approximately 10% and about 30% relative survival benefit today. Without compromising treatment efficacy, emphasis should also be put on minimizing adverse events related to therapy.

Since 1957 chemotherapy in colorectal cancer has been and still is based on 5-fluorouracil (5-FU). The 5-FU combinations have however developed since then. We performed a study (n=28) with a novel combination of raltitrexed administered intravenously every three weeks with carmofur (an oral 5-FU prodrug) divided in 3 daily oral doses over 2 weeks, which has been proven feasible at single agent doses (3.0 and 300mg/m^2 respectively) and had a promising efficacy, with a 44% response rate (RR). The dose limiting adverse events were mostly predictable from single agent experience and included diarrhoea, anorexia, anaemia, mucositis, fatigue, thrombocytopenia and neutropenia. The combination of fatigue with non-neutropenic fever not responsive to antibiotics, flu-like symptoms and anorexia was unexpected however, and has not been thoroughly evaluated.

In a longitudinal study we looked at clinical inflammatory symptoms and signs in 52 patients treated with the combination of raltitrexed and carmofur. The majority of patients had fever (>37.0°C in 75%) and fatigue (grade ≥1 in 94%) accompanied by anorexia with slight nausea, and flu-like symptoms with headache and myalgia. Serum C-reactive protein (CRP) level increased >10 mg/L in 94% and the erythrocyte sedimentation rate (ESR) >10 mm/h in 87%. In a cross-sectional one cycle study systemic inflammatory composite score (SICS, based on fever, fatigue, CRP, IL-6, IL-8, TNFα) was calculated in single agent raltitrexed, raltitrexed and carmofur, and 5-FU based chemotherapy groups, and was higher in patients treated with raltitrexed (P<.0001). The median cycle day 7 score was higher than the precycle score in patients treated with either raltitrexed and carmofur (9.5 vs. 2.5, P=.0003) or single-agent raltitrexed (6 vs. 3, P=.0004), whereas in patients given 5-FU based regimens the score remained unaltered (1.5 vs. 1.5). These findings suggest that raltitrexed treatment might be associated with systemic inflammation.
In the adjuvant setting colorectal cancer patients (n=150) were randomised to 5-FU and leucovorin (LV) as bolus injections or continuous infusion. Assessing all adverse events, even those related to the vascular access device (VAD), showed that continuous modality was better tolerated than bolus injection ($P<.0001$). Significantly less hospitalisations (78 vs. 205 days, $P=.045$), diarrhoea (Gr 3-4 in 10% vs. 44%, $P<.0001$), stomatitis (Gr 3-4 in 22% vs. 57%, $P<.0001$) and neutropenia (14% vs. 29%, $P=.029$) were encountered in the continuous arm, but on the other hand more palmoplantar erytrodyssesthesia (PPE Gr 2-3 in 44% vs. 13%, $P=.0009$) and VAD complications (occurred at insertion in 3% and during use in 7%, with replacement needed in 3%). A second randomisation was performed and 1/3 were allocated to no dietary supplements, 1/3 to *Lactobacillus rhamnosus* GG (LGG) and 1/3 to LGG and nutritional supplement containing fibre (Fibre, Novasource GI control®). Less diarrhoea (Gr 3-4 in 21% vs. 37%, $P=.027$) and abdominal discomfort were seen in patients receiving LGG (Gr 2-3 in 2% vs. 12%, $P=.025$). The addition of fibre to 1/3 of patients did not reduce gastrointestinal toxicity any further compared with patients receiving no supplements or LGG ($P=.13$). Thus, LGG given during 5-FU based chemotherapy may reduce gastrointestinal symptoms.

Mucosal injury associated with 5-FU and LV treatment may induce secondary lactose intolerance. Fifty-three (35%) of the 150 patients receiving this therapy had hypolactasia during chemotherapy as compared with 24% at baseline ($P<.0001$) and 26% after treatment. The continuous infusion modality induced more secondary hypolactasia than the bolus modality ($P=.006$). Patients with hypolactasia had significantly worse flatulence (including borborygia and bloating) than normolactasians (Gr 1-2 in 70% vs. 62%, $P=.0004$), which was significant for both bolus injection ($P=.0064$) and continuous infusion subgroups ($P=.018$). In the bolus injection subgroup, patients with hypolactasia had significantly worse diarrhoea during treatment (Gr 3-4 in 64% vs. 32%, $P<.0001$ at baseline or Gr 3-4 in 44% vs. 36%, $P=.014$ during adjuvant treatment). Hypolactasia at baseline ($P=.10$) or post treatment ($P=.32$) was not associated with the nutritional status. Hypolactasia during treatment was associated with an impaired nutritional status (SGA B (moderate) or C (severe malnutrition) in 42% of hypolactasians, 28% of borderliners and 18% of normolactasians ($P<.0001$).

In conclusion, raltitrexed combined with carmofur is feasible and the combination has a promising efficacy in the treatment of metastatic colorectal cancer. The systemic inflammatory reaction associated with raltitrexed administration is relatively common and the differential diagnosis with infections requires considerable expertise. In 5-FU and LV adjuvant treatment, the continuous infusion modality is more tolerable, even with VAD related complications included, than true bolus injection administration. Probiotic LGG supplement during chemotherapy may reduce treatment induced diarrhoea and abdominal discomfort. Mucosal injury in 5-FU and LV therapy induces reversible secondary lactose intolerance, which may impair treatment tolerability and nutritional status.
4. INTRODUCTION

Colorectal cancer is the third most common cancer worldwide (Parkin et al., 1999). In Finland over 2000 new cases per year are diagnosed (Anonymous, 2004). Surgery with a curative intent is possible in three quarters of the patients, but despite apparently curative surgery about half of the patients subsequently develop incurable recurrent disease (Galandiuk et al., 1992). The prognosis of colorectal cancer is slowly getting better, but still colorectal cancer is the fourth most common cause of cancer death (Pisani et al., 1999). Survival in Dukes’ A (growth to bowel wall) colorectal cancer is good with 5-year survival over 90% (Jarvinen et al., 1988). Unfortunately only 20% of cases are diagnosed at this stage. Forty-one percent is diagnosed at stage Dukes’ B (growth through muscularis propria) and 13% at Dukes’ C (lymph node metastases) with a 5-year survival of 69% and 43% respectively (Jarvinen et al., 1988). Twenty-five percent of colorectal cancers are diagnosed at stage Dukes’ D with distant metastases and the prognosis is poor with less than 5% surviving 5 years (Jarvinen et al., 1988).

Adjuvant treatment addressing micrometastases and local relapses with 5-fluorouracil (5-FU) based chemotherapy and radiotherapy has been attempted since the 1960s. Disease free survival (DFS) and overall survival (OS) has consistently been increased, since the first significant reports in 1986, with adjuvant treatment as chemotherapy, radiation or chemoradiation (Douglass et al., 1986; Douglass & Stablein, 1990). Prognosis is also improved by development of surgical techniques. An active liver, lung and even peritoneal metastases surgery gives 5-year survival rates of 27%-45% (Fortner et al., 1984; Iwatsuki et al., 1986; Elias et al., 2001). Total mesorectal excision in rectal cancer results in much better local control, but also in increased survival (Heald & Ryall, 1986; Sjodahl, 2001). Treatment of metastatic colorectal cancer has developed markedly since the 1980s when 5-FU biomodulation and treatment modalities were developed. New chemotherapeutics with irinotecan, oxaliplatin, oral 5-FU derivates, raltitrexed, EGFR and VEGF inhibitors etc alone or in combination have increased survival up to 21.5 months compared with 5 months in patients treated with best supportive care (Glimelius et al., 1992; Scheithauer et al., 1993; Glimelius et al., 1995; Hurwitz et al., 2003; Tournigand et al., 2004).

The development of colorectal cancer treatment yields significant survival benefit, but results in increased treatment related morbidity, possibly at the cost of quality of life. New treatment modalities are expensive and thus they are an economic challenge. The present studies were
designed to develop colorectal cancer treatment with a special emphasis on treatment related safety and mechanisms of toxicity.
5. REVIEW OF LITERATURE

5.1. Development and efficacy of chemotherapy in colorectal cancer

Chemotherapy for metastatic colorectal cancer has been investigated since the 1960s. A very wide variety of chemotherapeutics has been investigated. Intercalating agents, vinca alkaloids, topoisomerase II inhibitors, taxanes and cisplatin, are not considered as the most effective agents, and will not be considered further. The MOF +/- STREP, consisting of methotrexate, vincristine, 5-FU +/- streptocotozin, which was very popular in the early 1980s is not recommended any more and will also not be reviewed further. 5-FU is an example of rational drug design. 5-FU based chemotherapy has been investigated since 1957 and is still the mainstay of chemotherapy for colorectal cancer.

5.1.1. 5-fluorouracil based chemotherapy

The basis for development of 5-FU was the observation that cancer cells utilize uracil more avidly than normal cells (Heidelberger et al., 1957). Uracil metabolism is affected by substituting the hydrogen atom in position five with a fluorine atom. Thymidine 5’- monophosphate (TMP) is a pyrimidine deoxyribonucleoside monophosphate, which after metabolism to thymidine 5’- triphosphate (ThTP), is essential for DNA replication and repair. An enzyme critical to the de novo synthesis of TMP is thymidylate synthetase (TS), which makes it an attractive target for anticancer therapeutic agents. The substrate for TS is deoxy uracil monophosphate (dUMP) and the cofactor for the reaction is 5,10-methylene tetrahydrofolate (5,10-CH₂FH₂). (Jackman et al., 1985)

Fluorinated pyrimidine-based inhibitors of TS have been known for many years. They need to be delivered into the cell as a base (5-FU) or nucleoside (5-fluoro-2-deoxyuridine; FdUrd, floxuridine) after which metabolism occurs to the TS inhibitory species, fluorodeoxy uracil monophosphate (FdUMP) (Jackman et al., 1985). However, 5-FU is extensively metabolised intracellularly to several anabolites other than FdUMP. 5-FU is incorporated via 5-FUTP into RNA, and via 5-FdUTP into DNA and these may be important additional determinants of its antitumour activity and toxicity (Jackman et al., 1985). There is in vitro evidence to suggest that the predominant cytotoxic activity of bolus 5-FU administration is an RNA effect, whereas continuous infusion works more via inhibition of TS (Aschele et al., 1992).
5.1.1.1. Importance of 5-FU scheduling

5-FU is a drug with a very short plasma half-life of approximately 11 minutes (MacMillan et al., 1978). The drug has cytotoxic activity mainly against cells in S-phase, therefore with bolus injections only a small proportion of cells would be susceptible and incorporation into RNA is probably the main mode of action (Fraile et al., 1980). In bolus 5-FU administration the reported response rates vary considerably from 3-45% but toxicity is usually acceptable with neutropenia and mucositis being most prominent (Conroy, 1998). The most common bolus 5-FU regimen was once a day for five days (daily-times-five), repeated every 3-5 weeks with RR up to 18%, time to progression (TTP) up to 6 months and OS up to 14.3 months (Conroy, 1998). Similar 5-FU doses as bolus (3-5 minutes) or short infusion (10-20 minutes) in combination with LV have been compared (n=203) and revealed significantly better efficacy as a bolus shot (RR 17% vs. 13%, \( P = .02 \)) and trend to better TTP (\( P = .07 \)) (Glimelius et al., 1998).

Protracted venous infusion (PVI) or intermittent continuous infusion of 5-FU has demonstrated better response rates than bolus therapy schemes but no survival benefit in single trials (Lokich et al., 1989; Leichman et al., 1995; Hansen et al., 1996; Rougier et al., 1997b). In a meta-analysis the objective response was 14% in bolus treatment and 22% in continuous infusion regimens and median survival was 11.3 months in bolus and 12.1 months in continuous infusion (Rougier et al., 1997a; Anonymous, 1998). In a randomized trial comparing six different treatment schedules, neutropenia, mucositis and diarrhoea were milder in protracted and continuous infusion schedules, but more palmoplantar erythrodysesthesia (PPE) was encountered (Lokich et al., 1989; Leichman et al., 1995).

Chronomodulation of continuous infusion 5-FU schedules have been investigated and a higher dose intensity is achieved with some response enhancement due to circadian variation in for example 5-FU degrading enzyme dihydropyrimidine dehydrogenase (DPD) activity (Levi et al., 1997; Mormont & Levi, 2003). In a phase II trial (n=100) with 5-FU and LV chronomodulated, the reported RR was 41%, TTP was 7 months and OS 17 months (Cure et al., 2002). 5-FU and LV, chronomodulated or flat, with addition of oxaliplatin has showed superior response rate (51% vs. 29%), and time to treatment failure (TTF; 6.4 vs. 4.9 months, \( P = .006 \)), but not increased survival (15.9 vs. 16.9 months) (Levi et al., 1997). Mitomycin C combined with 5-FU as flat protracted venous infusion or chronomodulated has been studied in patients with colorectal cancer (n=320) and showed a trend for inferior RR (40% vs. 29%; \( P = .0582 \)), and non-significant median failure
free survival (8.3 vs. 10.7; \( P=.14 \)) and OS differences (17.6 vs. 16 months), in chronomodulation (Price et al., 1999).

Besides the schedule, another important factor could be the dose-intensity of the drug (Hryniuk et al., 1987). A very high dose-intensity can be achieved with weekly 24-48 hour infusions, which might be a major reason for the higher activity as opposed to bolus injections. In 24 hour infusion weekly, the maximum tolerated dose was 2600mg/m\(^2\) (Ardalan et al., 1988), RR was 13% and median OS 15 months (Lokich et al., 1989). The maximum tolerated dose for 48 hours once a week is 3500mg/m\(^2\) (Diaz-Rubio et al., 1990) and in advanced colorectal cancer the RR was 38.5% and median OS was 12 months (Diaz-Rubio et al., 1994).

### 5.1.1.2. Biochemical modulation of 5-fluorouracil

The anti-cancer efficacy of fluorouracil can be increased by various means enhancing cytotoxicity or reducing host toxicity. 5-FU has been combined with inhibitors of de novo pyrimidine synthesis, which act by depleting the natural uridine nucleotide pools. The most promising have been N-phosponoacetyl-L-aspartate (PALA) (Martin et al., 1983) and Brequinar sodium (Pizzorno et al., 1992). In a randomized study no enhanced efficacy with PALA addition was noted (Buroker et al., 1985). The combination of 5-FU with inhibitors of purine synthesis, 6-methylmercaptopurine ribonucleoside, O-diaxoaecetyl-L-serine, and 6-diazo-5-oxo-L-norleucine has also been investigated but they did not achieve wider clinical acceptance (Soberro, 1998).

Antifolates, methotrexate and trimetrexate given prior to 5-FU combinations enhance 5-FU incorporation into RNA and DNA and TS inhibition (Soberro, 1998). In a meta-analysis of 8 trials with 1178 patients the RR was higher 10% vs. 19% \((P<.05)\) but only a moderate survival benefit (9.1 vs. 10.7 months) was encountered for single 5-FU vs. methotrexate biomodulation (Anonymous, 1994). The combination of two phase III studies \((n=746\) in total) (Blanke et al., 2002; Punt et al., 2002b) with 5-FU and LV with or without trimetrexate showed a non-significant increase in median OS (13.0 vs. 14.6 months, \( P=.15 \)) and in median progression free survival (PFS; 4.4 vs. 5.4 months, \( P=.07 \)) (Punt et al., 2002a). Interferon (IFN) has also been combined with 5-FU in several trials and biochemical, molecular, pharmacologic and immunomodulatory synergistic mechanisms have been proposed (Grem, 1996). The large randomized trials have failed to confirm any clinical usefulness and in most studies toxicity has been worse with interferon (Raderer & Scheithauer, 1995; Kohne et al., 1998; Thirion et al., 2001). High-dose thymidine,
levamisole and dipyridamole have also been used without any significant benefit (Buroker et al., 1985; Kohne et al., 1995).

The most common approach has been to combine fluorouracil with LV (LV; folinic acid, 5-formyl-tetrahydrofolate). The ternary complex, of 5-FU metabolized to 5-FdUMP, TS and LV metabolized to 5,10-methyl tetrahydrofolate (5,10-meTHF) pentaglutamated, is very slowly reversible, thus inducing cell death (Fraile et al., 1980). In single trials high dose LV vs. 5-FU alone significantly enhanced RR, but not OS (Majewicz et al., 1984; Petrelli et al., 1987; O'Connell, 1989; Labianca et al., 1991). According to a meta-analysis, 23% of patients with metastatic colorectal cancer demonstrated an objective response with the combination of LV and 5-FU, whereas only 11% responded to 5-FU alone, $P<.0001$, without any significant survival benefit (Anonymous, 1992b). In advanced colorectal cancer the low dose LV 5-day 4-weekly "Mayo" regimen has yielded better survival than the regimen with high dose LV (O'Connell, 1989). Metastatic colorectal cancer trials have not revealed any significant difference in toxicity (20-25% grade (Gr) 3-4 toxicity) nor in RRs between LV doses (Poon et al., 1989; Labianca et al., 1997). Given the considerable difference in cost between the dosages, low dose LV is recommended in a “daily-times-five regimen”.

Infusional high-dose 5-FU can also be modulated with LV to achieve superior results (Anderson et al., 1989). High-dose continuous infusion 5-FU + LV has been compared with 5-FU + interferon (and 5-FU + LV + IFN, which was stopped due to toxicity), and yielded a significantly higher response rate (44% vs. 18%) and better survival (16.6 months vs. 12.7 months) in 5-FU + LV than 5-FU + IFN (Kohne et al., 1998). Weekly 24-hour continuous infusion of 5-FU has been compared with the same regimen combined with LV or with the Mayo bolus regimen and showed better PFS for the 24-hour regimen with LV but slightly more diarrhoea (Kohne et al., 2003). The Spanish research group has not however, achieved superior results with oral LV added to a high dose continuous 5-FU infusion (Diaz-Rubio et al., 1994). A study with 433 patients with advanced colorectal cancer, compared bolus and infusional 5-FU 2000mg/m2 plus LV 400mg/m2, 48 hour infusion, every two weeks (de Gramont schedule), with standard bolus 5-FU plus low-dose LV (Mayo) and showed a significantly higher RR of 32.6% for continuous infusion vs. 14.4% for the bolus regimen (de Gramont et al., 1997a). The median PFS was 28 weeks vs. 22 weeks ($P=.0012$) and a trend for OS benefit was seen ($P=.067$).
Subsequently the “de Gramont” regimen mentioned above was simplified. In “folfuafort” LV 500mg/m2 is given over 2 hours days 1 and 2 followed by 5-FU 1.5-2g/m2 over 24 hours also on days 1 and 2. every two weeks (Beerblock et al., 1997). In a phase II trial in advanced colorectal cancer with 101 patients (98 evaluable) the RR with “folfuafort” was 33.7%, median PFS 8 months and median OS 18 months. Grade 3-4 toxicity was experienced in 15% (Beerblock et al., 1997). In the “simplified de Gramont” regimen LV 400mg/m2 was given as a 2-hour infusion day 1 followed by 5-FU bolus 400mg/m2 and a 48-hour continuous infusion of 5-FU administered at a dose of 2.4-3.0g/m2/48 hours. The treatment was repeated every 15 days (de Gramont et al., 1997b). A phase II study with the “simplified de Gramont” regimen in metastatic colorectal cancer showed a RR of 37% and a good safety profile with only 16 % grade 3-4 toxicities (de Gramont et al., 1997b).

5.1.2. Oral 5-fluorouracil derivates
Oral 5-FUs are prodrugs, which are enzymatically activated to 5-FdUMP in the target cells, proliferating tissues and in the liver. Tegafur (florafur, 1-(2-tetrahydrofuranyl)-5-fluorouracil) is a lipophilic prodrug of 5-FU with good bioavailability and a long plasma half-life, although it has unacceptable neurotoxicity in intravenous administration and subsequently oral administration is preferred (Conroy, 1998; Sobrero, 1998). This drug has achieved wide clinical acceptance in Japan and Russia and it has shown similar activity to other 5-FU derivates (Conroy, 1998).

Biomodulation with oral low-dose LV has been found feasible and in phase II efficacy in colorectal cancer has been noted (Nogue et al., 1995; Nogue et al., 1996). Further combination studies are warranted. UFT is a combination of tegafur and uracil in a 1:4 molar ratio thus inhibiting 5-FU degradation and with a smaller equieffective dose of tegafur neurotoxicity is diminished (Conroy, 1998). In phase II studies the RR has been 25% in single agent treatment and 39% in LV combinations (Conroy, 1998). A randomised phase III comparison, with UFT and LV vs. bolus 5-FU and LV (Mayo regimen), showed equivalent survival and efficacy (RR 12% vs. 15%, $P=.232$) and better tolerability in the UFT arm (Pazdur et al., 1999). A second study with the same randomisation showed equivalent TTP (3.4 vs. 3.3 months), RR (11% vs. 9%) and OS (12.2 vs. 11.9 months) (Carmichael et al., 1999). S-1 is a further development of tegafur where it is combined with 5-chloro-2,4-dihydroxyxymidine inhibiting 5-FU degradation in the liver and potassium oxonate inhibiting digestive tract phosphorylation in a molar ratio of 1:0.4:1 aiming at reduced toxicity and increased efficacy. In phase II trials in metastatic colorectal cancer patients (n=63 and 47) the RR was 35% and 24% respectively, with main adverse reactions as
myelosuppression and gastrointestinal (GI) toxicities (Ohtsu et al., 2000; Van den Brande et al., 2003).

Doxifluridine is enzymatically activated in the intestine and tumour, but its clinical use has been limited by diarrhoea (Sobrero, 1998). Therefore capecitabine was developed and has three enzymatic steps for activation rendering it unactively absorbable in the intestine and thus diarrhoea has been diminished. Three different treatment schedules have been investigated in a randomized phase II trial showing the most promising safety and efficacy profile for 2 weeks administration without LV every three weeks (Van Cutsem et al., 2000). In a randomized study comparing capecitabine with 5-FU and LV (Mayo regimen) the RR was 24.8% vs. 15.5% (P= .005), TTP as 4.3 and 4.7 months (P=.72) and median OS was 12.5 and 13.3 months (P=.974) (Hoff et al., 2001). In a similar European study RR was 18.9% for capecitabine and 15.0% for 5-FU/LV (Mayo), median TTP was 5.2 and 4.7 months (P=.65) and OS was 13.2 and 12.1 months (P=.33) (Van Cutsem et al., 2001b). Thus equivalence with bolus 5-FU/LV with an improved tolerability was achieved.

Eniluracil is an effective inhibitor of DPD, which is the first degradative enzyme in the pathway of 5-FU. In two randomized phase III trials (n=531 and 981), oral eniluracil and 5-FU have been compared with intravenous 5-FU and LV (Mayo regimen). The hazard ratio for PFS was 0.831 (CI95% 0.69-1.00) and for OS 0.77 (CI95% 0.62-0.95, 47.4 vs. 63.7 weeks) thus eniluracil appeared inferior to Mayo 5-FU and LV (Van Cutsem et al., 2001a). The second trial did not show statistically inferior results for eniluracil but did not reach the protocol defined criteria for survival equivalence hazard ratio (HR) for OS 0.880; (CI95%, 0.75-1.03)(Schilsky et al., 2002). Median duration of PFS was inferior in the eniluracil group (20.0 vs. 22.7 weeks, P=.01)(Levin et al., 2001). So far DPD inhibitory agents have not shown fulfilment in the early promises by this group of drugs (Schmoll, 2003).

5.1.2.1. Carmofur
Carmofur (1-hexylcarbomoyl-5-fluorouracil; HCFU) is an oral derivate of 5-fluorouracil. It is metabolised to FdUMP and acts as intravenous 5-FU. In advanced colorectal carcinoma the RR has been 12-50% (Koyama, 1981; Koyama, 1982; Grohn et al., 1990). The recommended dose of carmofur as a single agent is 300-600mg/m2 daily as continuous medication (Koyama, 1981; Sawada et al., 1983). Carmofur is well absorbed gastrointestinally and food does not alter the

Carmofur has been widely used alone and in combinations especially in Japan and Finland, (many publications in Japanese and thus English abstracts have been used). In treatment of metastatic disease acceptable results have been achieved, alone or in combinations, in gastric cancer (Koyama, 1982; Grohn et al., 1990), and combined with immunomodulators and mitomycin C (Nakanishi, 1986; Osawa et al., 1996) (Nakanishi, 1986; Osawa et al., 1996) or interferon 2α (Grohn et al., 1994). In colorectal cancer (Koyama, 1982; Niimoto et al., 1987; Grohn et al., 1990), in pancreas cancer (Grohn et al., 1990; Kajanti & Pyrhonen, 1991) and breast cancer acceptable results have been seen (Koyama, 1982; Kusama et al., 1995). In ovarian (Harada et al., 1986)(Terabe et al., 1986; Ono et al., 1997; Nakamura et al., 2001; Ono et al., 2001; Sakamoto et al., 2001a; Sakamoto et al., 2001b; Kotake et al., 2002)(Nakazato et al., 1986; Yamamura et al., 1988)(Ono et al., 1997; Nakamura et al., 2001)(Ikeda et al., 1994)cancer the combination with cyclophosphamide, and hexamethylmelamine (Sipila et al., 1989) has been feasible. In head and neck cancer carmofur has been combined with radiotherapy (Harada et al., 1986).

Carmofur has also been tested in the adjuvant treatment of colorectal cancer (with promising results) (Terabe et al., 1986; Ono et al., 1997; Nakamura et al., 2001; Ono et al., 2001; Sakamoto et al., 2001a; Sakamoto et al., 2001b; Kotake et al., 2002) and combined with mitomycin C (Ito et al., 1996a; Ito et al., 1996b; Sakamoto et al., 1999), in gastric cancer combined with mitomycin C and 5-FU (with acceptable results) (Nakazato et al., 1986; Yamamura et al., 1988), in hepatocellular cancer (with poor results) (Ono et al., 1997; Nakamura et al., 2001), in urogenital cancer (with questionable results) (Nishio et al., 1987) and in ovarian cancer (with promising results) (Ikeda et al., 1994). In Japan wide acceptance has been achieved in adjuvant treatment in colorectal cancer (Ito et al., 1996a; Ito et al., 1996b; Sakamoto et al., 2001a; Sakamoto et al., 2001b; Sakamoto et al., 2004).

5.1.3. Raltitrexed

Raltitrexed (Tomudex®; ZD 1694) was developed to be a specific TS inhibitor (The Institute of Cancer Research and Zeneca Pharmaceuticals). It is an (quinazoline) analogue of the folate cofactor rather than the pyrimidine substrate (Jackman et al., 1995). Thus the problem of incorporation into nucleic acids can be overcome and an increase in dUMP is achieved (Jackman
et al., 1985). Raltitrexed utilises the reduced folate carrier for uptake into cells and is a substrate for folylpolyglutamate synthetase (Cunningham et al., 1995; Jackman et al., 1996). In phase I trials raltitrexed has shown activity in ovarian, breast, colorectal, pancreatic, and non-small cell lung cancer (Jackman et al., 1995). The recommended dose has been 3mg/m² every three weeks (Clarke et al., 1994; Sobrero, 1997b).

Raltitrexed has been found to have comparative efficacy in treatment of metastatic colorectal cancer as compared with the standard 5-FU and LV (Mayo regimen) (Cunningham et al., 1995; Zalcberg et al., 1996; Cocconi et al., 1998). In one study the Mayo regimen appeared to be somewhat more efficient than raltitrexed (Pazdur & Vincent, 1997). In single raltitrexed therapy median TTP was 3.1-4.8 months, OS was 9.7-11.2 months and RR was 14-26% (Cunningham et al., 1995; Zalcberg et al., 1996; Pazdur & Vincent, 1997; Cocconi et al., 1998).

The anticancer efficacy of raltitrexed has been enhanced by combining it with one or more, synergistically acting drugs. Combinations with irinotecan (Carnaghi et al., 2002; Lewis et al., 2002; Aparicio et al., 2003), oxaliplatin (Comella et al., 2000a; Fizazi et al., 2000; Scheithauer et al., 2001a; Scheithauer et al., 2001b; Casciu et al., 2002; Comella et al., 2002; Neri et al., 2002; Martoni et al., 2003), mitomycin C (Rosati et al., 2003) and 5-FU (Schwartz et al., 1999; Comella et al., 2000b; Caponigro et al., 2001a; Caponigro et al., 2001b; Comella et al., 2002) have been used.

In pre-clinical cell line studies synergy has been demonstrated when raltitrexed administration is concomitant or preferably followed by 5-FU, but not in reverse order (Jackman et al., 1999; Kano et al., 2000) (oral communication Johnston P in January 1998). 5-FU and raltitrexed both act on TS, which is a key enzyme in the de novo synthesis of thymidine triphosphate required for DNA synthesis. Raltitrexed is a pure inhibitor and 5-FU has additional effects as well. 5-FU has shown anti-tumour activity in raltitrexed resistant sub clones (Johnston et al., 1995). Clinical trials, with raltitrexed combined with 5-FU, have been performed mainly in the treatment of colorectal cancer. In a three arm first-line randomized comparison (n=159) raltitrexed and 5-FU/LV showed comparable RR and TTP with methotrexate and 5-FU/LV, but inferior RR and TTP compared with irinotecan and 5-FU/LV (Comella et al., 2000b). Other results include: response rates of 25% and 23% with combinations of raltitrexed/5-FU/LV as first-line treatment and oxaliplatin/raltitrexed/5-FU/LV as second-line treatment in metastatic colorectal cancer, respectively (Caponigro et al.,
2001a). In phase I studies, the combination of bolus and continuous 5-FU with raltitrexed have been found feasible (Schwartz et al., 1999). RR was promising in first-line treatment with the infusional 5-FU/raltitrexed regimen, where 53% of patients responded, and in the bolus combination RR was 12.5% in a phase I study and stabilizations were seen in second line after 5-FU progression (Dragnev et al., 1998; Schwartz et al., 1999). In second- and third-line treatment raltitrexed has shown limited efficacy after 5-FU failure (Tsavaris et al., 2002a; Tsavaris et al., 2002b), but generally 5-FU has not been efficient after raltitrexed failure (Farrugia et al., 1998).

Raltitrexed as a radiosensitizer has been studied in the neoadjuvant and postoperative chemoradiation setting. Raltitrexed as concomitant chemotherapy during preoperative radiotherapy in chemonaive patients with stage II/III rectal cancer (n=45) has been found feasible at a single agent dose of 3.0g/m² every 19 days. This combination gave a clinical response in 80% of the cases (Valentini et al., 2001). In a postoperative chemoradiation setting in Dukes’ B and C patients (n=22) a combination of 2.6g/m² raltitrexed every three weeks with standard radiotherapy was feasible (Botwood et al., 2000). A similar dosing was found feasible in conjunction with preoperative radiotherapy in a second trial (n=20) in patients with unresectable or recurrent rectal cancer (James et al., 2003a).

5.1.4. **Combination chemotherapy with 5-fluorouracil and leucovorin**

Randomized trials have studied 5-FU with or without LV combined with mitomycin C, irinotecan and oxaliplatin. Protracted venous infusion of 5-FU has been administered with or without mitomycin C in a randomized trial (n=200). Overall RR was 54% vs. 38% (P=.024), median TTF was 7.9 vs. 5.4 months (P=.033) and median OS was 14 vs. 15 months (P=ns) in 5-FU combined with mitomycin C vs. 5-FU alone, respectively (Ross et al., 1997).

Irinotecan combined with 5-FU and LV has shown superior efficacy and overall survival (Douillard et al., 2000; Saltz et al., 2000). In a three arm first-line study (n=683) patients were assigned to receive either bolus irinotecan/5-FU/LV (ILF), bolus 5-FU/LV (Mayo) or weekly irinotecan alone. ILF was superior compared with Mayo, PFS was 7.0 vs. 4.3 months (P=.004), confirmed RR was 39% vs. 21% (P<.001), and OS 14.8 vs. 12.6 months (P=.04). Results for irinotecan alone were similar to Mayo (Saltz et al., 2000). Continuous infusion of 5-FU and LV weekly or every two weeks has been tested with and without irinotecan in a first-line study (n=387) and showed superiority in combination. The RR for evaluable patients was 49% vs. 31%
(P<.001 and 35% vs. 22%, P<.005 by intention to treat) and TTP was 6.7 vs. 4.4 months (P<.001), and OS was longer, 17.4 vs. 14.1 months (P=.031) (Douillard et al., 2000).

Oxaliplatin has been used in combination with 5-FU and LV as flat or chronomodulated infusion. In a phase III study (n=420) patients received 5-FU and LV as continuous infusion (de Gramont regimen) with or without 85mg/m² oxaliplatin. RR was 50.7% vs. 22.3% (P=.0001), median TTP 9.0 vs. 6.2 months (P=.0003), but OS was not significantly better 16.2 vs. 14.7 months (P=.12) (de Gramont et al., 2000). In a second trial, presented in abstract form, weekly 24 hour infusion of 5-FU was combined with oxaliplatin and compared to 5-FU and LV (Mayo) schedule (n=252) and gave a higher RR (48.3% vs. 22.6%), TTP (7.9 vs. 5.3 months, P<.0001) and OS (20.4 vs. 16.1 months) (Grothey et al., 2003). Oxaliplatin combined with continuous 5FU and LV (FOLFOX) treatment was significantly superior (n=795) to irinotecan combined with weekly bolus 5-FU and LV (ILF), and to the combination of irinotecan and oxaliplatin (IROX) (Goldberg et al., 2004). was 45% vs. 31% vs. 35%, median TTP was 8.7 vs. 6.9 vs. 6.5 months and OS was 19.5 vs. 15.0 vs. 17.4 months. Chronomodulated 5-FU and LV with or without oxaliplatin has been studied in a French trial (n=200) (Giacchetti et al., 2000). More objective responses were encountered in combination treatment (53% vs. 16%; P<.001), a longer median TTF (8.7 vs. 6.1 months, P=.048), but similar OS was seen (19.4 vs. 19.9 months; P=n.s.).

Simplified de Gramont continuous infusion of 5-FU and LV has been randomly combined with irinotecan (FOLFIRI) or oxaliplatin (FOLFOX) and showed similar efficacy (n=226) (Tourmigand et al., 2004). Median OS was 21.5 months in patients allocated to FOLFIRI and then FOLFOX vs. 20.6 months in patients allocated to FOLFOX followed by FOLFIRI. In first-line treatment FOLFIRI and FOLFOX had an externally reviewed RR of 56% vs. 54%, median TTP of 8.5 vs. 8.0 months and in second-line RR was 4% vs. 15% and median TTP 2.5 vs. 4.2 months.

Results of trials with 5-FU and LV and irinotecan with or without epidermal growth factor receptor (EGFR) and vascular endothelial growth factor (VEGF) inhibitors have been presented. Cetuximab is a chimeric monoclonal antibody against the EGFR. In a large, randomized, open-label, multicentre study (n=329) in adult patients with irinotecan-refractory, metastatic colorectal cancer expressing EGFR, cetuximab 400 mg/m² initial dose followed by 250 mg/m² weekly plus irinotecan (various doses) produced a greater rate of partial response (17.9% vs. 9.9%) and disease control, and increased TTP (126 vs. 45 days) compared with cetuximab monotherapy; survival
was similar in both groups (Cunningham et al., 2003). The same dosage of cetuximab combined with irinotecan, fluorouracil and LV (various regimens) produced partial responses in 43-58% and stable disease in 32-52% of patients with treatment-naive metastatic colorectal cancer expressing EGFR in three small, open-label trials (n=65 in total) (Rosenberg et al., 2002; Schöfﬂski et al., 2002; Raoul et al., 2003).

Bevacizumab is a monoclonal antibody to VEGF. A phase II randomized trial (n=104) in first line metastatic colorectal cancer compared low and high dose bevacizumab combined with 5-FU and LV bolus to 5-FU and LV alone (Kabbinavar et al., 2003). The low-dose and high-dose arms had higher RR (40% vs. 24% vs. 17%), median TTP (9.0 vs. 7.2 vs. 5.2 months) and OS (21.5 vs. 16.1 vs. 13.8 months), than the 5-FU and LV control arm. Feasibility has been shown for the combination of bevacizumab and FOLFOX (n=223) (Benson et al., 2003) and for ILF (n=92) (Giantonio et al., 2003). A randomized placebo controlled study (n=813) showed higher RR (44.8% vs. 34.8%, P=.0004), longer median PFS (10.6 vs. 6.2 months, P<.001) and OS (20.3 vs. 15.6 months, P<.001) in the ILF combined with bevacizumab arm compared with ILF alone (Hurwitz et al., 2004b). Compared with the ILF arm, patients with 5-FU and LV combined with bevacizumab (n= approximately 100) had an improvement in objective response rate, duration of response, and survival without significant toxicity, which requires further investigations (Hurwitz et al., 2004a).

Gefitinib in combination with FOLFOX has shown promising efficacy in a phase I-II study in first and second line treatment (Cho et al., 2003).

5.1.5. **Adjuvant treatment in colon cancer**

5.1.5.1. **Chemotherapy**

In the adjuvant treatment of colon cancer the first study showing evidence of improved survival was an NSABP C-01 trial where treatment with MOF (Methyl-CCNU, vincristine and 5-FU) showed a 67% survival at 5 years vs. 55% in the untreated group (Wolmark et al., 1988).

One of the most striking results was seen in the American intergroup study that showed that the combination of 5-FU and levamisole, compared to levamisole or to surgery alone improved the overall survival in Dukes’ C cancer (Moertel et al., 1990). The results of this trial have been
updated and show (with a median follow-up of 6.5 years) a decrease in relapse of 40\% (P<.0001) and a decrease in mortality of 33\% (P=.0007) (Moertel et al., 1995a). Similar results are seen in a US (n=262) and Dutch (n=1029) study comparing 12 months of 5-FU and levamisole to surgery alone (Laurie et al., 1989; Taal et al., 2001).

**Table 1**  
Studies with 5-FU +/- levamisole +/- leucovorin vs. surgery alone control arm in adjuvant treatment of colon cancer

<table>
<thead>
<tr>
<th>Study</th>
<th>Dukes’ stage</th>
<th>Patients N</th>
<th>Duration months</th>
<th>Effect on relapse</th>
<th>Effect on mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>5-fluorouracil+ levamisole</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>NCCTG and Mayo Clinic</td>
<td>C</td>
<td>262</td>
<td>12</td>
<td>Reduced</td>
<td>Reduced</td>
</tr>
<tr>
<td>(Laurie et al., 1989)</td>
<td></td>
<td></td>
<td></td>
<td>P=.02</td>
<td>P=.05</td>
</tr>
<tr>
<td>Intergroup study (Moertel et al., 1995a)</td>
<td>C</td>
<td>929</td>
<td>12</td>
<td>Reduced 41%, P&lt;.0001</td>
<td>Reduced 33%, P=.006</td>
</tr>
<tr>
<td>(Moertel et al., 1990)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dutch (Taal et al., 2001) Colon &amp; rectal cancers</td>
<td>B &amp; C</td>
<td>1029</td>
<td>12</td>
<td>Not reported</td>
<td>Reduced 25%, P=.0007</td>
</tr>
<tr>
<td><strong>5-fluorouracil+ leucovorin</strong></td>
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<tr>
<td>NSABP C-03 (Wolmark et al., 1993)</td>
<td>B &amp; C</td>
<td>1081</td>
<td>11</td>
<td>Reduced 30%, P=.004</td>
<td>Reduced 32%, P=.003</td>
</tr>
<tr>
<td>Italian etc (Francini et al., 1994)</td>
<td>B &amp; C</td>
<td>239</td>
<td>11</td>
<td>Reduced 35%, P=.005</td>
<td>Reduced 34%, P=.004</td>
</tr>
<tr>
<td>IMPACT (Labianca et al., 1995)</td>
<td>B &amp; C</td>
<td>1493</td>
<td>6</td>
<td>Reduced 35%, P&lt;.0001</td>
<td>Reduced 22%, P=.03</td>
</tr>
<tr>
<td>Intergroup (O’Connell et al., 1997)</td>
<td>B &amp; C</td>
<td>317</td>
<td>6</td>
<td>Reduced 34%, P=.001</td>
<td>Reduced 30%, P=.01</td>
</tr>
<tr>
<td><strong>5-fluorouracil protracted venous infusion</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>AXIS (James et al., 2003b) Colon &amp; rectal cancers</td>
<td>A, B, (2551 curatively)</td>
<td>3583</td>
<td>0.25</td>
<td>Absolute difference 2.9%, P=.15</td>
<td>Absolute difference 1.7%, P=.329</td>
</tr>
</tbody>
</table>

In the adjuvant setting, the IMPACT meta-analysis of the Canadian, French and Italian colon cancer studies comparing 5-FU and LV with surgery alone has reported a statistically significant 5\% improvement in 3-year survival. Dukes’ C survival without relapse was 56\% vs. 44\% in the control group and in Dukes’ B the difference was not significant, 79\% vs. 76\% (Labianca et al., 1995). In an update of the trial the survival advantage of adjuvant therapy withstood and no significant impact on quality of life from chemotherapy was noted (Zaniboni et al., 1998). The NSABP successor study C-03 compared 5-FU/LV vs. MOF in Dukes’ B and C colon cancer and reported a significant survival benefit for 5-FU/LV at three years with an equal benefit for Dukes’ B and C (Wolmark et al., 1993). In a meta-analysis the combination of 5-FU and LV reduced the risk of death by 29\% as compared to untreated controls after curative surgery of colorectal cancer, whereas 5-FU alone reduces the risk of death by only 6\% (Gray, 1997).
Results from two large US adjuvant studies that accrued over 5400 patients and compared 5-FU/LV with 5-FU/levamisole and with the combination of these drugs and also for 6 vs. 12 months, did not reveal significant differences among the various regimens (Haller et al., 1997; Wolmark et al., 1999). The NSABP C-04 indicated in pair-wise comparisons an advantage for 5-FU/LV over 5-FU/levamisole (Wolmark et al., 1999). The intergroup trial showed that 6 months of adjuvant therapy with 5-FU/LV appears to be as effective as 12 months of 5-FU+levamisole (Haller et al., 1997). Furthermore the joint American-Canadian trial (n=891) suggested that 6 months of chemotherapy appeared sufficient and that levamisole did not add much to 5-FU/LV but increased toxicity (O'Connell et al., 1998). The Quasar study could not verify any survival benefit for either high dose vs. low dose LV with 5-FU, nor for the addition of levamisole to 5-FU and LV treatment (Anonymous, 2000). Similarly, the German study showed a survival advantage for 5-FU and LV over 5-FU and levamisole in 680 Dukes’ C colon cancer patients (Porschen et al., 2001; Arkenau et al., 2003). In a study with 180 Dukes’ C colon cancer patients no survival advantage for treatment with 5-FU/LV for 6 or 12 months could be seen over 5-FU and levamisole for 12 months (Dencausse et al., 2002).

The studies mentioned above verify the effect on relapse and survival in colon Dukes’ C patients, but generally no significant survival benefit for Dukes’ B patients. In the Dutch study with 463 colorectal patients in stage II, a significant survival benefit was noted for this subgroup, 78% vs. 70% (Taal et al., 2001). However, the individual studies were insufficiently powered to detect a treatment benefit in Dukes’ B patients, a 4% survival advantage would need approximately 4000 patients to be randomized (Rao & Cunningham, 2003). The IMPACT B2 study, which combined data from patients with Dukes' B colon cancer in five separate trials, failed to show a statistically significant benefit of adjuvant 5-FU/LV compared with surgery alone (Anonymous, 1999b). Neither did the Intergroup trial show any benefit for 318 patients with Dukes’ B stage although recurrence rate was reduced by 31% (Moertel et al., 1995b). An analysis of four separate studies (NSABP) compared the benefit of adjuvant treatment in Dukes' B patients with that in Dukes' C patients and showed similar relative reductions in mortality and disease-free survival in Dukes' B and in Dukes' C patients (Mamounas et al., 1999). The Liver Infusion Meta-Analysis Group also reported similar relative benefits from a portal vein infusion of 5-FU-based chemotherapy in Dukes’ B and Dukes' C patients (Anonymous, 1997b). A US population survey in 3151 medicare beneficiaries with Dukes’ B colon cancer revealed a median survival of 75% in 2291 non-treated
patients vs. 78% for 860 treated patients (mostly 5-FU and LV claims were noted), with a
unadjusted risk ratio of 0.80 (CI95%, 0.68-0.95) and adjusted risk ratio of 0.91 (CI95%, 0.77-1.09)
(Schrag et al., 2002). A meta-analysis of all Dukes’ B patients included could verify the position
of adjuvant chemotherapy in Dukes’ B colon cancer (Buyse & Piedbois, 2001).

Based on the encouraging results from advanced colorectal treatment, regarding efficacy and
toxicity profile, 5-FU infusional treatments have been started in the adjuvant setting. Three studies
with protracted venous infusion have been published. In an English trial on 716 Dukes’ B and C
colorectal cancer patients were randomized to 5-FU and LV (Mayo) vs. PVI of 5-FU for 12 weeks,
they reported no 5-year OS differences (73.7% vs. 74.6%) but relapse free survival benefit in
colon Dukes’ B (72% vs. 80%) and in rectal cancer patients (55% vs. 75%) (Saini et al., 2003). A
Canadian study with 1078 patients randomized to Mayo or PVI of 5-FU during 24 weeks of 27
was closed at interim analysis because superiority of PVI could not be verified (Poplin et al.,
2000). A favourable toxicity profile for PVI was demonstrated in both of these studies (Poplin et
al., 2000; Saini et al., 2003). In the AXIS study a 7-day PVI of 7g of 5-FU did not increase disease
free or overall survival in all or curatively resected Dukes’ A-D colorectal cancer patients (Table
1). A trend for disease free survival benefit was noted in colon cancer patients (James et al.,
2003b). In a GERCOR study (n=905) patients were randomized to 48 hour bolus and continuous
infusion of 5-FU and LV once a fortnight vs. short infusions of 5-FU and LV every four weeks
and showed no significant difference in disease free survival (hazard ratio 1.042 (CI95%, 0.814-
1.335) in treatment arm comparison (Andre et al., 2003). A second randomization for 24 vs. 36
weeks of adjuvant treatment also did not show any difference in DFS (hazard ratio 0.942, CI95%
0.735-1.21) (Andre et al., 2003).

Oral 5-FU based regimens, i.e. capecitabine etc are under evaluation. In a
meta-analysis (n=9819) patients with oral 5-FU, tegafur, capecitabine and UFT have been compared
with surgery alone patients and showed significantly better DFS and OS for Dukes’ B and C
patients (Sakamoto et al., 2001a; Sakamoto et al., 2004).

The encouraging results achieved with the combination of irinotecan or oxaliplatin with 5-FU
based intravenous and oral regimens have led to several studies also in the adjuvant setting. In
abstract form the results of the MOSAIC study have been presented. In this study patients
(n=2246) have been randomized to 5-FU and LV with the de Gramont schedule or the same
regimen combined with oxaliplatin and the combination gave a 3-year disease free survival advantage of 72.9% vs. 78.2% ($P=.0002$) in Dukes’ B and C colon and high rectal tumours (Andre et al., 2004).

5.1.5.2. Miscellaneous adjuvant therapies

Immunotherapies in adjuvant treatment have been studied since the 1970s. Active specific immunotherapy with an autologous tumour cell Bacillus Calmette Guerin vaccine as adjuvant treatment in stage II colon cancer ($n=254$) showed a longer recurrence free period and a risk reduction for recurrence, but no overall survival advantage (Vermorken et al., 1999). A similar design was used in a US study were 412, mainly stage II patients (stage III in 115), were randomized to active specific immunotherapy vs. resection alone with no significant DFS ($P=.078$) or OS survival ($P=.12$) benefit.

Edrecolomab is a murine (17-1A) monoclonal antibody that increased OS by 32% in Dukes’ C colorectal cancer patients when compared with observation (Riethmüller et al., 1998). In a successive trial Dukes’ C patients were randomized to 5-FU and LV (Mayo) for 6 months with or without five edrecolomab infusions or to single edrecolomab. This trial showed inferior OS or DFS for single edrecolomab infusions, and no benefit in the combined edrecolomab and chemotherapy arm (Punt et al., 2001).

Immunomodulatory interferon α has been studied in combination with 5-FU with no distinct survival benefit over 5-FU +/- LV +/- levamisole in adjuvant treatment for Dukes’ B2 and C patients. In a Greek study ($n=322$) 5-FU combined with interferon α did not give any survival advantage over 5-FU and LV, but it did enhance treatment related toxicity (Gennatas et al., 2002). In the FOGT-1 ($n=813$) study the 5-FU/interferon α and 5-FU/levamisole combinations showed inferior survival to 5-FU/LV/levamisole, with exaggerated treatment related toxicity and treatment discontinuation in the interferon arm (Staib et al., 2001).

Portal vein infusion was first tested in 1979 and based on these promising results further studies were introduced (Taylor et al., 1979). NSABP C-02 tested intraportal adjuvant 5-FU chemotherapy for a week vs. surgery alone and results showed better DFS (69% vs. 60%, $P=.02$) and OS (78% vs. 70%, $P=.07$) at 5 years (Wolmark et al., 1990). In an EORTC and a Mayo clinic study adjuvant 5-FU portal vein infusion was not linked to increased survival (Beart et al., 1990;
Rougier et al., 1998). In a meta-analysis of 4000 patients included in 10 trials the absolute survival advantage associated with portal vein infusion at 5 years was 4.7% (standard deviation = 1.2%, $P=0.006$) (Anonymous, 1997b).

Intraperitoneal chemotherapy has been attempted often in combination with intravenous chemotherapy. In an Austrian study ($n=241$), intraperitoneal and intravenous chemotherapy with 5-FU and LV was compared to intravenous 5-FU and levamisole, in Dukes’ C patients ($n=196$) a 4-yearDFS (77.3% vs. 57.5%; $P=0.0015$) and OS benefit (83% vs. 65%; $P=0.0005$) was noted in the combination arm (Scheithauer et al., 1998). A French study ($n=267$) tested intraperitoneal 5-FU during 6 days shortly after resection in stage II and III colon cancer. Slight but non-significant improvements in DFS (68% vs. 62%) and OS (74% vs. 69%) were seen (Vaillant et al., 2000).

### 5.1.6. Adjuvant treatment in rectal cancer

Good surgery is the mainstay of curative treatment in colorectal cancer. Total mesorectal excision (TME) has improved survival and diminished local relapses markedly in rectal cancer and can be considered standard procedure in the majority of cases (Heald & Ryall, 1986; Wibe et al., 2002). With TME type of surgery the national survival and local recurrence rate of rectal cancer in Norway has changed markedly from 55% and 28% respectively, in the pre-TME era, to 71% and 8% in the later time period after a median of 39 months follow up without routine adjuvant treatment (Wibe et al., 2003). With a five-year follow up the overall survival and recurrence rates were 59% and 15% in the lower third, 62% and 13% in the middle third and 69% and 9% in the highest third of the rectum (Wibe et al., 2004).

In most countries, including Finland, the results of rectal cancer are not yet “adequate” after surgery alone (Pakkastie et al., 1995). Patients with rectal cancer are still at risk of local and systemic relapse. Adjuvant therapy should address both these problems. The prognosis in Dukes’ A rectal cancer is good with more than 80% surviving 5 years (Bosman, 2002) and generally surgery is sufficient. Five-year survival has been 60-65% in Dukes’ B and 25-30% in Dukes’ C cancer (Bosman, 2002). Radiotherapy addresses the local recurrence, and thereby also late seeding distant metastases, and improves survival.
5.1.6.1. Radiotherapy

Several trials of preoperative or postoperative radiotherapy vs. surgery alone have shown a decrease in the local recurrence rate, but a survival benefit in only a few preoperative trials (Anonymous, 1997a; Martling et al., 2001)(Table 2). Postoperative radiotherapy alone has not affected survival.

Table 2  Studies with preoperative or postoperative radiotherapy in rectal cancer with surgery alone control arm

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Treatment schedule Gy/fractions/days</th>
<th>Treatment field</th>
<th>Survival ratio at 2-5 years, surgery vs. XRT %</th>
<th>P</th>
<th>Curative vs. all</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Preoperative radiotherapy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UK/MRC (Anonymous, 1984)</td>
<td>564</td>
<td>5/1/1</td>
<td>Pelvis</td>
<td>47</td>
<td>50</td>
<td>ns</td>
</tr>
<tr>
<td>VASOG I (Higgins et al., 1975)</td>
<td>557</td>
<td>20/10/14</td>
<td>Pelvis</td>
<td>-</td>
<td>46</td>
<td>ns</td>
</tr>
<tr>
<td>VASOG II (Higgins et al., 1986)</td>
<td>453</td>
<td>20-25/10/14</td>
<td>Pelvis</td>
<td>32</td>
<td>43</td>
<td>ns</td>
</tr>
<tr>
<td>Norway (Dahl et al., 1990)</td>
<td>314</td>
<td>31,5/18/24</td>
<td>Pelvis –L2</td>
<td>50</td>
<td>50</td>
<td>-</td>
</tr>
<tr>
<td>EORTC (Gerard et al., 1988)</td>
<td>259</td>
<td>34,5/15/19</td>
<td>Pelvis –L2</td>
<td>59</td>
<td>69</td>
<td>.08</td>
</tr>
<tr>
<td>Stockholm (Anonymous, 1990a)</td>
<td>679</td>
<td>25/5/7</td>
<td>Pelvis –L2</td>
<td>50</td>
<td>55</td>
<td>-</td>
</tr>
<tr>
<td>Stockholm II (Martling et al., 2001)</td>
<td>481</td>
<td>25/5/7</td>
<td>Pelvis</td>
<td>39</td>
<td>46</td>
<td>&lt; .03</td>
</tr>
<tr>
<td>Swedish (Anonymous, 1997a,b)</td>
<td>1168</td>
<td>25/5/7</td>
<td>Pelvis –L2</td>
<td>48</td>
<td>58</td>
<td>.004</td>
</tr>
<tr>
<td>(Reis Neto et al., 1989)</td>
<td>66</td>
<td>40/20/28</td>
<td>Pelvis</td>
<td>29</td>
<td>71</td>
<td>-</td>
</tr>
<tr>
<td>(Kligerman et al., 1972)</td>
<td>31</td>
<td>46/23/31</td>
<td>Pelvis –L2</td>
<td>25</td>
<td>41</td>
<td>-</td>
</tr>
<tr>
<td>Dutch etc (Kapiteijn et al., 2001)</td>
<td>1861</td>
<td>25/5/7</td>
<td>Pelvis</td>
<td>81.8</td>
<td>82.0</td>
<td>.84</td>
</tr>
<tr>
<td><strong>Postoperative radiotherapy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSABP R-01 (Fisher et al., 1988)</td>
<td>368</td>
<td>46-51/26-29/35-38</td>
<td>Pelvis</td>
<td>43</td>
<td>40</td>
<td>ns</td>
</tr>
<tr>
<td>Denmark (Balslev et al., 1986; Bentzen et al., 1988)</td>
<td>494</td>
<td>50/25/49 (14 break)</td>
<td>Post pelvis</td>
<td>No difference</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>EORTC (Arnaud et al., 1997)</td>
<td>172</td>
<td>46/13/30-38</td>
<td>Pelvis</td>
<td>45</td>
<td>57</td>
<td>ns</td>
</tr>
</tbody>
</table>
Survival benefit (58% vs. 48%; $P=0.004$ and 46% vs. 39%; $P<0.03$) has been seen in short preoperative radiotherapy 25/5Gy with surgery within a week vs. surgery alone (Anonymous, 1997a; Martling et al., 2001), a schedule which does not downstage the tumour (Marijnen et al., 2001) or compensate for positive margins (Marijnen et al., 2003). The local recurrence rate has been about 30% and has more than halved in preoperative radiotherapy arms (Table 3). Even in combination with optimal TME surgery with a low local recurrence rate (8.2%) the 25/5Gy radiotherapy further reduces recurrences (2.4%), although it does not affect the DFS or OS, with a 2-year follow up (Kapiteijn et al., 2001).

Table 3  Local and distant recurrence rates in rectal cancer with preoperative and postoperative radiotherapy.

<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>Local recurrence %</th>
<th>Distant recurrence %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Surg</td>
<td>CT</td>
<td>XRT</td>
</tr>
<tr>
<td><strong>Pre vs. postoperative radiotherapy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Swedish (Frykholm et al., 1993)</td>
<td>471</td>
<td>-</td>
<td>12</td>
</tr>
<tr>
<td><strong>Preoperative</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stockholm (Anonymous, 1990)</td>
<td>849</td>
<td>23</td>
<td>-</td>
</tr>
<tr>
<td>Curative Swedish (Anonymous, 1997a)</td>
<td>679</td>
<td>25</td>
<td>-</td>
</tr>
<tr>
<td>(Anonymous, 1994)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>London (Anonymous, 1984)</td>
<td>468</td>
<td>24</td>
<td>-</td>
</tr>
<tr>
<td>EORTC (Gerard et al., 1988)</td>
<td>466</td>
<td>35</td>
<td>-</td>
</tr>
<tr>
<td>Curative Dutch etc (Kapiteijn et al., 2001)</td>
<td>341</td>
<td>30</td>
<td>-</td>
</tr>
<tr>
<td>Postoperative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Netherlands (Arnaud et al., 1997)</td>
<td>172</td>
<td>33</td>
<td>-</td>
</tr>
<tr>
<td>NSABP (Fisher et al., 1988)</td>
<td>555</td>
<td>25</td>
<td>21</td>
</tr>
<tr>
<td>GITSG 7175 (Anonymous, 1985; Douglass et al., 1986; Thomas &amp; Lindblad, 1988)</td>
<td>227</td>
<td>24</td>
<td>27</td>
</tr>
<tr>
<td>NCCCTG/ Mayo (Krook et al., 1991)</td>
<td>204</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NCCCTG (O’Connell et al., 1994)</td>
<td>680</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Surg= surgery, CT=chemotherapy, XRT=radiotherapy
In a meta-analysis of 8507 rectal cancer patients in 22 trials, preoperative radiotherapy (at biologically effective doses ≥30Gy) reduces risk of local recurrence and death from rectal cancer. Postoperative radiotherapy also reduces local recurrence. Short preoperative radiation schedules seem to be at least as effective as longer schedules (Anonymous, 2001).

5.1.6.2. Chemoradiation

The combination of radiotherapy and chemotherapy has demonstrated effects on disease free survival and overall survival in most studies (Douglass et al., 1986; Fisher et al., 1988; Kook et al., 1991; O’Connell et al., 1994; Tveit et al., 1997) (Table 4).

Table 4  Postoperative chemoradiation studies in rectal cancer

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients n</th>
<th>Treatment schedule</th>
<th>Local relapses %</th>
<th>DFS ratio at 4-5 years, %</th>
<th>OS ratio at 2-5 years, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Fisher et al., 1988)</td>
<td>555</td>
<td>Surg</td>
<td>25</td>
<td>30</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>surg+xrt</td>
<td>16</td>
<td>34</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>surg+MOF</td>
<td>21</td>
<td>42</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$P=.06$</td>
<td>$P=.006$</td>
</tr>
<tr>
<td>(Douglass et al., 1986; Stablein, 1990)</td>
<td>202</td>
<td>surg</td>
<td>24</td>
<td>45</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td></td>
<td>surg+MF</td>
<td>27</td>
<td>53</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td></td>
<td>surg+xrt</td>
<td>20</td>
<td>53</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td></td>
<td>surg+xrt+MF</td>
<td>11</td>
<td>70</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$P=.08$</td>
<td>$P=.009$</td>
</tr>
<tr>
<td>(Mansour et al., 1991)</td>
<td>237</td>
<td>surg+xrt</td>
<td>Not reported</td>
<td>40</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td></td>
<td>surg+MF</td>
<td>45</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>surg+xrt+MF</td>
<td>46</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>(Kook et al., 1991)</td>
<td>204</td>
<td>surg+xrt</td>
<td>25</td>
<td>38</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td></td>
<td>surg+xrt+MF</td>
<td>14</td>
<td>58</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$P=.04$</td>
<td>$P=.002$</td>
</tr>
<tr>
<td>(O’Connell et al., 1994)</td>
<td>660</td>
<td>surg+xrt+FU</td>
<td>Not reported</td>
<td>53</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>surg+xrt+cFU</td>
<td>63</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$P=.11$</td>
<td>$P=.01$</td>
</tr>
<tr>
<td>(Tveit et al., 1997)</td>
<td>144</td>
<td>surg</td>
<td>30</td>
<td>50</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td></td>
<td>surg+xrt+FU</td>
<td>12</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$P=.01$</td>
<td>$P=.01$</td>
</tr>
<tr>
<td>(Wolmark et al., 2000)</td>
<td>694</td>
<td>surg+FU/MOF</td>
<td>13</td>
<td>46</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td></td>
<td>surg+FU/MOF+xrt</td>
<td>8</td>
<td>64</td>
<td>64</td>
</tr>
</tbody>
</table>

DFS=disease free survival, surg=surgery, xrt=postoperative radiotherapy, MF=methyl-CCNU & 5-fluorouracil, MOF=methyl-CCNU & vincristine & 5-fluorouracil, FU=5-fluorouracil bolus, cFU=continuous protracted venous infusion 5-fluorouracil during 5 weeks of radiotherapy, TME surg=surgery with the total mesorectal excision technique.
Studies performed by the GITSG and NCCTG-Mayo reveal a benefit to overall postoperative survival using adjuvant 5-FU and semustine (Methyl-CCNU) with radiotherapy (Douglass et al., 1986; Krook et al., 1991). In these trials combined modality adjuvant treatment with radiotherapy and chemotherapy following surgery also resulted in local failure rates lower than with radiotherapy or chemotherapy alone. Methyl-CCNU is not commercially available, and previous studies have linked this drug to increased renal toxicity and leukaemia.

Two trials have confirmed that the addition of Methyl-CCNU was not an essential component of the combined modality therapy, and that 5-FU alone plus radiation therapy was at least equally effective and may be considered standard (O'Connell et al., 1994; Wolmark et al., 2000). In the NSABP R-02 study (n = 694) patients with Dukes’ B or C rectal cancer received postoperative adjuvant chemotherapy alone (females 5-FU+LV and males MOF or 5-FU+LV) or chemoradiation. Postoperative radiotherapy resulted in no beneficial effect on disease-free survival (P=.90) or overall survival (P=.89), although it reduced the cumulative incidence of locoregional relapse from 13% to 8% (P=.02). Males with 5-FU+LV had a better DFS (55% vs. 47%; P=.009), but not OS (65% vs. 62%; P=.17) than with MOF (Wolmark et al., 2000). The English AXIS study randomized patients (n=762) to preoperative or postoperative radiotherapy in conjunction with a 7-day 5-FU PVI vs. surgery alone. In this study treatment compliance was low and survival equivalent (approximately 50%, HR 0.98, CI95%, 0.81-1.18) in adjuvant radiotherapy+-/5-FU vs. surgery alone arm (James et al., 2003b).

The NCCTG-Mayo study reported a considerable 36% reduction in cancer mortality (Krook et al. 1991). Again, it is however, unclear whether the benefit seen in this small series was inflated by chance, or was due to a synergistic benefit from giving infusional 5-FU with radiotherapy. The continuous infusion may be important since a third study by this group found better survival with protracted venous infusion compared to bolus 5-FU when given concomitantly with radiotherapy (O’Connell et al. 1994). In a US study (n= 1695), randomization to postoperative chemoradiation with 5-FU, 5-FU and levamisole, 5-FU and LV or 5-FU and LV and levamisole chemotherapy for 6 months was performed. The OS and DFS were similar in drug arm comparison (Tepper et al., 2002). The local recurrence rate was 14% (9% in low-risk and 18% in high-risk patients). Overall, 7-year survival rates were 70% and 45% for the low-risk and high-risk groups, respectively (Tepper et al., 2002).
Optimal scheduling of postoperative chemoradiation has been investigated. In a study by the PAR group, rectal cancer patient (n=218) were randomized to postoperative radiotherapy vs. radiotherapy and sequential 5-FU and levamisole (low compliance 59%) and showed no significant difference in DFS ($P= .66$) or OS ($P= .18$)(Cañiero et al., 2003). In another study, 308 rectal cancer patients received 6 months of postoperative chemoradiation with 5-FU and LV. Radiation therapy (45 Gy/1.8 Gy) was allocated to start on cycle day 1 or on day 36. In this study better DFS (81% v. 70% at 4 years; $P= .043$) and lower local recurrence rates (23 vs. 38 cases, $P= .047$) were seen in the early starting group. OS showed no significant difference at 4 years (84% vs. 82%; $P= .387$) (Lee et al., 2002).

Long preoperative radiotherapy 45-55/1.8-2 Gy with a 4-7 week interval to operation has been used in downstaging of rectal tumours that were not primarily radically operable (Bosset et al., 2000). Two randomized UK trials (n=284 and 279) have addressed the effect of preoperative radiotherapy given as 20/5 Gy or 40/2 Gy in tethered or fixed rectal tumours. In both trials a reduction in local recurrence was seen, but no overall survival benefit (Marsh et al., 1994; Anonymous, 1996). In a Swedish trial (n=70) patients were allocated to preoperative radiotherapy vs. chemoradiotherapy and 64% vs. 74% were radically resectable, and of these 44% had a local recurrence in the radiotherapy arm vs. 17% in the chemoradiotherapy (+5-FU, LV and methotrexate) group ($P= .05$). Local DFS was 38% vs. 66% in radiotherapy and chemoradiation arm respectively, at 5 years ($P= .03$ log-rank test). Five-year OS was 18% vs. 29% ($P= .30$) (Frykholm et al., 2001). The generally used chemotherapy agents in this setting have been 5-FU as bolus or protracted venous infusion possibly combined with LV (Minsky et al., 1991a; Minsky et al., 1991b; Rich, 1997; Janjan et al., 1998). In an Italian phase II study (n=70) PVI during preoperative radiotherapy showed a promising pCR rate of 22% and combined with continuous infusion of the de Gramont schedule a promising 4-year OS estimation of 79% and DFS estimation of 61% (Luppi et al., 2003). Newer chemotherapeutic agents, for example capecitabine, raltitrexed and oxaliplatin, show promise in this entity, but evidence from randomized trials is not yet present. In a multicentre phase I study the feasibility of capecitabine in combination with radiotherapy was proven (Souglakos et al., 2003) and further studies are underway. A phase II study with oxaliplatin in combination with 5-FU and LV showed a RR of 75% (15% pCR) given preoperatively in conjunction with radiation (Gerard et al., 2003).
The effects of chemotherapy in advanced colon and rectal cancers are generally similar. The current area of interest is the adjuvant chemotherapy regimen with 5-FU biomalodulated with LV, perhaps even to be combined with irinotecan or oxaliplatin, as in colon cancer, combined with pre- or postoperative radiotherapy. The US-NIH consensus conference recommended postoperative radiation and chemotherapy in adjuvant treatment of Dukes’ B and C rectal cancer (NIH 1990). Since then, new approaches have been made to assess the role of preoperative vs. postoperative radiation in combination with chemotherapy. The consensus conference in Paris 1994 recommended preoperative radiotherapy for T3 and T4 tumours (Conférence de Consensus 1995). The Third international conference: Perspectives in Colorectal Cancer Dublin 2001, recommended adjuvant treatment in Dukes’ B and C rectal cancer as postoperative chemoradiation, preoperative short (25/5Gy) or long radiation (45-50.4/1.8-2Gy)+/-5-FU based chemotherapy (Van Cutsem et al., 2002).

5.1.6.3. Interferon in adjuvant therapy for rectal cancer

Addition of immunomodulatory interferon α to 5-FU and LV according to the Mayo schedule in combination with radiotherapy did not increase disease-free survival or overall survival in Dukes’ B and C rectal cancer patients (Gennatas et al., 2003). Similarly, no additional reduction in recurrences and death were seen with interferon α in addition to 5-FU and radiation in the FOGT-2 study (Staib L personal communication 2004).

5.1.7. Adjuvant treatment after curative resection of colorectal metastases

Evidence supporting routine use of intravenous adjuvant treatment after curative resection of liver or lung metastases is pending, but adjuvant chemotherapy may be beneficial for a proportion of patients with resected metastases. The 5-year survival after liver resection for colorectal metastases is about 25-40% (Girard et al., 1996; Fong et al., 1997; Adam, 2003; Fusai & Davidson, 2003). Retrospective and prospective small patient series show some survival benefit for addition of intravenous or hepatic arterial infusion (HAI) chemotherapy. In 235 non-randomized patients and 256 resections 5-year survival was 36% in the non-adjuvant chemotherapy group vs. 53% in patients who received postoperative 5-FU and LV based chemotherapy (Figuera et al., 2001). Similar results were seen in a French study were 48 patients had received neoadjuvant treatment compared with 23 that had immediate liver resection. The 3-
and 5-year OS were improved (67% vs. 52% and 39% vs. 21%) and fewer extended hepatectomies were required (39 of 48 vs. 23 of 23; \(P = .027\)) (Adam et al., 2003a).

HAI has gained substantial interest in this setting (Kemeny & Fata, 2001). In a phase I-II study the 2-year survival increased from 65% historically, to 89% in patients that received HAI flouxuridine and dexamethasone in combination with intravenous irinotecan postoperatively (Kemeny et al., 2003). Interleukin-2, mitomycin C and 5-fluorouracil as HAI post liver resection gave a 78% OS at 28.5 months (Okuno et al., 1996). 5-FU as HAI for 6 months postoperatively gave a 50% OS at 33 months and fewer recurrences compared with historical controls (Curley et al., 1993).

Five randomized studies that address the usefulness of adjuvant therapy in the postresective setting have been reported. In two studies reported in abstract form (n=162 and 109) systemic intravenous 5-FU and LV for 6 months was administered to patients, post liver or lung resection. In these underpowered studies no significant DFS nor OS was reported (Langer et al., 2002; Portier et al., 2002) (Table 5).

**Table 5 Randomized studies in colorectal cancer with chemotherapy vs. liver/lung resection alone.**

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Randomization</th>
<th>Duration months</th>
<th>DFS</th>
<th>OS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5-year</td>
<td></td>
</tr>
<tr>
<td>(Portier et al., 2002)</td>
<td>162</td>
<td>res</td>
<td>-</td>
<td>24%</td>
<td>44%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>res+5-FU/LV</td>
<td>6</td>
<td>33%</td>
<td>51%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(P = \text{ns})</td>
<td>(P = \text{ns})</td>
</tr>
<tr>
<td>(Langer et al., 2002)</td>
<td>109</td>
<td>res</td>
<td>-</td>
<td>35%</td>
<td>47%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>res+5-FU/LV</td>
<td>6</td>
<td>45%</td>
<td>57%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(P = \text{ns})</td>
<td>(P = \text{ns})</td>
</tr>
<tr>
<td>German (Lorenz et al., 1998)</td>
<td>226</td>
<td>res</td>
<td>-</td>
<td>13.7 months</td>
<td>34.5 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>res+HAI (5-FU/LV)</td>
<td>6</td>
<td>14.2 months</td>
<td>40.8 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(P = \text{ns})</td>
<td>(P = \text{ns})</td>
</tr>
<tr>
<td>US (Kemeny et al., 2002)</td>
<td>109</td>
<td>res</td>
<td>-</td>
<td>25%</td>
<td>34 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>res+HAI (FUDR) +iv 5-FU</td>
<td>6</td>
<td>46%</td>
<td>47 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(P = .04)</td>
<td>(P = .19)</td>
</tr>
<tr>
<td>US (Kemeny et al., 1999)</td>
<td>156</td>
<td>res+5-FU/LV</td>
<td>6</td>
<td>60%</td>
<td>72 (49%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>res+HAI (FUDR+DXM)+iv 5-FU/LV</td>
<td>6</td>
<td>90%</td>
<td>86 (61%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(P &lt; .001)</td>
<td>(P = .03)</td>
</tr>
</tbody>
</table>

Res = resection, HAI = hepatic arterial infusion, CT = systemic intravenous chemotherapy

In a German study 226 patients were randomized to resection alone vs. resection plus postoperative 6 months of HAI of 5-FU and LV. Compliance with chemotherapy was low. Median
survival was 34.5 months for patients with adjuvant therapy vs. 40.8 months for control patients. The median time to progression was 14.2 months for the chemotherapy group vs. 13.7 months for the control group (Lorenz et al., 1998). In two US studies (n=156 and 109), patients at the time of resection of hepatic metastases from colorectal cancer, were randomized to receive six cycles of hepatic arterial infusion with fluorouridine and dexamethasone plus intravenous fluorouracil and LV vs. resection alone or same systemic chemotherapy. The hepatic DFS at 2- and 4-years was significantly better in the HAI and systemic treatment arm (90% vs. 60% and 67% vs. 43%) (Kemeny et al., 1999; Kemeny et al., 2002) (Table 5). OS at 2 years was significantly better in one of the studies (Kemeny et al., 1999). Approximately 10% of patients with metastases from colorectal cancer are primarily resectable, the rest have a poor 5-year survival of less than 5% (Bosman, 2002). New strategies, such as downstaging chemotherapy, portal vein embolization and two-stage hepatectomy and repeat resections may increase the resectability rate by 15% (Fusai & Davidson, 2003). Increased resectability has been seen in patients following preoperative chemotherapy. In a French study 53 (16%) out of 330 unresectable cases were rendered resectable with 5-FU+LV and oxaliplatin chronomodulated and the 5-year survival was 40% (Bismuth et al., 1996). In a second French study (n=151) the combination of 5-FU+LV+oxaliplatin chronomodulated had a PR of 59% and a resection attempt was possible in 51% with a 5-year survival rate of 50% (Giacchetti et al., 1999). In a study (n=31) giving intravenous irinotecan, 5-FU and LV combined with pirarubicin as HAI in non-resectable liver metastases, the RR was 48% and liver resection was made possible in 11 patients (35%). Median OS was 20.5 months vs. not reached and median DFS was 9.1 vs. 20.2 months in patients with completely vs. non resected metastases (Zelck et al., 2003). An interesting feature, with neoadjuvant treatment before curative resection attempt has been seen in a French patient series, were patients with PD chemotherapy response had a 0% 3-year survival compared with 58% in patients that were downstaged and 45% in stabilized patients (Adam et al., 2003b).

Repeat liver resections give a comparative survival benefit as the first resection and should be attempted when feasible (Adam et al., 2003a; Taschieri et al., 2003). The EORTC ongoing study (aim n=330) compares liver resection to pre and postoperative 5-FU+LV combined with oxaliplatin. The feasibility study showed a significant downstaging of the tumour especially after 6 cycles of chemotherapy compared with 3 cycles and the chemotherapy given before liver resection was feasible (Lorenz et al., 2003).
5.2. Tolerability of chemotherapy in colorectal cancer

The aim of cancer therapy is to kill living cancer cells. Unfortunately the discrimination between benign and malignant cells is not selective, thus the main adverse events in cancer treatment are based on unintended damage in rapidly dividing benign cell lines in for example bone marrow, mucosa, hair roots and dermis. Typically, chemotherapeutic agents have a very narrow therapeutic ratio. Fortunately, the capacity for renewal is greater in normal than in neoplastic tissues making cancer therapy feasible.

5.2.1. Toxicity of palliative chemotherapy

5.2.1.1. Intravenous 5-FU with and without LV

The main toxicities of 5-fluoropyrimidines consist of mild to moderate nausea, haematologic toxicity in red, white and platelet cell lines, moderate to severe oro-gastro-intestinal toxicity and mild alopecia. The toxicity profile varies significantly with schedule, dose and route of administration. In a meta-analysis comparing 5-FU bolus (n=612) vs. PVI (n=607) a significantly lower incidence of haematologic toxicity was seen in PVI administration (Gr 3-4 in 31% vs. 4%), but more hand-foot syndrome (all grades, 13% vs. 34%) and similar frequencies of non-haematologic toxicity (diarrhoea, stomatitis, nausea and vomiting) 14 vs. 13% (Anonymous, 1998).

5-FU efficacy is enhanced by biochemical modulation with LV, and the toxicity is also altered (Table 6). The dose intensity of LV does not markedly alter the toxicity at the doses used clinically (Labianca et al., 1997). The monthly 5-FU and low dose LV iv push (Mayo) has been compared with weekly 5-FU and low dose LV boluses (Roswell Park). The toxicity profiles of these two regimens are slightly different (Buroker et al., 1994). Nowadays the Mayo regimen is often considered somewhat too toxic (Vincent et al., 2002). In general, continuous infusion regimens have a favourable toxicity profile as compared with the bolus Mayo regimen. The German AIO 5-FU 24-hour continuous regimen has been compared with the addition of LV and with the Mayo regimens, and revealed a slight efficacy benefit for the continuous 5-FU+LV with a similar overall toxicity as in the Mayo arm (Kohne et al., 2003). Once a fortnight LV5FU2 regimen has revealed a RR and TTP benefit over the Mayo regimen with a favourable overall toxicity profile (Gr 3-4 in 11% vs. 24%, P=.0004) (de Gramont et al., 1997a).
Table 6  Tolerability of 5-fluoropyrimidine-based chemotherapy in metastatic colorectal cancer.

<table>
<thead>
<tr>
<th>Study</th>
<th>Monthly 5FU+LV low dose as short infusion</th>
<th>Monthly 5FU+LV high dose as short infusion</th>
<th>Weekly 5FU+LV low dose as bolus</th>
<th>Weekly 5FU+LV high dose as bolus</th>
<th>Monthly 5FU+LV as short infusion</th>
<th>Weekly 5FU+LV as cont. infusion</th>
<th>Monthly 5FU+LV as bolus+ cont. infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Event Gr 3-4 (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucositis</td>
<td>5</td>
<td>7</td>
<td>24</td>
<td>2</td>
<td>11</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>5</td>
<td>10</td>
<td>18</td>
<td>32</td>
<td>9</td>
<td>6</td>
<td>22</td>
</tr>
<tr>
<td>Nausea</td>
<td>2</td>
<td>3</td>
<td>9</td>
<td>5</td>
<td>3</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Leukopenia/ Neutropenia</td>
<td>1</td>
<td>3</td>
<td>29</td>
<td>5</td>
<td>7</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Infection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>PPE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Alopecia Gr 2-3</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>21</td>
<td>31</td>
<td>1</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Hospitalizations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Fatalities</td>
<td>2.7</td>
<td>1.1</td>
<td>1.2</td>
<td>1.2</td>
<td>0.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5.2.1.2. Oral 5-FU derivates

In general oral 5-FU prodrugs have a favourable toxicity profile resembling that protracted venous administration (Anonymous, 1998) (Table 7). In a study that compared the Mayo bolus regimen with capecitabine, less stomatitis, hospitalizations and infections were seen in the capecitabine arm (Van Cutsem et al., 2001b). Oral eniluracil and 5-FU caused less stomatitis, neutropenia and infections compared with the Mayo regimen (Schilsky et al., 2002).

Carmofur has a different toxicity profile as compared to the other oral prodrugs mentioned. Toxicity, including grades 1-4, appears in 20-43% of the patients and is generally tolerable, and consisted of urinary frequency (4.3%), hot flushes (12.2%) and urgent defecation (1.1%), which are more common with high doses and recent treatment initiation. Nausea & vomiting (3.6%), abdominal pain, diarrhoea (3.7%), anorexia (4.3%), asthenia (10%), anaemia, leucopenia, thrombocytopenia and an antabus type of reaction in association with alcohol intake (3.8%) have also been recorded (Nishio et al., 1987; Kajanti & Pyrhonen, 1991; Kusama et al., 1995). A severe but very rare (<.01%) toxicity is leukoencephalopathy and carmofur treatment should be withdrawn in case of neurologic symptoms; dizziness, light-headed feeling, numbness, dysarthria, staggering of gait or amnesia (Yasue et al. 1985, Furuya et al 1987).
Table 7  Tolerability of oral 5-fluoropyrimidines

<table>
<thead>
<tr>
<th>Study</th>
<th>(Hoff et al., 2001) n=605</th>
<th>(Douillard et al., 2002) n=816</th>
<th>(Schilsky et al., 2002) n=981</th>
<th>(Koyama, 1981; Grohn et al., 1990; Ito et al., 1996a; Ito et al., 1996b) n=119-149</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regimen</td>
<td>Monthly 5FU+LV as short infusion?</td>
<td>2 weeks 3 weekly capecitabine</td>
<td>Monthly 5FU+LV as short infusion?</td>
<td>Monthly 5FU+LV as short infusion?</td>
</tr>
<tr>
<td>Mucositis</td>
<td>16 3</td>
<td>19 1</td>
<td>12 1</td>
<td>1</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>14 15</td>
<td>16 21</td>
<td>16 19</td>
<td>0-9</td>
</tr>
<tr>
<td>Nausea</td>
<td>5 4</td>
<td>10 13</td>
<td>7 3</td>
<td>3-4</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>26 3</td>
<td>56 1</td>
<td>47 5</td>
<td>0-3</td>
</tr>
<tr>
<td>Infection/Fever</td>
<td>1 1</td>
<td>13 0</td>
<td>10 0</td>
<td>Rare</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>0 1</td>
<td>2 0</td>
<td>2 3</td>
<td>1</td>
</tr>
<tr>
<td>Neurotoxicity</td>
<td>2 1</td>
<td>4 6</td>
<td>0.1</td>
<td>0</td>
</tr>
<tr>
<td>PPE</td>
<td>1 18</td>
<td>0 0</td>
<td>22 4</td>
<td>0</td>
</tr>
<tr>
<td>Alopecia Gr 1-3</td>
<td>21 6</td>
<td>22 4</td>
<td>2.0 0.8</td>
<td>Rare</td>
</tr>
<tr>
<td>Overall</td>
<td>41 43</td>
<td>22 4</td>
<td>2.0 0.8</td>
<td>Rare</td>
</tr>
<tr>
<td>Hospitalizations</td>
<td>11 20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fatalities</td>
<td>1.0 0.7</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5.2.1.3. Raltitrexed

Raltitrexed has an acceptable overall safety profile, and has some advantages over the Mayo regimen in terms of less severe neutropenia and mucositis (Sobrero, 1997a) (Table 8). The most frequent dose limiting toxicities reported are fatigue, gastrointestinal toxicity (diarrhoea), and haematological toxicity (mainly neutropenia)(Cunningham et al., 1996; Pazdur & Vincent, 1997; Cocconi et al., 1998). Reversible rises in liver transaminases, rash and fever are also seen. In randomized phase III studies an unacceptable toxic death rate (1.9%) was found when the drug was used in the adjuvant setting (Petacc1) and as first-line treatment for metastatic disease (6%) (Anonymous, 1999a; Maughan et al., 2002). This was mainly attributed to combined haematological and gastrointestinal toxicity in conjunction with protocol violations and neglected dose reductions (Anonymous, 1999a; Maughan et al., 2002).
Table 8  Tolerability of raltitrexed chemotherapy in metastatic colorectal cancer

<table>
<thead>
<tr>
<th>Study</th>
<th>(Cunningham et al., 1996) n=439</th>
<th>(Pazdur &amp; Vincent, 1997) n=427</th>
<th>(Cocconi et al., 1998) n=495</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regimen</td>
<td>3 weekly raltitrexed 5FU+LV as bolus</td>
<td>3 weekly raltitrexed 5FU+LV as short infusion?</td>
<td>3 weekly raltitrexed 5FU+LV as short infusion?</td>
</tr>
<tr>
<td>Event Gr 3-4 (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucositis</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>14</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Nausea</td>
<td>13</td>
<td>13</td>
<td>10</td>
</tr>
<tr>
<td>Leukopenia</td>
<td>14</td>
<td>18</td>
<td>6</td>
</tr>
<tr>
<td>Fever</td>
<td>3</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Infection</td>
<td>5</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>4</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Anaemia</td>
<td>9</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>Transaminases</td>
<td>10</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>Fatigue</td>
<td>6</td>
<td>18</td>
<td>5</td>
</tr>
<tr>
<td>Fatalities</td>
<td>3.6%</td>
<td>2.8%</td>
<td>Not reported</td>
</tr>
</tbody>
</table>

5.2.1.4. 5-FU+/LV combination chemotherapy

The addition of irinotecan or oxaliplatin carries the toxicity profile of the underlying 5-FU regimen to the profile of the added drug (Table 9). Typical toxicity of irinotecan consists of diarrhoea, nausea, neutropenia, cholinergic syndrome and alopecia at higher doses (Cunningham et al., 1998). Oxaliplatin may cause neurotoxicity, nausea, and usually moderate haematologic and oro-gastro-intestinal toxicity (Levi et al., 1993; Machover et al., 1996). The combination of bolus or weekly continuous 5-FU and LV with irinotecan causes increased diarrhoea, neutropenia (one study), nausea and infections (Douillard et al., 2000; Saltz et al., 2000). Oxaliplatin combined with the Nordic regimen, consisting of bolus 5-FU and LV, has a feasible toxicity profile (Sorbye et al., 2004). In comparison to continuous 5-FU and LV with and without oxaliplatin neurotoxicity, neutropenia and nausea are increased in the combination (de Gramont et al., 2000). Comparing FOLFOX and FOLFIRI, patients had higher overall Gr 3-4 toxicity in the FOLFOX arm, especially neurotoxicity, and thrombocytopenia and neutropenia were more frequent. Conversely, febrile neutropenia, nausea, stomatitis and fatigue were more common in the FOLFIRI arm (Tournigand et al., 2004). Gain with some pain is evident in efficient combinations.
Table 9  Tolerability of 5-FU and LV combination chemotherapy in metastatic colorectal cancer.

<table>
<thead>
<tr>
<th>Study</th>
<th>(Saltz et al., 2000) n=225+219</th>
<th>(Douillard et al., 2000) n=288</th>
<th>(de Gramont et al., 2000) n=420</th>
<th>(Tournigand et al., 2004) n=226</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regimen</td>
<td>Monthly 5FU+LV as short infusion</td>
<td>Weekly 5FU+LV as cont. infusion</td>
<td>Weekly 5FU+LV as cont. infusion + irinotecan</td>
<td>2 weekly 5FU+LV as bolus+ cont. infusion + oxaliplatin simplified</td>
</tr>
<tr>
<td>Mucositis</td>
<td>17</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>13</td>
<td>23</td>
<td>26</td>
<td>5</td>
</tr>
<tr>
<td>Nausea</td>
<td>4</td>
<td>10</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Leukopenia/Neutropenia</td>
<td>66</td>
<td>54</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Infection</td>
<td>15</td>
<td>9</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Neurotoxicity</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>PPE</td>
<td></td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Alopecia Gr 2-3</td>
<td></td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td></td>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>

The toxicity profile of the single agent, the dosing and schedule is thus carried to the combination chemotherapy. Carmofur has a unique well-tolerated profile in oral administration, which theoretically could be well combined with the raltitrexed related toxicity, to achieve the preclinical synergistic effects of the combination.

5.2.2. Toxicity of adjuvant 5-FU based chemotherapy

In adjuvant treatment toxicity is of higher concern, especially fatalities in patients potentially cured. The first efficient combinations in adjuvant treatment were MOF consisting of methyl-CCNU, vincristine and 5-FU with a risk for haematologic toxicity, alopecia, diarrhoea, myeloproliferative disorders and leukaemia (Fisher et al., 1988; Wolmark et al., 1993; Wolmark et al., 2000). Levamisole has been combined with 5-FU+/LV (Tepper et al., 1997a; Wolmark et al., 1999). Levamisole, alone or in combination, is linked to mild nausea, diarrhoea and leukopenia (Laurie et al., 1989) and to a small risk of leukoencephalopathy and hepatic damage (Moertel et al., 1993; Luppi et al., 1996).

The 5-FU (with varying doses) and LV combination has shown a tolerable toxicity profile, especially when administrated as a short infusion compared with a bolus shot (Table 10). The
highest toxicities reported are in the NCI-CTC study with a true bolus delivery and weekly toxicity reporting by nurses or research staff (Labianca et al., 1995). Haematologic toxicity is underestimated in many studies due to missing nadir full blood count measures. Most common toxicity in bolus 5-FU and LV is stomatitis, diarrhoea, nausea/vomiting and neutropenia (Table 10). A rare but potentially severe toxicity seen in these studies in neurological toxicity reported in 2% of patients with 5-FU+levamisole and in 1% in 5FU+LV and 5-FU+LV+levamisole (Wolmark et al., 1999).

Table 10 Tolerability of intravenous 5-fluoropyrimidine based adjuvant chemotherapy

<table>
<thead>
<tr>
<th>Study</th>
<th>(Porschén et al., 2001) n=680</th>
<th>(Labianca et al., 1995) n=444 GIVIO+ n=134 FFCD / n=195 NCI-CTC</th>
<th>(Francini et al., 1994) n=118</th>
<th>(Wolmark et al., 1999) n=691+696</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regimen</td>
<td>Monthly 5FU as short infusion</td>
<td>Monthly 5FU+LV as short infusion? Monthly 5FU+LV as bolus</td>
<td>Monthly 5FU+LV as bolus</td>
<td>Weekly 5FU+LV as bolus? Weekly 5FU+LV as bolus?</td>
</tr>
<tr>
<td></td>
<td>infusion + levamisole</td>
<td>reported</td>
<td>reported</td>
<td>+ levamisole</td>
</tr>
<tr>
<td>Event Gr 3-4 (%)</td>
<td>0 2</td>
<td>5 37 4</td>
<td>20 (Gr 2-4)</td>
<td>2 1</td>
</tr>
<tr>
<td>Mucoctis</td>
<td>2 4</td>
<td>16 47 4</td>
<td>21 (Gr 2-4)</td>
<td>25 27</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>1 5</td>
<td>9 18 5</td>
<td>0 (Gr 2-4)</td>
<td>5 7</td>
</tr>
<tr>
<td>Nausea</td>
<td>2 &gt;1</td>
<td>Not recorded</td>
<td>13 (Gr 2-4)</td>
<td></td>
</tr>
<tr>
<td>Leukopenia/</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutropenia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infection</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPE</td>
<td></td>
<td></td>
<td>3 (Gr 2-4)</td>
<td></td>
</tr>
<tr>
<td>Alopecia Gr 2-3</td>
<td>1 1</td>
<td>4 16 1</td>
<td>18 (Gr 2-4)</td>
<td></td>
</tr>
<tr>
<td>Other toxicity</td>
<td>6 4</td>
<td>0.3?</td>
<td>35</td>
<td>36</td>
</tr>
<tr>
<td>Overall</td>
<td>0 0</td>
<td></td>
<td>0.6</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Prolonged administration of 5-FU has resulted in more tolerable regimens, with same efficacy (5.1.1.1, 5.1.1.2), compared with intravenous 5-FU and LV bolus or short infusion (Table 11). The continuous infusion arm was better tolerated with less stomatitis, diarrhoea, neutropenia and overall toxicity in comparison with short infusion of 5-FU and LV (Andre et al., 2003). Protracted venous infusion of 5-FU was compared with bolus 5-FU and LV (Mayo) and tolerability of treatment was better in the PVI arm with less stomatitis, diarrhoea, alopecia and neutropenia (Saini et al., 2003). Hickman line function was assessed in 342 patients and serious complications (including pneumothorax, sepsicaemia and thrombosis) were seen in 9% and mild to moderate (including entry site infection and pain) in 36%. Line replacement was necessary in 9% (Saini et al., 2003).
Table 11  Tolerability of 5-fluoropyrimidine based adjuvant chemotherapy orally or as a prolonged infusion.

<table>
<thead>
<tr>
<th>Study</th>
<th>(Andre et al., 2003) n=902</th>
<th>(Sami et al., 2003) n=716</th>
<th>(Schethauer et al., 2003) n=1987</th>
<th>(de Gramont et al., 2003) n=2246</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regimen</td>
<td>Monthly 5FU+LV 2 weekly 5FU bolus short infusion +LV</td>
<td>Monthly 5FU+LV 12 weeks 5FU bolus + cont inf +LV</td>
<td>Monthly 5FU+LV 2 weeks 3 weekly bolus? capecitabine</td>
<td>2 weekly 5FU+LV as bolus+ cont infusion + oxaliplatin</td>
</tr>
<tr>
<td>Event Gr 3-4 (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucositis</td>
<td>7</td>
<td>20</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>9</td>
<td>16</td>
<td>13</td>
<td>2</td>
</tr>
<tr>
<td>Nausea</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Leukopenia/ Neutropenia</td>
<td>16</td>
<td>56</td>
<td>26</td>
<td>5</td>
</tr>
<tr>
<td>Infection</td>
<td>1</td>
<td>6</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Neurotoxicity</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>PPE</td>
<td>0</td>
<td>4</td>
<td>1</td>
<td>18</td>
</tr>
<tr>
<td>Alopecia Gr 2-3</td>
<td>0</td>
<td>14</td>
<td>22 (Gr1-2)</td>
<td>5</td>
</tr>
<tr>
<td>Overall</td>
<td>26</td>
<td>11</td>
<td>6 (Gr 1-2)</td>
<td>5</td>
</tr>
<tr>
<td>Hospitalizations</td>
<td>10</td>
<td>8</td>
<td>More</td>
<td>Less</td>
</tr>
<tr>
<td>Fatalities</td>
<td>0.5</td>
<td>0.9</td>
<td>0</td>
<td>0.3</td>
</tr>
</tbody>
</table>

5-FU and LV as a bolus according to the Mayo or Roswell Park schedule has been considered as standard adjuvant treatment in colon cancer. The true bolus administration causes quite high overall toxicity, especially oro-gastro-intestinal and haematologic toxicity. In adjuvant treatment high treatment related toxicity is of concern. The favourable toxicity profile of continuous infusion administration in metastatic colorectal cancer, and the development of modified easily administrable schedules, made the comparison of standard adjuvant Mayo treatment with the simplified de Gramont regimen attractive.

5.2.2.1. 5-FU based chemoradiation

5-FU is a radiosensitizer often used in chemoradiation trials in rectal cancer. 5-FU has been used as a single bolus and causes diarrhoea, leukopenia and dermatitis as main toxicities during chemoradiation (Krook et al., 1991; O'Connell et al., 1994; Tveit et al., 1997). Enhanced efficacy has been seen with protracted venous infusion during radiation compared with bolus administration, causing less neutropenia but some more diarrhoea (O'Connell et al., 1994).

Combinations with 5-FU and LV with and without levamisole are feasible (Wolmark et al., 2000), but increased toxicity is seen in the triplet combination (Tepper et al., 1997b; Tepper et al., 2002).
## Table 12  Tolerability of adjuvant 5-FU based postoperative chemoradiation

<table>
<thead>
<tr>
<th>Study</th>
<th>(Anonymous, 1992c) n=210</th>
<th>(Krook et al., 1991) n=104</th>
<th>(Tveit  et al., 1997) n=187</th>
<th>(O'Connell et al., 1994)n=220+214 during radiation</th>
<th>(Wolmark et al., 2000)n=260+263</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regimen</td>
<td>Monthly 5FU+ radiation</td>
<td>Monthly 5FU+ radiation</td>
<td>Twice 2 weekly 5FU bolus +radiation</td>
<td>5FU bolus + radiation</td>
<td>Weekly 5FU bolus + LV (+/- LV) + radiation</td>
</tr>
<tr>
<td>Mucositis</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>20</td>
<td>0</td>
<td>14</td>
<td>24</td>
<td>31</td>
</tr>
<tr>
<td>Nausea</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Leukopenia/ Neutropenia</td>
<td>18</td>
<td>0</td>
<td>11</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Infection</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Alopecia Gr 2-3</td>
<td>53</td>
<td>50</td>
<td>0</td>
<td>&lt;1</td>
<td>37</td>
</tr>
<tr>
<td>Overall</td>
<td>2</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Small bowel obstruction</td>
<td>1.0</td>
<td>0.9</td>
<td>0</td>
<td>0</td>
<td>0.3</td>
</tr>
<tr>
<td>Fatalities</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Preliminary tolerability results (n=628) for a preoperative vs. postoperative chemoradiation study indicate that overall Gr 3-4 toxicity is low (<15%). Grade 3-4 diarrhoea was seen in 11% vs. 14%, erythema, nausea (<3% in each arm) and leukopenia (<3% in each arm) were the next common toxicities. Postoperative complication rates were similar (Sauer et al., 2001).

Long-term toxicity is seen in the chemoradiation arms. Small bowel obstruction, requiring operation or other therapeutic interventions, is seen in 2-4% of patients (Krook et al., 1991; Anonymous, 1992c; O'Connell et al., 1994; Tveit et al., 1997). All 50 men assessed had normal urinary function after therapy for rectal cancer and all sexually active patients (n = 24) in the TME group and 9 of the 11 in the preoperative radiotherapy and TME surgery had normal ejaculation (P=.09) (Bonnel et al., 2002).

The survival benefits observed, with postoperative chemoradiation, preoperative radiotherapy and with continuous 5-FU administration during radiotherapy, therefore made a comparison with bolus vs. continuous 5-FU administration during radiotherapy attractive. Combining radiotherapy, with a chemotherapy regimen with less toxicity and hospital constraints, was challenging.
5.2.3. Systemic inflammatory reactions with chemotherapy

The human immune system is based on innate, non-specific responses and acquired, specific responses (Table 13). The innate immunity is present prior to the invasion of microorganisms or other triggers and the response is non-specific because it cannot discriminate between different intruders. The inducible more sophisticated mechanisms of the specific immunity responses act side by side.

Table 13 Innate and acquired immunity

<table>
<thead>
<tr>
<th></th>
<th>Innate immunity</th>
<th>Acquired immunity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells involved</td>
<td>Monocytes, Macrophages</td>
<td>T lymphocytes</td>
</tr>
<tr>
<td></td>
<td>Granulocytes</td>
<td>B lymphocytes</td>
</tr>
<tr>
<td></td>
<td>Endothelial cells</td>
<td>IL-2, IL-6, IL-10, IFNγ</td>
</tr>
<tr>
<td></td>
<td>Natural Killer cells</td>
<td>Production of specific antibodies</td>
</tr>
<tr>
<td></td>
<td>Fibroblasts</td>
<td>Production of specific reactive T cells</td>
</tr>
<tr>
<td>Messengers</td>
<td>Cytokines, such as TNFα, IL-1β, IL-6, IL-8, IL-10</td>
<td>Nitric oxide (NO)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Complement</td>
</tr>
<tr>
<td>Results</td>
<td>Non-specific defence</td>
<td>Production of specific antibodies</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Production of specific reactive T cells</td>
</tr>
</tbody>
</table>

The specific immunity can be divided into humoral and cellular immunity. Humoral immunity is mediated by antibodies, which are able to recognize antigens and are produced by B-lymphocytes. Cellular immunity is mediated by T-lymphocytes, which are able to recognize and eliminate antigen-expressing cells, where antigens have been processed and presented in the context of molecules of the major histocompatibility complex I and II. The cells of the immune system, such as monocytes, macrophages, granulocytes and lymphocytes are normally present as circulating cells in blood and lymph, as collections in the lymphoid organs and as matured cells in the tissues. The ability of the cells to migrate, circulate and exchange between compartments is essential for the immune response. The communication between these components in the different immune responses is dependent on the release of and interaction with cytokines, seen as messengers in the regulation, balancing this very complex mechanism.

Cytokines (cyto=cell, kinesis=movement) are soluble glycoproteins or proteins acting as messengers between cells. They can be produced and secreted by many different cells. The first cytokines that were discovered were thought to be messengers between leukocytes and thus named interleukins (IL). To induce an effect cytokines bind to their specific receptors on the cell membrane. This complex of cytokine and receptor then initiates the intracellular responses like upregulation of transcription factor activity inducing cell proliferation, release of other cytokines
and induction of apoptosis signals. The specific activity is thus dependent on the cytokine, the receptor and cell type, which it binds to. Cytokines can function as autocrine, paracrine and blood borne endocrine messengers inducing functions in the cell itself, in the neighbouring or distant cells. Cytokines can be divided into four categories of function: 1) mediators of innate immunity, 2) regulators of lymphocyte activation, growth and differentiation, 3) regulators of immune-mediated inflammation and 4) stimulators of immature myeloid proliferation and differentiation.

Monocytes and their mature counterparts’ macrophages have an essential initiating role in the local innate immune response (Figure 1). As a reaction to a local toxin, for example bacteria, the tissue macrophages and damaged endothelial cells produce mediators to activate the innate immunity. This leads to phagocytosis of immunoglobulin or complement-opsonized microorganisms by phagocytic cells (local inflammatory response). Activated monocytes/macrophages and neutrophils produce pro-inflammatory cytokines, such as tumour necrosis factor α (TNFα), IL-1β, IL-6 and IL-8 (Nathan et al., 1980). To regulate unwanted effects of the pro-inflammatory reaction, a counter-reaction, with anti-inflammatory cytokine release is simultaneously initiated. Anti-inflammatory cytokines such as IL-10 and IL-1RA are released, and receptor shedding, such as soluble TNFR or the type II IL-1R, occurs. If cytokines are released in the circulation a systemic inflammatory response is initiated. A balance must be obtained, because excessive cytokine amounts, causing systemic inflammatory reaction, can be harmful, even lethal.

**Figure 1**  **Local and systemic inflammatory response**

![Diagram of local and systemic inflammatory response]

- Local inflammatory response
  - pro-inflammatory: TNFα, IL-1β, IL-6, IL-8, NO
  - anti-inflammatory: IL-10, IL-1RA, STNFR, TGFβ

- Systemic inflammatory response
  - pro-inflammatory: TNFα, IL-1β, IL-6, IL-8, NO
  - anti-inflammatory: IL-10, IL-1RA, STNFR, TGFβ

Main cellular source: tissue macrophages-endothelial cells
Main cellular source: monocytes, neutrophils, endothelial cells, fibroblasts
In systemic inflammatory response the majority of active cytokines are produced by monocytes, neutrophils, endothelial cells and fibroblasts (Nathan et al., 1980; Schindler et al., 1990). The clinical picture is the result of a balance between pro-inflammatory and anti-inflammatory responses, both locally and/or systemically (Anonymous, 1992a; Bone, 1996b).

Three different clinical pictures are currently described (Bone, 1996c).
1. Systemic inflammatory response syndrome (SIRS) occurs when proinflammatory responses dominate anti-inflammatory responses. SIRS in adults is defined as at least two of the following conditions present: 1) temperature >38°C or <36°C, 2) heart rate >90 beats/minute, 3) respiratory rate >20 breaths/minute or PaCO₂ <32 mmHg and 4) white blood count >12 or <4x10⁹/l or >10% immature (band) forms. Elevated TNFα, IL-1β, and IL-6 are found in SIRS. Sepsis is defined as SIRS with documented infection.

2. Compensatory anti-inflammatory response syndrome (CARS) occurs when anti-inflammatory responses dominate the pro-inflammatory responses. CARS is also called immune paralysis and anergy. It is characterized by altered monocyte function, impaired antigen presenting ability and reduced ability to produce pro-inflammatory cytokines. CARS is characterized by elevated IL-10 levels, decreased in vitro TNFα and IL-6 production and down regulated HLA-DR expression on monocytes.

3. Mixed anti-inflammatory response syndrome occurs when features of both SIRS and CARS are present.

5.2.3.1. Pro-inflammatory and anti-inflammatory cytokines

Tumour necrosis factor α (TNFα) was previously called cachectin. The main stimulus for TNFα production is lipopolysaccharide, but agents such as viruses, IL-1, IFNγ and granulocyte-macrophage colony-stimulating factor also induce its secretion (Beutler et al., 1986; Balkwill, 1989). Monocytes and macrophages are the main cell source, but other cells known to produce it include T-lymphocytes and natural killer cells (Wallach, 1997). TNFα has a very broad spectrum of activity. It can produce cytotoxicity by induction of apoptosis and necrosis of multiple cell lines and tumour types (Couriel et al., 2000). TNFα also induces a large number of proteins: nuclear factors such as NF-κB, nitric oxide synthetase, cell surface molecules, including ICAM-1, IL-2 receptor α, HLA class I and II proteins and secreted proteins such as IL-1, IL-6, IL-8, interferon β, granulocyte-macrophage colony-stimulating factor, platelet-derived growth factor, urokinase...
plaminogen activator, and TNFα itself (Couriel et al., 2000). The action is concentration dependent and at low concentrations the effects are local, with upregulation of adhesion molecules, increasing adhesion of neutrophils and monocytes, and their transmigration through the vascular endothelium cells resulting in local accumulation of leukocytes. In higher amounts TNFα is able to give rise to the systemic inflammatory response, alone or together with IL-1β (De Bont, 1999). At very high concentrations in the circulation TNFα may cause a septic shock, which can be lethal (Cannon et al., 1990). Chronic administration in mice induces cachexia and it seems at least partly responsible for cancer-related cachexia (Beutler & Cerami, 1988a; Beutler & Cerami, 1988b).

Interleukin-1β (IL-1β) was formerly known as the lymphocyte-activating factor. A variety of cells can produce it including monocytes, macrophages, dendritic cells, natural killer cells, endothelial cells and fibroblasts (Dinarello et al., 1986). It is a potent inducer of fever (Dinarello et al., 1986) and it induces many cytokines of the inflammatory cascade when it circulates in high concentrations (Dinarello et al., 1986; Dinarello, 1987; Dinarello et al., 1987). The systemic effects of IL-1β are similar to those of TNFα, but it can neither produce tissue damage, upregulate MHC class II molecules, lyse cells nor cause lethal reactions on its own (Dinarello, 1988). At lower concentrations it induces local inflammatory reactions.

Interleukin 1 receptor antagonist (IL-1RA) is a naturally occurring antagonist of IL-1β. IL-1RA complex with the IL-1 receptor does not induce cell activation, as opposed to IL-1β complexes. It is secreted by the same cells as IL-1β but regulated by different mechanisms (Arend et al., 1991; Andersson et al., 1992).

Interleukin 6 (IL-6) was formerly known as the hepatocyte-stimulating factor. It is produced by mononuclear phagocytes, endothelial cells and fibroblasts (Hooper et al., 1998). It is a highly multi-functional cytokine with both pro-inflammatory and anti-inflammatory properties. It can induce acute phase protein production in hepatocytes, such as fibrinogen and CRP (De Bont, 1999). It is also important in the growth support of late B-lymphocyte differentiation (Denizot et al., 1995; Denizot et al., 1996). The IL-6 receptor has two subunits, one for binding IL-6 (α unit) and the other for signalling (β unit). The receptor can be shed from the cell membrane to downregulate IL-6 activities (De Bont, 1999).
Interleukin 8 (IL-8), previously called neutrophil activating protein, is a proinflammatory cytokine. It is produced by monocytes, lymphocytes, granulocytes and endothelial cells (De Bont, 1999). Its main function is to act as a neutrophil chemo-attractant (Baggiolini et al., 1989). It belongs to the chemokine family (chemotactic cytokine = chemokine). IL-8 infusions are able to induce rapid mobilization of haematopoietic stem cells (Laterveer et al., 1995). IL-8 can induce angiogenesis and support tumour growth and on the other hand endothelial cell derived IL-8 can suppress tumour growth by induction of apoptosis (Koch et al., 1992; Terui et al., 1998).

Interleukin 10 (IL-10) is an anti-inflammatory cytokine produced by activated monocytes and macrophages (De Bont, 1999). IL-10 inhibits the production of TNFα, IL-1β, IL-6 and IL-8 and IL-10 itself (de Waal Malefyt et al., 1991a). Moreover it downregulates MHC class II and ICAM-1 expression (de Waal Malefyt et al., 1991b). The net effect is an inhibition of T-cell mediated immune response.

5.2.3.2. Cytokines in the treatment of cancer

William Coley was probably the first to investigate tumour necrosis factor as a potential antitumour agent in the early twentieth century. He administered Coley toxins, which were gram + and - bacterial products later found to be lipopolysaccharides, to patients with inoperable cancer and reported some responses (Starnes, 1992). Lipopolysaccharides were found to cause hemorrhagic necrosis of transplanted tumours in mice (Shear, 1944), and they were also found to induce an endogenous factor later named as TNF (Carswell et al., 1975).

Attempts to use TNFα as a chemotherapeutic agent have been carried out since the 1980s. The systemic use of TNFα caused considerable toxicity with fever, severe hypotension, and organ failure, but cancer response rates were disappointing (Creagan et al., 1988; Sherman et al., 1988; Spriggs et al., 1988; Feldman et al., 1992; Tomita et al., 1998). These trials led to attempts of isolated limb or liver perfusion with TNFα in metastatic melanoma (Rossi et al., 2002) and inoperable soft tissue sarcoma (Eggermont et al., 1996; Di Filippo et al., 1999), and later also in the treatment of hepatic metastases (de Vries et al., 1998; Lindner et al., 1999). The combination of chemotherapeutic agents with TNFα may give higher RRs (Watanabe et al., 1988; Tomita et al., 1998).

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The mode of action with TNFα has been clarified over the past years leading to a novel anticancer therapy. The interaction between TNFα and TNF-R1 has been shown to generate a signal that activates a caspase II subfamily-dependent apoptosis (Utaisincharoen et al., 1999). The TNF-related apoptosis-inducing ligand (TRAIL) has been defined as a typical member of the TNF ligand family, shown to induce apoptosis by activating the death receptors TRAIL-R1 and TRAIL-R2. TRAIL has attracted great attention in recent years as a promising anti cancer reagent because recombinant soluble TRAIL derivatives induce apoptosis in a broad range of tumour cells (Wajant et al., 2002).

5.2.3.3. Chemotherapy triggered cytokine release

One mechanism for chemotherapy related toxicity might be an inflammatory reaction with cytokine release triggered by chemotherapeutic agents themselves. Fever, fatigue, myalgia, arthralgia, pulmonary toxicity and transient hepatic transaminase reactions, even lethal multiorgan failure etc may result from cytokine release. Non-neutropenic fever within 72 hours of chemotherapy administration, which is not linked to microbial infection and that responds poorly to antimicrobials, has been documented. Such a fever reaction has traditionally been linked to cytarabine and bleomycin administration. Recently, also paclitaxel and oxaliplatin have been described as potential initiators for inflammatory reactions. The toxicity profile of these drugs could partly be described by drug induced cytokine activation.

Cytarabine is an arabinose nucleoside. The toxicity profile consists of myelosuppression, mild gastrointestinal symptoms, cutaneous changes, transient hepatic enzyme rise and pulmonary complications (Chu et al., 2001). “The ara-C syndrome” that occurs in 33-50% of patients on prolonged administration of the drug, is characterized by fever, fatigue, muscle, bone, abdominal and chest pain (Castleberry et al., 1981). In these patients no infections, neither presence of pyrogens, could be documented. The reaction could be inhibited by corticosteroids. The likely explanation has been a hypersensitivity reaction. Recently, cytokine activation in conjunction with cytarabine treatment has been documented (Chiche et al., 1993; Briasoulis & Pavlidis, 2001; Ek et al., 2001; Ek & Abrahamsson, 2004). In one study 13 of the 16 patients treated with cytarabine developed fever a median of 30 hr following treatment initiation. At 12 hr after cytarabine administration levels of TNFα were elevated followed by a rise in IL-6, IFNα, and IL-1ra, peaking at the onset of fever. Thereafter these serum levels slowly declined, whereas low IL-10 levels became detectable (Ek et al., 2001).
Bleomycin toxicity is characterized by hypersensitivity reactions and pulmonary changes varying from mild radiologic changes to lethal pulmonary fibrosis, which may occur in 10% of patients. Fever or hyperpyrexia is found in about 20-50% of patients. Joint pain and cutaneous changes are also seen (Cheson 2001). There is some evidence for a TNFα activation after administration of bleomycin (Sleijfer et al., 1998). Similarly, cytokine activation has been measured in bleomycin lung damage suggesting an inflammatory reaction triggered by chemotherapy itself (Zhang et al., 1997; Ortiz et al., 1998; Smith et al., 1998).

The platinum compound oxaliplatin is generally well tolerated with neurotoxicity as the DLT, but it has also been linked with fever reactions, pulmonary changes and anaphylaxia. In fever reactions TNFα and IL-6 activation have been observed (Ulrich-Pur et al., 2000; Tonini et al., 2002). This syndrome has recurred on consequent cycles, and is amendable with corticosteroids (Ulrich-Pur et al., 2000; Tonini et al., 2002).

Paclitaxel is a antimicrotubuline agent and its typical toxicity consist of myelosuppression and hypersensitivity reactions (Rowinsky & Tolcher, 2001). Fatigue, flu-like symptoms and fever have been documented with cytokine activation 3 days after paclitaxel administration (Pusztai et al., 2004).

Raltitrexed has a generally manageable toxicity profile. Although gathering evidence from single agent raltitrexed and the combination with carmofur showed non-neutropenic fever, fatigue and flu-like reaction in combination with transient transaminase rise, pulmonary reactions and rash. The similarity of interferon and interleukin-2 therapy related toxicity was identified and the thought of a cytokine burst in conjunction with raltitrexed therapy was to be studied. At this time only one study with cytokine activation in conjunction with chemotherapy had been published (Chiche et al., 1993).
5.2.4. Chemotherapy or radiotherapy induced secondary hypolactasia

5.2.4.1. Hypolactasia and lactose intolerance

Lactose is a disaccharide consisting of glucose and galactose, and it is found in milk and dairy products. The ability to digest lactose is dependent on an enzyme (lactase-phlorizin hydrolase) in the small intestinal brush border. Hypolactasia is present if the lactase activity in the small intestinal mucosa is low. The discrepancy between the amount of lactose to be digested and the hydrolyzing capacity of the lactase enzyme is called lactose malabsorption. Reasons for lactose malabsorption are congenital lactase deficiency (almost total lack of lactase, alactasia), hypolactasia (“general” lactose malabsorption) and secondary hypolactasia (due to reversible injury to the mucosa in the gastrointestinal tract).

Hypolactasia with malabsorptive symptoms is called lactase intolerance. Normolactasia is lactase activity persistence as moderate or high. Lactase activity has a genetically determined pattern in mammals with high activity from late gestation to early childhood, where after it declines to adult levels (Sahi & Launiala, 1978; Flatz, 1987). Lactase deficiency is a common autosomal recessive condition resulting in decreased intestinal lactose degradation. A -13910 T/C (and possibly -22018 G/A) single nucleotide polymorphism near the lactase phenylalanine hydrolase gene is reported to be strongly associated with adult lactase non-persistence (Kuukkanen et al., 2003). An association to milk aversion and reduced milk calcium intake has been observed in C/C genotypes (Obermayer-Pietsch et al., 2004), but is not conclusively associated with lactase intolerance. The hypolactasia figures vary widely in different populations (Scrimshaw & Murray, 1988). In Finland hypolactasia is present in approximately 17% of the population, which is considerably higher than the incidence in the other Nordic countries (about 5%) (Sahi, 1994). The decline in lactase activity is a normal pattern in about 75% of the adult population in the world (Sahi, 1994).

Symptoms of lactose intolerance are loose stools, abdominal bloating and pain, flatulence, nausea, diarrhoea and borborygmia. Loose stools and diarrhoea is caused by unabsorbed carbohydrate that causes secretion of fluid and electrolytes until osmotic equilibrium is reached (Launiala, 1968; Christopher & Bayless, 1971). The abdominal distension and cramps are thought to have their origin in the small intestine (Christopher & Bayless, 1971) or in the colon (Jouet et al., 2002). The production of hydrogen depends on colonic acidity. Reduced hydrogen excretion and symptoms have been reported after continuous lactose consumption (Hertzler & Savaiano, 1996).
5.2.4.2. Diagnostics in hypolactasia

The diagnosis of lactose intolerance is inexact due to varying symptoms. Hypolactasia and low lactase activity can be measured directly from small intestinal biopsies, but lactase activity may vary along the small intestine. Indirect diagnostic methods are less invasive. Primary hypolactasia can be diagnosed with a genetic test that detects the -13910 T/C single nucleotide polymorphism close to the lactase gene in chromosome 2. The C/C genotype is linked to hypolactasia and lactose malabsorption and the C/T and T/T types with lactase persistence (Kuokkanen et al., 2003). Secondary hypolactasia cannot be measured by genetic testing.

Indirect measures of lactase activity include measures of lactose hydrolysation to glucose and galactose, which can be measured from exhalation air, urine and blood. A common method is to measure blood glucose levels at 20 minute intervals for 1-2 hours after a load of 50g of lactose (Peuhkuri et al., 2000). A blood glucose level increase of less than 1.1mmol/l is considered as hypolactasia, and an increase of more than 1.6 mmol/l as normolactasia. Increase of 1.1-1.6 can be considered as a borderline finding. The sensitivity and specificity of indirect methods can however be questioned (Newcomer et al., 1975).

Lactose tolerance tests with ethanol addition have been used. Thus galactose metabolism can be inhibited and galactose measured from urine or blood (Isokoski et al., 1972). Further developments of the method have proven non-ethanol galactose measurement methods feasible (Arola, 1988). Measurement of breath hydrogen response after an oral load of lactose is based on the principle that unhydrolysed lactose is fermented by the colonic microflora, producing hydrogen and methane, which are measurable eventually from exhalation air (Levitt, 1969). The hydrogen test has been found quite reliable, but it carries certain difficulties, for example with the colonic bacterial flora and factors affecting the equilibrium, for example antibiotics (Arola, 1994).

5.2.4.3. Secondary hypolactasia during chemo- and radiotherapy

Gastrointestinal mucosal injury is common during 5-FU-based chemotherapy. Most common symptoms are diarrhoea and stomatitis, which are common especially following bolus administration of 5-FU. 5-FU and LV bolus treatment may also be associated with substantial small bowel toxicity (Fata et al., 1999). 5-FU and LV cause mucosal injury in the brush border
(microvilli) of the small bowel epithelium (Decker-Baumann et al., 1999). The microvilli contain enzymes responsible for hydrolysis and absorption of dietary carbohydrates including lactose, but little is known about the frequency of chemotherapy-induced lactose intolerance.

In one study chemotherapy given for acute lymphatic leukemia was found to cause reversible alteration in D-xylene and lactose absorption in a proportion of children studied (2 out of 16, and 4 out of 12, respectively) (Halton et al., 1993). In 2 other studies chemotherapy-induced lactose malabsorption was found in 11 (55%) and in 9 (33%) out of the 20 and 27 children treated with various types of anti-cancer drugs, respectively (Hyams et al., 1982; Pettoello-Mantovani et al., 1995). In one study where 27 adult patients were treated with various chemotherapy regimens, 5 patients had symptom-free hypolactasia prior to chemotherapy and 8 hypolactasia early post-treatment, and of these 8 patients only 3 had gastrointestinal symptoms compatible with lactose intolerance. Based on these findings the authors concluded that lactose intolerance is rare in chemotherapy patients (Parnes et al., 1994). Similarly, pelvic radiotherapy caused hypolactasia in only one of the 20 adults investigated (Ruppin et al., 1987). Hence, the evidence based on small patient numbers and variable treatment regimens suggests that chemotherapy-related lactose intolerance is relatively uncommon and might have limited clinical significance.

5.2.5. Use of probiotics and fibre to alleviate chemotherapy induced toxicity

5-fluorouracil based chemotherapy may cause haematologic, dermal and mucosal damage with significant morbidity. Diarrhoea and stomatitis occur in more than 50% of the patients treated. Methods to diminish adverse events include growth factors, cooling of hair roots and mouth mucosa during chemotherapy infusions, and cytoprotective and symptomatic drugs. Oral supplements with probiotic bacteria and fibre have not been extensively studied in alleviation of chemo- or radiotherapy induced gastrointestinal toxicity.

5.2.5.1. Probiotic bacteria

Probiotics are living microbial products that enhance health when given to humans or animals (Schrezenmeir & de Vrese, 2001). A probiotic is typically a specific bacterial strain or combination of strains with documented beneficial effect tested when tested in humans. The most common strains are the lactobacilli, which are anaerobic gram-positive chain-forming rods and part of the normal flora in the entire gastrointestinal tract. Most common Lactobacilli species are
the *L. acidophilus*, *L. casei*, *L. paracasei*, *L. rhamnosus*, *L. plantarum*, *L. pseudoplasticum* and *L. reuteri*. *Lactobacillus rhamnosus* GG (LGG) is a thoroughly studied lactobacillus strain with probiotic effects.

### 5.2.5.1.1. *Lactobacillus GG*

When given orally LGG colonizes the GI tract temporarily for about a week (Alander et al., 1997; Alander et al., 1999) and adheres to the intestinal mucus (Ouwehand et al., 1999) and the human colon tissue epithelium (Alander et al., 1997; Alander et al., 1999). Consumption of LGG leads to increased amount of beneficial anaerobic bacteria especially bifidobacteria and lactobacilli in the bowel flora (Benno et al., 1996; Apostolou et al., 2001).

Colonization resistance is the ability of the normal bacterial flora to inhibit adhesion and colonization of external bacteria and it is based on chemical, physiobiologic, and immunologic factors, mostly outlined by anaerobic bacteria. This resistance can be broken for example by antibiotics and other medication and may lead to diarrhoea and other GI symptoms. In animals LGG has improved colonization resistance, inhibited colonization of harmful microbes (Naaber et al., 1998; Apostolou et al., 2001), and diminished translocation of gut microbes to the bloodstream and internal organs (Dong et al., 1987). In patients with opportunistic *Clostridium difficile* infection LGG has been a successful treatment (Gorbach et al., 1987; Biller et al., 1995; Bennet et al., 1996; Pochapin et al., 1998; Pochapin, 2000). Another mechanism in osmotic diarrhoea treatment could be stabilization of the bacterial flora, which can be seen for example as a non-increased urease activity due to inhibition of overgrowth of urease producing bacteria (Isolauri et al., 1994). Patients with shigellosis have a disturbed microbial flora, and during treatment with antibiotics and LGG, or LGG only, had normal anaerobic counts, whereas patients treated with antibiotics only still had a significantly disturbed flora at the end of treatment (Sepp et al., 1995). Similar trends were seen in patients treated for salmonella and shigella infections. Microbial flora disturbances diminished during LGG administration as measured by formation of short chain fatty acids (SCFA) (Siigur et al., 1996).

Oral LGG activates innate, aspecific immunity and acquired, specific immunologic responses. In cell line studies LGG has induced proinflammatory cytokine production (Miettinen et al., 1996; Miettinen et al., 1998) and initiated mediators active in bacterial identification and cell communication (Kaila et al., 1995; Miettinen et al., 2000). In animal studies oral LGG
consumption has induced higher T and B lymphocyte counts (Kirjavainen et al., 1999), and in humans LGG has activated immune responses in healthy persons but suppressed immunologic hyperactivity in patients with cow milk allergy or in those with autoimmune diseases such as rheumatoid arthritis (Majamaa & Isolauri, 1997; Pelto et al., 1998; Pelto et al., 1999; Hatakka et al., 2001; Kalliomaki et al., 2001; Rautava et al., 2002). Co-administration of LGG with oral rotavirus and salmonella typhi vaccination has led to improved antibody formation (higher IgA and IgM titers) (Isolauri et al., 1995; He et al., 2000). Administration of LGG during rotavirus diarrhoea induced innate immunity (increased IgG, IgA and IgM production) and acquired immunity (through increased amount of antibody-forming cells). In LGG group 90% had rotavirus specific IgA at 3 weeks post vaccination as compared with 46% in placebo group (Kaila et al., 1992). Other postulated mechanisms in diarrhoea could be inducible nitric oxide formation (Korhonen et al., 2001), and prevention and treatment of damages of the intestinal mucosa (Mack et al., 1999; Banaszak et al., 2001; Mattar et al., 2001; Banaszak et al., 2002; Khaled et al., 2003; Mack et al., 2003).

LGG has been studied in prevention of diarrhoea. In a randomized double blind study in poor Peruvian children the incidence of diarrhoea, especially adenovirus related, was diminished (Oberhelman et al., 1999). Similarly, in a Polish study in non-diarrhoea hospitalized children LGG prevented clinical but not microbial rotavirus diarrhoea (Szajewska et al., 2001). The most common indication for probiotics has been prevention of antibiotic associated diarrhoea and abdominal pain. In five of six studies identified LGG reduced adverse events during antibiotic treatment significantly. Of these two studies were performed in children receiving antibiotics predominantly due to respiratory infections (Arvola et al., 1999; Vanderhoof et al., 1999), one study in healthy adults receiving erythromycin (Siitonen et al., 1990) and 2 in patients who received triple treatment for Helicobacter pylori eradication (Armuzzi et al., 2001a; Armuzzi et al., 2001b). The only study with a negative result was performed in hospitalized patients with complex antibiotic therapy for severe infections (Thomas et al., 2001). In two randomized studies LGG has been investigated in prevention of traveller’s diarrhoea. In a Finnish study travellers received LGG during a trip to two destinations in Turkey, and in one destination LGG prevented diarrhoea symptoms (Oksanen et al., 1990). In a second study Americans travelling to Asia, Africa, Central- and South-America received LGG which was found to prevent diarrhoea (incidence of diarrhoea 3.9% vs. 7.4%, \(P=.05\)) (Hilton et al., 1997).
LGG was successful in treatment of diarrhoea in eleven studies. These studies were predominantly performed in children with rotavirus diarrhoea, and consequently LGG shortened and relieved diarrhoea (Isolauri et al., 1991; Kaila et al., 1992; Isolauri et al., 1994; Kaila et al., 1995; Majamaa et al., 1995; Raza et al., 1995; Pant et al., 1996; Guarino et al., 1997; Shornikova et al., 1997; Rautanen et al., 1998; Guandalini et al., 2000) and shortened virus carriage time (Isolauri et al., 1991; Kaila et al., 1992). In two studies carried out in Asia LGG shortened the duration of acute diarrhoea, independent of microbial origin, provided that the cases had not bloody stools (Raza et al., 1995; Pant et al., 1996). In two European studies and one Russian study LGG reduced duration of diarrhoea also in non-rotavirus infections (Guarino et al., 1997; Shornikova et al., 1997; Guandalini et al., 2000).

5.2.5.1.2. Probiotics during chemo- and radiotherapy

Three studies that investigated the use of probiotics during pelvic radiotherapy have been published. In a small, randomized study (n=24) in gynaecologic cancers prevention of intestinal side effects by live Lactobacillus acidophilus cultures was studied. Lactobacilli appeared to prevent radiotherapy-associated diarrhoea (Salminen et al., 1988). The effect of Lactobacillus rhamnosus vs. placebo on radiation-induced diarrhoea was studied in Hungary (n=206). The Lactobacillus group had less bowel movements ($P<.10$) and favourable faeces consistency rated by the investigators than the group who received placebo ($P<.05$). Diarrhoea grade and faeces consistency showed a statistically highly significant treatment-by-time interaction at the second half of the study ($P<.001$) (Urbancsek et al., 2001). In a randomized study highly concentrated freeze-dried living bacteria (VSL/3) were administered to reduce these side effects in 190 patients receiving radiotherapy to the pelvic area. Gastrointestinal toxicity was found in 51% vs. 31% (Gr 3-4 in 30% vs. 7%), and 5 vs. 2 patients stopped treatment due to enteritis in the radiotherapy alone and VSL/3 group, respectively (Delia et al., 2002a).

No studies of probiotics and chemotherapy use in humans have been identified. One study investigated changes in the oral and intestinal microfloras in rats given iv 5-FU for 6 days and effects of adding Lactobacillus plantarum 299v in the drinking water. 5-FU treatment increased the number of facultative anaerobes and gram-negative rods in the oral cavity and in the large intestine. Bacterial translocation both to the cervical and mesenteric lymph nodes increased in numbers after 5-FU treatment. Lactobacillus improved food intake, body weight and reduced the
raise of facultative anaerobes in 5-FU-treated rats, but did not reduce bacterial translocation nor diarrhoea (Von Bultzingslowen et al., 2003).

Probiotics have emerging potential in treatment of oro-gastro-intestinal disturbances. The major drawback of 5-FU treatment is diarrhoea, stomatitis and other gastrointestinal disturbances, which in conjunction with neutropenia may lead to substantial morbidity. Not much is known about probiotics, especially the thoroughly documented *Lactobacillus* GG, in reduction of oro-gastro-intestinal toxicity during 5-FU chemotherapy or chemoradiation.

### 5.2.5.2. Hydrolysed guargum fibre

Present interest in fibre stems from the middle of the nineteenth century when the preoccupation of the Victorians with their bowel habits led many physicians to declaim the virtues of bran. By 1909 Arthur Hurst had singled out fibre as the principal component of bran responsible for its colonic effects (Hertz, 1909). The principal mode of action was defined as the capacity to absorb and retain water in the gut (Porges, 1928). Later studies concluded that it was the chemical stimuli, which arose from the destruction of hemicelluloses and cellulose by the intestinal bacterial flora, one of these products being lower volatile fatty acids, that were the primary mode of action (Williams & Olmsted, 1936). After a long period of silence, fibres regained interest in the 1970s. The fibres were now shown to speed up the intestinal transit time, dilute gut contents and favourably affect the microbial flora (Burkitt, 1971).

All fibres increase the faecal output and elements that make up the faeces. Wheat bran increases the output with 5.4g per added gram of fibre fed, fruits and vegetables with 4.7g, gums and mucilages with 3.7g, cellulose with 3.5g and pectin with just 1.2g (Cummings, 2001). This is due to the different physicochemical properties of the dietary fibres, particle size and chemical composition of fibre polysaccharides being the most important. A neglected component of stool, which affects composition, is the bacteria. Because bacteria resist dehydration, they most likely retain their water against the forces of the colonic mucosa, during the passage. The principal mode of action of fibres are 1) to increase faecal bulk by retaining water in the undegradable cellular structure, 2) to increase microbial growth, faecal mass and short chain fatty acid (SCFA) production when fibres are extensively degraded by bacteria, 3) to shorten transit time thereby increasing bacterial growth and reducing colonic water absorption, 4) and to increase gas (H₂, CH₄, CO₂) production (Cummings, 2001; Kobayashi & Fleming, 2001). SCFAs, are also referred
to as volatile fatty acids, include mainly acetate, propionate and butyrate. They are produced by the colonic microflora, when bacteria hydrolyze and ferment undegradable fibres and carbohydrates (Vanderhoof, 1998). The fibre source affects the SCFA production. Water soluble fibres, for example inulin, isphagula, senna and guar fibre, produce more SCFA than bran and cellulose that contain indispensible compounds (Kobayashi & Fleming, 2001). SCFAs are an important source of energy for the colonocytes (Roediger, 1982) and they stimulate water and sodium absorption (Ruppin et al., 1980; Roediger & Rae, 1982) thus reducing diarrhoea.

Fibres are used in prevention and treatment of a wide variety of disorders, for example constipation, diabetes, hyperlipidemia, colonic neoplasia, colon diverticulosis and inflammatory bowel disease, as discussed by Spiller (Spiller, 2001). Fibre has aroused interest in treatment of diarrhoea especially during enteral feeding, infectious diarrhoea and antibiotic related diarrhoea (Meier et al., 2003). Fibre has been used in enteral feeding to reduce diarrhoea via the mechanisms outlined above (Nakao et al., 2002). In children with infectious diarrhoea (Brown et al., 1993; Vanderhoof et al., 1997) and antibiotic associated diarrhoea (Burks et al., 2001) soy fibre significantly shortened duration of diarrhoea. Partially hydrolyzed guargum is known to prevent and treat diarrhoea (Homann et al., 1994) and constipation (Chuang et al., 1992), to reduce symptoms of irritable bowel syndrome (Parisi et al., 2002) and to prolong the colonic transit time (Meier et al., 1993).

Fibre supplementation pre and post methotrexate administration has been investigated in rats and it resulted in non-significant reduction in diarrhoea incidence (Funk & Baker, 1991b; Funk & Baker, 1991a; Chevreau & Funk-Archuleta, 1995). The dietary fibre chitosan was given to sarcoma bearing mice receiving 5-FU without affecting antitumour efficacy of 5-FU. Chitosan reduced leukopenia, prevented mucosal injury and delayed the onset of diarrhoea (Kimura & Okuda, 1999). The ability of fibre to reduce chemotherapy related gastrointestinal toxicity in humans has not been studied and the postulated synergistic effect of fibre and probiotics (Meier et al., 2003) has not been evaluated. Thus, LGG in combination with partially hydrolyzed guargum fibre is worthy of investigation in patients who receive 5-FU based chemotherapy, which commonly causes an abundance of oro-gastro-intestinal toxicity.
6. **AIMS OF THE PRESENT STUDY**

1. To find out the maximal tolerated dose (MTD) of 3-weekly administered raltitrexed that can be given in combination with carmofur orally days 1-14. Secondary aims were efficacy and toxicity of this treatment regimen.

2. To investigate clinical markers and the systemic inflammatory composite score (SICS; based on fever, fatigue, CRP, IL-6, IL-8, TNFα) in single raltitrexed, raltitrexed and carmofur and 5-FU based chemotherapy.

3. To compare toxicity profiles and adverse events of bolus and infusional 5-FU based treatment regimens in adjuvant treatment of colorectal cancer, and to study the effects of *Lactobacillus rhamnosus* and partially hydrolyzed guargum fibre.

4. To study the frequency and effect of 5-FU based treatment induced secondary hypolactasia and lactose intolerance on treatment related toxicities.
7. MATERIAL AND METHODS

Four studies, which are the basis of this thesis, were performed in the Department of Oncology at Helsinki University Central Hospital. For studies I and II 92 patients with metastatic colorectal cancer were entered between March 1998 and December 1999. For studies III and IV 160 patients with completely resected colorectal cancer were accrued between November 1997 and April 2001.

7.1. Study design, patient selection and evaluation

Study I was an open non-randomized phase I study, which was designed to define the MTD and recommended dose of raltitrexed combined with carmofur. Patients with advanced or metastatic colorectal cancer were eligible for inclusion using an arbour design manner (n=28).

Studies II were open and non-randomised, assessing the inflammatory reaction with raltitrexed chemotherapy in patients with metastatic colorectal cancer. A longitudinal study assessed fever, fatigue and any changes in the serum CRP level, as well as the effect of dexamethasone, in a longitudinal study in 52 patients receiving raltitrexed and carmofur. A cross-sectional study assessed the severity of systemic inflammation during one cycle of treatment with single raltitrexed (n=20), raltitrexed combined with carmofur (n=20) or during 5-FU based chemotherapy (n=20).

Study III was an open, randomized, two by three factorial design study in patients (n=150) with radically operated Dukes’ B, C or D colorectal cancer. Toxicity was evaluated in patients randomized to 5-FU and LV for 6 months according to the bolus Mayo schedule for 5 consecutive days repeated every 4 weeks or to the simplified de Gramont continuous infusion schedule with 48 hours treatment every 2 weeks. Rectal cancer patients also received loco-regional radiotherapy. A second randomization was performed to assess the efficacy of nutritional supplements on oro-gastro-intestinal toxicity and 1/3 of the patients were allocated to nutritional guidelines, 1/3 to nutritional guidelines and Lactobacillus rhamnosus GG (LGG) supplement and 1/3 to nutritional guidelines, LGG and partially hydrolyzed guargum fibre (Fibre).

Study IV assessed hypolactasia and lactose intolerance in patients who received similar adjuvant treatment as was given in study III. Lactose tolerance was studied prior to, during and after, six months of adjuvant treatment. Impact of hypolactasia on treatment tolerability and nutritional status was evaluated.
Table 14  Study design in studies I-IV

<table>
<thead>
<tr>
<th></th>
<th>Study I</th>
<th>Study II</th>
<th>Study II</th>
<th>Study III</th>
<th>Study IV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=28</td>
<td>n=52</td>
<td>n=60</td>
<td>n=150</td>
<td>n=150</td>
</tr>
<tr>
<td>Study design</td>
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<td>Open non-randomised study</td>
<td>Open non-randomised study</td>
<td>Randomised to adjuvant treatment *</td>
<td>Randomised to adjuvant treatment *</td>
</tr>
<tr>
<td>Study patients</td>
<td>Consecutive eligible patients</td>
<td>Consecutive eligible patients</td>
<td>Consecutive eligible patients</td>
<td>Consecutive eligible patients</td>
<td>Consecutive eligible patients</td>
</tr>
<tr>
<td>Chemotherapy regimen</td>
<td>Raltitrexed and carmustine</td>
<td>Raltitrexed and carmustine</td>
<td>Raltitrexed and carmustine (n=20), raltitrexed single (n=20) or 5-FU based (n=20)</td>
<td>5-FU and LV as bolus injection (Mayo) or as continuous infusion (simplified de Gramont)</td>
<td>5-FU and LV as bolus injection (Mayo) or as continuous infusion (simplified de Gramont)</td>
</tr>
<tr>
<td>Nutritional supplement</td>
<td></td>
<td></td>
<td></td>
<td>1/3 no, 1/3 LGG</td>
<td>1/3 no, 1/3 LGG</td>
</tr>
<tr>
<td>Study objective</td>
<td>Define MTD and recommended dose of combined raltitrexed and carmustine **</td>
<td>Study the frequency of fever, fatigue, and an increased serum CRP level and effect of dexamethasone longitudinally</td>
<td>Compare the severity of systemic inflammation during one cycle of treatment in three treatment regimens</td>
<td>Compare treatment tolerability in bolus vs. continuous modality and in nutritional supplement arms</td>
<td>Study frequency of secondary hypolactasia and its effects on treatment tolerability and nutritional status</td>
</tr>
</tbody>
</table>

*Patients were randomized, with the minimisation technique with 1/6 chance and stratification according to gender, primary and Duke's stage, to postoperative adjuvant chemotherapy in colorectal cancer or chemoradiation in rectal cancer if distal margin of tumour was below the peritoneal fold, with 5-FU and LV chemotherapy either as bolus injection (Mayo regimen) or continuous infusion (simplified de Gramont). In addition to nutritional guiding, the participants were randomly assigned to receive or not to receive in a 2:1 fashion *Lactobacillus rhamnosus* GG (LGG) or 1:2 fashion fibre-containing nutritional support, Novasouce GI control (Fibre).

** The dose of raltitrexed was first escalated from 1.5 mg/m² to 3.0 mg/m² while keeping the carmustine dose fixed at 300 mg/m², after which the raltitrexed dose was fixed at 3.0 mg/m² and the carmustine dose, in turn, was escalated. According to an arbour design, 1, 3, 6, 9, and 9 patients were entered at the dose levels 1 to 5, respectively. DLT was defined as dose level where >35% of patients got DLT. Recommended dose was MTD-I
Table 15  Inclusion criteria in studies I-IV

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
<th>Study I</th>
<th>Study II</th>
<th>Study III</th>
<th>Study IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>18-75</td>
<td>18-75</td>
<td>18-75</td>
<td>18-75</td>
</tr>
<tr>
<td>WHO PS</td>
<td>0-2</td>
<td>0-2</td>
<td>0-2</td>
<td>0-2</td>
</tr>
<tr>
<td>Colorectal cancer Disease stage</td>
<td>Measurable locally advanced or metastatic</td>
<td>Histologically confirmed colorectal cancer</td>
<td>Locally advanced or metastatic</td>
<td>Radically operated (R0) Dukes’ B, C or D. No distant metastases</td>
</tr>
<tr>
<td>Other cancer than CRC</td>
<td>No, except for adequately treated CIS of cervix or non-melanoma skin cancer</td>
<td>≤ One line of therapy</td>
<td>Allowed</td>
<td>No previous treatment</td>
</tr>
<tr>
<td>Previous cancer therapy</td>
<td>&gt;4.0 E9/l</td>
<td>&gt;4.0 E9/l</td>
<td>&gt;1.5E9/l</td>
<td>&gt;1.5E9/l</td>
</tr>
<tr>
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<td>&gt;100E9/l</td>
<td>&gt;100E9/l</td>
<td>&gt;100E9/l</td>
</tr>
<tr>
<td>Platelets</td>
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<td>&gt;100E9/l</td>
<td>&gt;100E9/l</td>
<td>&gt;100E9/l</td>
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<tr>
<td>Liver function tests</td>
<td>Alanine transerase or alkaline phosphatase ≤ 2.5 times ULN, unless liver metastases</td>
<td>Alkaline transerase or alkaline phosphatase ≤ 2.5 times ULN, unless liver metastases</td>
<td>Alkaline phosphatase ≤ 2.5 times ULN</td>
<td>Alkaline phosphatase ≤ 2.5 times ULN</td>
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<tr>
<td>Serum creatinine</td>
<td>&lt; ULN</td>
<td>&lt; ULN</td>
<td>&lt; 1.5x ULN</td>
<td>&lt; 1.5x ULN</td>
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<tr>
<td>Other diseases</td>
<td>Metabolic, neurologic or psychiatric disease that is incompatible with chemotherapy, serious thromboembolic event which is currently under treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fertility</td>
<td>No pregnancy or lactation, nor absence of adequate contraception in fertile patients</td>
<td>Raltitrexed +/- capecitabine. 5-FU based (Mayo, simplified de Gramont or capecitabine), No infections days 0-9 of the cross-sectional cycle.</td>
<td>No missing oral lactose tolerance tests. Received more than half of scheduled therapy</td>
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</tr>
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<td>Treatment factors</td>
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<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Written informed consent</td>
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Table 16  Patient evaluation in studies I-IV

<table>
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<tr>
<td><strong>At baseline</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anamnesis and Status*</td>
<td>Within 2 weeks</td>
<td>Within 2 weeks</td>
<td>Within 1 week</td>
<td>Within 3 weeks</td>
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<td>Imaging studies</td>
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<tr>
<td></td>
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<td>Imaging studies of metastatic sites within 4 weeks</td>
<td>Imaging studies to define disease stage</td>
<td>Imaging studies to exclude metastases</td>
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<tr>
<td>Serum biochemistry**</td>
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<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Other investigations</td>
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<tr>
<td></td>
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<td>QLQ©</td>
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<td>Oral lactose tolerance test and questionnaire, QLQ© and SGA©</td>
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<tr>
<td></td>
<td></td>
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<td>3 weekly</td>
<td>1 monthly</td>
</tr>
<tr>
<td><strong>During chemotherapy</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Anamnesis and status</td>
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<td>Yes</td>
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<td>Imaging of metastatic sites 9 weekly§</td>
<td>Imaging of metastatic sites 9 weekly§</td>
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<td>Prior to each cycle and at day 7-10</td>
<td>At day 7 and 14</td>
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<td>Prior to each cycle</td>
<td>Prior to each cycle</td>
<td>Prior to each cycle</td>
</tr>
<tr>
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<td>Prior to each cycle</td>
<td>Prior to each cycle</td>
<td>Prior to each cycle</td>
</tr>
<tr>
<td>Other investigations</td>
<td>Body temperature as needed.</td>
<td>SICS assessment at day 7 and 14. Infection diagnostics§§</td>
<td>Prior to each cycle based on patient diary</td>
<td>Oral lactose tolerance test and questionnaire at 4 months</td>
</tr>
<tr>
<td></td>
<td>2-3 monthly</td>
<td>2-3 monthly</td>
<td>Once</td>
<td>2-6 monthly</td>
</tr>
<tr>
<td><strong>After chemotherapy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anamnesis and status*</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Imaging</td>
<td></td>
<td></td>
<td>Imaging studies to define disease stage</td>
<td>Imaging studies to exclude metastases</td>
</tr>
<tr>
<td></td>
<td>Imaging studies of metastatic sites</td>
<td>Imaging studies to define disease stage</td>
<td>Imaging studies to exclude metastases</td>
<td>Imaging studies to exclude metastases</td>
</tr>
<tr>
<td>Full blood counts</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Serum biochemistry**</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Other investigations</td>
<td>QLQ©</td>
<td>QLQ©</td>
<td>SICS assessment and QLQ© and SGA©</td>
<td>Oral lactose tolerance test and questionnaire, QLQ© and SGA©</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Anamnesis and status include: Full medical history, height, weight, WHO PS and physical examination.
**Serum biochemistry includes: alkaline phosphatase, alanine and aspartate aminotransferase, bilirubin, creatinine, electrolytes (Na/K/Ca), ESR, CRP and carcinoembryogenic antigen.
***Toxicity was graded according to the Common Toxicity Criteria of the National Cancer Institute of Canada.
Patients had a personal booklet were they recorded and rated toxicity, general wellbeing, number of good quality of life days and resource utilization during each cycle of chemotherapy.
Table 17  Grading of the SICS sub scores in the cytokine study

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Grade 0</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever, °C*</td>
<td>&lt;37.1</td>
<td>37.1-38.0</td>
<td>38.1-40.0</td>
<td>&gt;40, &lt;24h</td>
<td>&gt;40, &gt;24h</td>
</tr>
<tr>
<td>Fatigue*</td>
<td>No change</td>
<td>Fall of 1 unit in the WHO PS</td>
<td>Fall of 2 units</td>
<td>Fall of 3 units</td>
<td>-</td>
</tr>
<tr>
<td>CRP, ng/L**</td>
<td>&lt;10</td>
<td>10-50</td>
<td>51-100</td>
<td>101-200</td>
<td>&gt;200</td>
</tr>
<tr>
<td>IL-6, ng/L**</td>
<td>&lt;10</td>
<td>10-50</td>
<td>51-150</td>
<td>151-300</td>
<td>&gt;300</td>
</tr>
<tr>
<td>TNFα, ng/L***</td>
<td>&lt;10</td>
<td>10-25</td>
<td>26-50</td>
<td>51-100</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

*According to National Cancer Institute of Canada common toxicity criteria  
** Modified from Takala et al., 1999

The lactose tolerance test was done after a 12-hour fast with an oral load of 50g of lactose with blood glucose concentration measured every 20 minutes for 40 minutes (Peuhkuri et al., 2000). A blood glucose level increase of less than 1.1mmol/L was considered to be compatible with the presence of hypolactasia. An increase of the blood glucose level of 1.1 to 1.6 mmol/L was considered as a borderline finding for lactase deficiency, and an increase of over 1.6 mmol/L as absence of lactase deficiency. A questionnaire for flatulence, abdominal distension, borborygmia, abdominal pain, eructation, headache, frequency and consistency of stool, was used for assessment of symptoms during the first 24 hours following the lactose tolerance test, and lactose intolerance was considered to be present when symptoms suggestive of lactose intolerance were present within the first 24 hours of lactose ingestion (Peuhkuri, 2000).

Response evaluation was performed according to the WHO criteria

Special attention was paid to infection diagnostics of febrile patients. In addition to careful clinical examinations, urine and blood samples were collected repeatedly for bacterial and fungal culture, antigen tests, and microbial serology. Conventional radiological imaging, ultrasound or computed tomographies were used to find infection foci.

7.2. Treatment regimens

In study I patients (n=28) received raltitrexed as a 15 to 30 minute intravenous infusion on day 1 of a 21-day cycle. Carmofur was administered orally 3 times a day on days 2 to 14 of the cycle. Cycles were repeated until disease progression or until dose limiting toxicity (DLT) occurred. If grade 4 neutropenia leukopenia or any other grade 3 or 4 toxicity was encountered, raltitrexed and carmofur doses were reduced by 50% in the next cycle. Subsequent doses could be increased to 75% of the starting dose provided that the toxicity did not recur. Treatment was discontinued for patients who had neurologic toxicity ≥grade 2 (except for constipation). Additional raltitrexed dose reductions were based on the worst grade of haematological toxicity and diarrhoea observed and based on the calculated creatinine clearance.

In study II, in the longitudinal component of the study patients (n=52) received raltitrexed and carmofur combined as in study I. The cross-sectional, one-cycle was component of the study comprised of 60 consecutive patients with metastatic colorectal cancer. Twenty patients were
treated with raltitrexed and carbomofur (they also participated in the longitudinal study); 20 patients received raltitrexed as a single-agent therapy; and 20 patients received 5-FU in combination with LV (the Mayo regimen or the simplified de Gramont regimen as described below), or single-agent carbomofur. Single-agent carbomofur was administered as 3-week cycles, where carbomofur 300 mg/m², divided in three daily doses, was first given orally for 14 days, followed by one week of rest.

In studies III and IV patients (n=150) received bolus 5-FU and LV according to the Mayo regimen, this consist of d,L-LV 20mg/m² (or l,L-LV 10mg/m²) and a 3-5 minute bolus of 5-FU 370-425mg/m² on days 1-5 of a 4 weekly cycle. In rectal cancer during chemoradiation, starting day 56, single 5-FU bolus 500mg/m² was given during days 1-3 on the first and fifth week of a 50.4 Gy pelvic radiation, in 1.8 Gy daily fractions over 5.5 weeks. Alternatively patients received their radiation 5 times 5 Gy over 5 days preoperatively without concomitant chemotherapy, followed by chemotherapy as in colon cancer. Continuous infusion 5-FU and LV was given as simplified de Gramont regimen with d,L-LV 400mg/m² (or l,L-LV 200mg/m²) as a 2 hour infusion followed by 5-FU bolus 400mg/m² and continuous infusion 3.0-3.6g/m², for 48 hours repeated every 14 days (de Gramont et al., 1997b). Rectal cancer patients received continuous infusion of 5-FU 225mg/m² (O’Connell et al., 1994) during the same radiation as described above. Total treatment time was 24 weeks with 6 cycles in bolus injection and 12 cycles in continuous infusion modality. LGG, *Lactobacillus rhamnosus GG* (ATCC 53103, Valio Ltd, Helsinki, Finland) was administered as gelatine capsules, which were swallowed as such or the content dissolved in cold drink, orally one capsule twice daily (dose was 1-2 x 10^10 colony-forming-units per day) during the six months of adjuvant treatment. Fibre-containing nutritional supplement (Novasource GI control®, Novartis Nutrition, Basel, Switzerland) was administered as 500 ml = 1 bottle, 550 kcal a day, guar gum 11g, during 8 days per month in the period of side effects and poor oral intake. In no supplements arm probiotics were prohibited during adjuvant treatment.

### 7.3. Patient demographics

Patients in all four studies had a median age close to 60 years, with a range from 31-77. The female and male ratio is equal across the studies. Overall patient characteristics are well balanced in the treatment arms. Individual data for each study is presented separately in Table 18.
Table 18  Patient characteristics in studies I-IV

<table>
<thead>
<tr>
<th>Study I</th>
<th>Study II Longitudinal</th>
<th>Study II Cross-sectional</th>
<th>Entire series</th>
<th>Study III Bolus injection</th>
<th>Study IV Cont. infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=28</td>
<td>n=52</td>
<td>n=20</td>
<td>n=150</td>
<td>n=75</td>
<td>n=75</td>
</tr>
<tr>
<td>Median age (range)</td>
<td>60 (40-73)</td>
<td>61 (41-75)</td>
<td>60 (31-75)</td>
<td>60 (31-75)</td>
<td>60 (31-75)</td>
</tr>
<tr>
<td>Male : Female</td>
<td>13 : 15</td>
<td>9 : 11</td>
<td>9 : 11</td>
<td>90 : 60</td>
<td>90 : 60</td>
</tr>
<tr>
<td>Cancer, Colon: Rectum</td>
<td>19 : 9</td>
<td>5 : 15</td>
<td>5 : 13</td>
<td>76 : 74</td>
<td>80 : 70</td>
</tr>
<tr>
<td>WHO PS 0 : 1 : 2</td>
<td>4 : 18 : 6</td>
<td>5 : 13 : 2</td>
<td>5 : 12</td>
<td>70 : 36</td>
<td>90 : 60</td>
</tr>
<tr>
<td>Duke’s B : C : D</td>
<td>Metastatic sites, 1:2:3+</td>
<td>5 : 10 : 5</td>
<td>5 : 10</td>
<td>37 : 38</td>
<td>80 : 70</td>
</tr>
<tr>
<td>Prior chemotherapy,</td>
<td>19 : 25 : 8</td>
<td>5 : 12 : 8</td>
<td>5 : 12</td>
<td>37 : 38</td>
<td>90 : 60</td>
</tr>
<tr>
<td>Total no of cycles</td>
<td>52</td>
<td>20</td>
<td>19 (1 infection)</td>
<td>20 : 42 : 13</td>
<td>40 : 26 : 24</td>
</tr>
<tr>
<td>Median no of cycles (range)</td>
<td>170</td>
<td>2.5 (1-7)</td>
<td>1 (1-10)</td>
<td>150 : 0 : 0 (Fibre was not feasible in 9)</td>
<td>15 : 0 : 0</td>
</tr>
<tr>
<td>Median dose intensity</td>
<td>Raltitrexed 94%</td>
<td>1 (1-10)</td>
<td>1 (1-10)</td>
<td>150 : 0 : 0 (Fibre was not feasible in 9)</td>
<td>15 : 0 : 0</td>
</tr>
<tr>
<td>5-FU based regimen</td>
<td>Carmofur 81%</td>
<td>80%</td>
<td>93%</td>
<td>150 : 0 : 0 (Fibre was not feasible in 9)</td>
<td>15 : 0 : 0</td>
</tr>
<tr>
<td>Bolus Mayo</td>
<td>Continuous Gramont</td>
<td>Oral Carmofur</td>
<td>Infection and antibiotics</td>
<td>Dexamethasone used</td>
<td>In fever prevention</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>8</td>
<td>9</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

7.4.  Statistical Methods

All statistical analyses were done with the StatView computer program, (SAS institute, Abacus concepts incorporation, Berkeley, CA, USA).

In Study I and II

In the longitudinal study part, repeated measures ANOVA statistics was used to examine changes in variables over time. In the cross-sectional study Mann-Whitney U-test or the Kruskall-Wallis test were used to compare groups for the difference between the day 0 (the baseline value before staring treatment) and day 7 (Δday 0 to 7) values, and the Wilcoxon signed rank test for paired
comparisons. All statistical significance tests were 2-tailed at the 5% level. Bonferroni correction for multiple comparisons was used when appropriate.

Study III and IV
Frequency tables were analyzed using the $X^2$-test, and non-normal distributions were analyzed with the Spearman rank correlation, Kruskall-Wallis, Wilcoxon signed rank and Mann Whitney’s U test. Repeated measures ANOVA and Kruskall Wallis test were used for treatment group comparisons. Odds ratio was calculated using the logistic regression model with added treatment modality (bolus vs. continuous), chemoradiation (chemotherapy only vs. chemoradiation) and nutritional supplement (No supplement vs. LGG or fibre). All P values were 2-tailed. Study was powered to detect a clinically relevant reduction in overall or diarrhoea frequency with a $\alpha = .05$ and $\beta = .80$, when $n=150$.

7.5. Ethical Considerations
The Ethical Review Board of the Helsinki University Hospital approved all four studies and a written informed consent was obtained from all patients. The National Agency for Medicines of Finland approved the use of the new drug combination raltitrexed and carmofur.
8. RESULTS

8.1. Phase I study with raltitrexed combined with carmofur (study I)

8.1.1. DLT in study with raltitrexed combined with carmofur

Twelve patients had DLT (Figure 2) of the 28 that were treated with the combination of raltitrexed and carmofur. The MTD was reached at the dose level 5 (raltitrexed 3.0 mg/m², carmofur 400 mg/m²), where 6 out of the 9 patients accrued had DLT. The recommended dose is thus raltitrexed 3.0 mg/m², carmofur 300 mg/m².

![Figure 2 DLT in raltitrexed and carmofur combined](image-url)
8.1.2. Adverse events in raltitrexed combined with carmofur

The spectrum of toxicity was somewhat unexpected. Several patients had severe fatigue, accompanied by anorexia, flu-like symptoms, and non-neutropenic fever, which was not responsive to antibiotics. The symptoms observed began 2 to 5 days after raltitrexed administration, and lasted for a median of 4 days. These symptoms occurred at all dose levels and constituted the DLT (>grade 2) in 10 patients (Figure 2).

In general, haematological toxicity was not severe. Grade 3 or 4 leukopenia and/or neutropenia lasting ≥2 days were observed in 3 (11%) patients, and 3 (11%) patients had febrile neutropenia (Figure 2). All but 1 (96%) of the 28 patients experienced at least grade 1 anaemia. Blood transfusions were given to 10 patients. Thrombocytopenia Gr 3 was observed in only 1 (4%) patient.

Diarrhoea and stomatitis Gr 3-4 occurred in 6 (21%) and 1 (4%) patient, respectively. Five (18%) patients had grade 3 nausea and vomiting. Neurological toxicity (dizziness, unsteady gait, dysarthria, amnesia) was detected in 3 (33%) patients at the highest dose level studied, with signs of leukoencephalopathy on computed tomography in 1 patient. All patients with neurologic symptoms had disease progression leading to death, but 2 patients could be followed up for 5 and 6 months after chemotherapy, during which time symptoms resolved almost completely. Other adverse events consisted of mild urinary frequency (n = 16), mild constipation (n = 9), partial alopecia (n = 1), elevated transaminase levels (n = 21) and transient grade 1 elevation of serum creatinine (n = 2).

8.1.3. Response evaluation in raltitrexed combined with carmofur

Of the 27 patients evaluable for response, 12 achieved a partial response (RR 44%; CI95%, 25-63%). In first-line patients (n = 22) RR was 50% (CI95%, 29-71%); stable disease (SD) was 36% and progressive disease (PD) 14%. The median TTP in first-line therapy was 5.2 months, and median OS 9.1 months. One of the 5 patients who received raltitrexed/carmofur as second-line treatment achieved a PR, whereas the remaining patients progressed. The median OS in this subgroup was 5.5 months.
8.2. Systemic inflammation during raltitrexed treatment  
(Study II)

8.2.1. Clinical markers of systemic inflammation

In the longitudinal study fever and/or fatigue occurred during 161 (75%) of the 214 cycles given to 52 patients (fever >37.0°C, n=39; >38.0°C, n=24).

Fatigue grade ≥1 was present in 49 (94%) patients accompanied by anorexia with slight nausea, and flu-like symptoms with headache and myalgia. Serum CRP level increased more than 10 mg/L in 49 (94%) and the erythrocyte sedimentation rate (ESR) more than 10 mm/h in 45 (87%) patients.

Body temperature followed a characteristic pattern, peaking 2 to 4 days after raltitrexed administration, and resolving usually within 4 days thereafter (range, 1 to 7 days). Fever was linked with a rise in CRP and ESR, which peaked on cycle days 7-8, and then returned to the precycle levels. The frequency of fever ($P=.0085$) and elevation of serum CRP ($P=.0014$) increased over the first 3 cycles of carboplatin plus raltitrexed treatment, whereas no marked change took place in the mean blood neutrophil levels ($P=.25$).

Figure 3  Changes in serum CRP level, body temperature, and blood neutrophil counts during one cycle of chemotherapy consisting either of raltitrexed plus carboplatin (n=20), single-agent raltitrexed (n=20), or a 5-FU based regimen (n=19)
In the one cycle cross-sectional study the post-infusion elevations of serum CRP and body temperature were highly significant as compared to the precycle values ($P=.0001$ and $<.0001$, respectively; Figure 3). CRP and fever peaked on cycle day 7 in carmofur+raltitrexed or single-agent raltitrexed arm (repeated measures analysis of variance $P=.021$ and $.084$, respectively), but no such elevations were observed in 5-FU based chemotherapy (Figure 3). No significant changes in neutrophil counts took place during the cycle ($P=.53$, Figure 3) and Gr 3-4 neutropenia was found in only 4 (7%) of the 59 patients, evenly distributed between treatment arms ($P=.89$). Elevated body temperature (range, from 37.0 to 40.1°C) occurred more frequently in patients who received single-agent raltitrexed or raltitrexed plus carmofur than in patients treated with 5-FU based regimens ($n=11$ vs. 11 vs. 1, $P=.0091$).

8.2.2. Effect of dexamethasone

In the longitudinal study, with combined raltitrexed and carmofur, 6 patients were given dexamethasone. A single dose of 10 mg intravenous dexamethasone given at the time of raltitrexed infusion failed to prevent the symptoms, but when 4.5 mg of dexamethasone t.i.d., tapered away over 10 days, was added, neither fever, fatigue, the flu-like symptoms nor elevations of CRP and ESR recurred during the subsequent chemotherapy cycles in all patients. Interestingly, 5 of the 6 patients evaluable for treatment response progressed after starting dexamethasone for prevention of the symptoms.

8.2.3. Systemic inflammatory composite score (SICS)

SICS calculated at baseline and at day 7 increased markedly in patients who received raltitrexed ($P<.0001$, Table 19). The median cycle day 7 score was higher than the precycle score in patients treated with either raltitrexed and carmofur (9.5 vs. 2.5, $P=.0003$) or single-agent raltitrexed (6 vs. 3, $P=.0004$), whereas in patients given 5-FU based regimens the score remained unaltered (1.5 vs. 1.5). The results remained essentially unchanged when patients with a documented infection during the cycle ($n=6$) and those who received dexamethasone as an antiemetic ($n=2$) were excluded (Table 19). Gender, age at diagnosis, the WHO PS, the number and extent of liver metastases were not significantly associated with the frequency of systemic inflammatory reaction.
Table 19  Markers of systemic inflammation in 59 patients in the cross-sectional study grouped by treatment arm. Medians on days 0 and 7 of the treatment cycle are shown.

<table>
<thead>
<tr>
<th>Marker §</th>
<th>5-FU</th>
<th>raltitrexed</th>
<th>raltitrexed + carmofur</th>
<th>P §§</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>day 0</td>
<td>day 0</td>
<td>day 7</td>
<td>day 7</td>
</tr>
<tr>
<td>CRP, mg/l</td>
<td>7</td>
<td>5.5</td>
<td>23</td>
<td>57</td>
</tr>
<tr>
<td>IL-6, ng/l</td>
<td>7</td>
<td>9</td>
<td>13</td>
<td>20</td>
</tr>
<tr>
<td>TNF-α, ng/l</td>
<td>9</td>
<td>9</td>
<td>9.5</td>
<td>10.5</td>
</tr>
<tr>
<td>IL-8, ng/l</td>
<td>7</td>
<td>7</td>
<td>22</td>
<td>21</td>
</tr>
<tr>
<td>Leukocytes, x10⁹/l</td>
<td>5.30</td>
<td>4.65</td>
<td>7.85</td>
<td>6.00</td>
</tr>
<tr>
<td>Neutrophils, x10⁹/l</td>
<td>3.19</td>
<td>3.22</td>
<td>4.52</td>
<td>4.18</td>
</tr>
<tr>
<td>Temperature, °C</td>
<td>36.5</td>
<td>36.4</td>
<td>36.5</td>
<td>37.25</td>
</tr>
<tr>
<td>Fever grade</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Fatigue QLQ</td>
<td>77.8</td>
<td>72.2</td>
<td>77.78</td>
<td>66.7</td>
</tr>
<tr>
<td>Fatigue grade</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>SICS exclusion §§</td>
<td>1.0</td>
<td>1.0</td>
<td>2.5</td>
<td>6</td>
</tr>
<tr>
<td>SICS</td>
<td>1.5</td>
<td>1.5</td>
<td>3</td>
<td>6</td>
</tr>
</tbody>
</table>

§ Markers in bold were used to calculate SICS (Table 17)  
§§ Kruskall-Wallis statistics for the difference between the pre-treatment value (day 0) and cycle day 7  
§§§ After exclusion of 2 patients with antiemetic dexamethasone and 6 patients with a documented infection, n=47  
* Wilcoxon signed rank p value <.05  
** Wilcoxon signed rank p value <.01  
*** Wilcoxon signed rank p value <.001  

8.3. Tolerability of adjuvant treatment in colorectal cancer (study III)

Adjuvant treatment was feasible with no deaths during therapy. Worst recorded toxicity of the patient was Gr 3-4 in 66% (98 out of 148) and commonly patients suffered from stomatitis (in 35%), diarrhoea (in 27%), neutropenia (in 22%), nausea (in 11%), and palmoplantar erythrodyssesthesia (PPE in 2%). Sixteen patients (11%) did not receive the full scheduled treatment, due to adverse events (7 cases, 6 in bolus arm), cancer relapse (5 cases) or non-cancer related diseases (4 cases). 5-FU dose intensity was higher in continuous (93% vs. 80%, P<.0001) than in bolus modality.

Thirty-six patients needed hospitalization during treatment for a total of 283 days, 205 days (median 10.5) in the bolus and 78 days (median 5) in the continuous arm (P=.045). VAD related complications took place in 10% (7 out of 73) of this patient series and led to 2 short hospitalizations and 2 VAD replacements (3%). Complications at insertion occurred in 7% and during VAD use in 3%. The number of outpatient visits to the clinic, including chemotherapy, heparinization of VAD, radiation, scheduled appointments, emergency and laboratory visits, was significantly lower in the continuous infusion arm, (median, 13 in the continuous arm vs. 36 in the
bolus arm, *P* < .0001). Only 6 patients (8%) came to the clinic for discontinuation of continuous infusion and heparinization of the VAD, whereas 61 (86%) were self-sufficient and 6 (8%) had help of friends or family.

### 8.3.1. Comparison of 5-FU and LV as bolus injection or continuous infusion

Overall toxicity was milder in patient receiving continuous infusion than following bolus administration, Gr 3-4 in 45% (33 out of 73) vs. 87% (65 out of 75) even when VAD related toxicity was included (*P* < .0001, OR 0.14, CI95% 0.06-0.31; Figure 4).

![Figure 4 Adverse events in study III. Above zero is bolus and below continuous modality, divided by chemotherapy and chemoradiation.](image)

Bolus injection treatment induced significantly more diarrhoea (Gr 3-4 in 44% vs. 10%, *P* < .0001; OR 0.11, CI95% 0.04-0.29), stomatitis (Gr 3-4 in 57% vs. 12%, *P* < .0001; OR 0.09, CI95% 0.04-0.22) and neutropenia (Gr 3-4 29% vs. 14%, *P* = .029; OR 0.38, CI95% 016-0.90) than continuous infusion treatment (Figure 4). Severe hand-foot syndrome was uncommon in both arms (Gr 3 4% vs. 0%, *P* = .98), but mild vs. moderate hand-foot syndrome was more common in the continuous infusion regimen (*P* < .0001; OR 5.2, CI95% 2.3-11.8, Figure 4). Alopecia Gr 2 was encountered in 24 patients (16%, *P* = .59 in comparison bolus vs. continuous arm). Anaemia (Gr 2-4 in 3%) and thrombocytopenia (Gr 2-4 in 2%) were rare with no difference between the treatment arms (*P* = .98 for both comparisons).

Every treatment cycle the patients recorded the number of “good quality of life days” in their diary, the maximum score being 168. The median number of “good quality of life” days was 102.
(60.7%) in the bolus arm and 125 (76.8%) in the continuous infusion arm (P<.0001; Figure 5). The correlation between the percentage “good quality of life days” as recorded by the patient and the worst toxicity as recorded by the physician was highly concordant (R= .272, P=.001).

Figure 5 The percentage of “good quality of life days” (with normal pre-treatment activity level and without impaired quality of life as recorded by patient in a diary) during adjuvant treatment, presented for each treatment arm (P<.0001).

Chemoradiation related adverse events were recorded during radiotherapy (approximately days 56-94 of the 168 day adjuvant treatment) and 3 months after finishing radiation. Significantly more toxicity was encountered in the bolus than the continuous infusion arm (Gr 3-4 in 59% vs. 33%, P=.0024). The most severe nonhaematologic toxicity was gr 3-4 in 56% of the bolus and in 30% of the continuous infusion patients (P=.048). Bolus injections during chemoradiation induced more diarrhoea than continuous infusion (P=.0039).

8.3.2. Toxicity and nutritional LGG and fibre supplements

Compliance with LGG therapy was excellent; all patients consumed their LGG doses timely. Fibre addition was not feasible in 9 cases (18%) due to taste aversion, and these patients had LGG only instead. All analyses were based on intention to treat. Bolus and continuous infusion treatments were evenly distributed between the two nutritional arms (P=.98). The 97 patients who received LGG had less diarrhoea than the 51 without LGG (Gr 3-4 in 21% vs. 37%, P=.027). Similarly, abdominal discomfort (with flatulence, borborygma and abdominal distension) was milder in patients with who consumed LGG supplement (Gr 2-3 in 2% vs. 12%, P=.025). Addition of fibre and LGG to 1/3 of patients did not reduce gastrointestinal toxicity additionally compared with patients receiving no supplements and LGG (P=.13). Diarrhoea Gr 3-4 frequency was 25% vs. 30% (P=.24).
8.4. Lactose intolerance during chemotherapy and –radiation (study IV)

The result of the lactose absorption test was abnormal in 36 (24%) of the 150 patients investigated prior to therapy and 97% had mild to severe gastrointestinal symptoms during the lactose tolerance test and on lactose consumption. Nine of the 18 (12% of all 150 patients investigated) patients, who had a borderline finding in the lactose absorption test (1.1-1.6 mmol/L), had mild to moderate gastrointestinal symptoms during the test. None of the 96 (64%) patients, who had normal lactose tolerance test result, had moderate or severe symptoms following lactose ingestion.

Fifty-three (35%) of the patients had hypolactasia during chemotherapy as compared with 24% at baseline ($P<.0001$, Figure 6). Fifty (94%) of the 53 patients with an abnormal test during treatment had mild, moderate or severe gastrointestinal symptoms following the test. This secondary hypolactasia was reversible upon discontinuation of therapy, with hypolactasia in 27% two months and 25% six months after treatment. In 2 secondary hypolactasia persisted when treatment continued beyond 6 months due to recurrent cancer.

![Figure 6](image)

**Figure 6** Hypolactasians, borderlines and normolactasians before, during and after treatment, also divided in bolus, continuous, chemotherapy and chemoradiation subgroups. Wilcoxon signed rank comparison before and during treatment.
The frequency of lactose absorption impairment during chemotherapy was more common in the continuous infusion than in the bolus injection arm ($P=.006$). In bolus modality, 19% (14 out of 73) had hypolactasia before and 25% during chemotherapy (18 out of 73, $P<.0001$), and in continuous modality 29% (22 out of 77) had hypolactasia before and 45% (35 out of 77, $P<.0001$) during treated (Figure 6). Addition of radiotherapy did not affect secondary hypolactasia figures ($P=.80$), neither did *Lactobacillus GG* ($P=.95$) nor did fibre containing nutritional support ($P=.87$).

### 8.4.1. Treatment tolerability in lactose intolerance during adjuvant treatment

Patients with hypolactasia had significantly worse flatulence (including borborygmmia and bloating) than normolactasians (Gr 1-2 in 70% vs. 62%, $P=.0004$) and this was significant for both bolus injection ($P=.0064$) and continuous infusion subgroups ($P=.018$). In the bolus injection subgroup patients with hypolactasia in the midst of adjuvant treatment had significantly worse diarrhoea during treatment (Gr 3-4 in 44% vs. 36%, $P=.014$). This difference was not significant for the entire group ($P=.22$) or the continuous infusion subgroup ($P=.18$). Similarly, hypolactasia at baseline predicted worse diarrhoea during treatment, (Gr 3-4 in 64% vs. 32%, $P<.0001$) in the bolus injection subgroup, but not in the continuous infusion subgroup ($P=.46$). Stomatitis was not significantly linked to hypolactasia during the treatment ($P=.66$).

### 8.4.2. Nutritional status in lactose intolerance during adjuvant treatment

Nutritional status was classified according to the SGA A, B or C depending on whether patients were well nourished, moderately malnourished or severely malnourished. Hypolactasia at baseline ($P=.10$) or post treatment ($P=.32$) was not associated with nutritional status. Hypolactasia in midst of treatment was associated with a worse nutritional status (SGA B or C in 42% of hypolactasians, 28% of borderlines and 18% of normolactasians, $P<.0001$). This association was significant in all subgroups, in bolus injection treatment (SGA B or C for hypolactasians, borderlines and normolactasians were 67% vs. 38% vs. 21% respectively, $P<.0001$), in continuous infusion treatment (SGA B or C in 29% vs. 21% vs. 13%, $P=.0003$), in chemotherapy only patients (SGA B or C in 28% vs. 16% vs. 13% of normolactasians, $P<.0001$) and in chemoradiation patients (SGA B or C in 62% vs. 46% vs. 30%, $P=.0041$). SGA grade was more impaired during treatment in patients with worse diarrhoea (SGA B or C in diarrhoea Gr 0-1 vs. 2-4 was 10% vs. 45% in $P<.0001$) and stomatitis (SGA B or C in stomatitis Gr 0-1 vs. 2-4 was 22% vs. 63%, $P<.0001$).
9. DISCUSSION

9.1. What does this mean for a clinician?

I am challenged with a case in March 2004. The patient is a 60-year old male with a previously unremarkable history, without regular medication. He is referred to me after right-sided hemicolecctomy. Pathology shows a moderately differentiated adenocarcinoma of the coecum extending trough the muscularis propria with 3 positive lymph nodes out of 12 investigated. The staging is pT3N1M0, MAC C2, Stage III or Dukes’ C.

Based on the knowledge from randomized studies this patient could benefit from adjuvant chemotherapy (Table 1). The overall effect on relapse is about 30-41% and on survival 22-34% with 6 months of 5-FU + LV or 12 months of 5-FU + levamisole compared with surgery alone. The absolute survival benefit is about 10-15%. Later studies indicate that there is no significant survival benefit from adding levamisole to 5-FU with or without LV (Haller et al., 1997; Tepper et al., 1997a; O'Connell et al., 1998; Wolmark et al., 1999; Anonymous, 2000). In colon cancer there is no conclusive evidence for the use of radiotherapy, peritoneal chemotherapy nor portal vein infusion in the adjuvant setting (Chapter 5.1.5.2). In rectal cancer preoperative radiotherapy with or without chemotherapy and postoperative chemoradiation have given survival benefit (Table 2, Table 3, Table 4). Thus I recommend him 6 months of adjuvant chemotherapy.

9.1.1. Safety and tolerability of adjuvant chemotherapy

Addressing safety and tolerability in the adjuvant setting is essential, when giving a potentially cured patient treatment, just in case he would be the one in ten benefiting. The mortality rate in adjuvant chemotherapy has ranged from 0-1.9% (Table 20) (Anonymous, 1999a; Study III). A mortality rate of ≤0.5% is acceptable to me. In an adjuvant study treatment related mortality was found in 1.9% of raltitrexed patients (Anonymous, 1999a). Therefore, raltitrexed chemotherapy cannot be recommended in the adjuvant treatment due to missing survival data after early closing of the pivotal study and the question marks on tolerability.
Table 20  Comparison of safety and efficacy of adjuvant 5-FU and LV given as bolus injection or continuous infusion

<table>
<thead>
<tr>
<th>Event Gr 3-4%</th>
<th>Study</th>
<th>(Labianca et al., 1995) n=444</th>
<th>(Andre et al., 2003) n=902</th>
<th>(Study III) n=150</th>
<th>(de Gramont et al., 2003) n=2246</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Monthly 5FU+LV short infusion</td>
<td>Monthly5FU+LV bolus short inf +LV</td>
<td>Monthly5FU bolus bolus +/- radiation</td>
<td>Monthly 5FU bolus bolus +cont inf +LV</td>
<td>2 weekly 5FU bolus bolus +cont inf +LV +OX</td>
</tr>
<tr>
<td>Mucositis</td>
<td>4-5</td>
<td>37</td>
<td>7  2</td>
<td>57  12</td>
<td>2  3</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>4-16</td>
<td>47</td>
<td>9  4</td>
<td>10  12</td>
<td>2  6</td>
</tr>
<tr>
<td>Nausea</td>
<td>5-9</td>
<td>18</td>
<td>3  1</td>
<td>14  29</td>
<td>5  41</td>
</tr>
<tr>
<td>Leukopenia/Neutropenia</td>
<td>Not recorded</td>
<td>Not recorded</td>
<td>1  2</td>
<td>19 (all) 8 (all)</td>
<td>0  1</td>
</tr>
<tr>
<td>Infection</td>
<td>1</td>
<td>76</td>
<td>16 7</td>
<td>19 (all) 8 (all)</td>
<td>0  1</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>Not recorded</td>
<td>Not recorded</td>
<td>0  0</td>
<td>15  17</td>
<td>5  5</td>
</tr>
<tr>
<td>PPE</td>
<td>1-4</td>
<td>16</td>
<td>26 11</td>
<td>87  45</td>
<td>5  5</td>
</tr>
<tr>
<td>Alopecia Gr 2-3</td>
<td>0.3?</td>
<td>0.5  0.9</td>
<td>0.5  0.9</td>
<td>0.5  0.9</td>
<td>0.5  0.9</td>
</tr>
<tr>
<td>Other toxicity</td>
<td>Overall Hospitalization</td>
<td>Fatalities</td>
<td>3 year</td>
<td>DFS %</td>
<td>OS %</td>
</tr>
</tbody>
</table>

The combinations of 5-FU with LV or levamisole have similar toxicity profiles (Table 10) (Porschen et al., 2001). It is evident that true bolus regimens of 5-FU and LV (Mayo regimen with 5-FU of 425mg/m²) lead to greater toxicity than regimens with smaller doses as short infusions (Table 20) (Labianca et al., 1995). In metastatic disease, the 5-FU administration time of 2-4 vs. 10-20 minute infusion has had a favourable impact on efficacy (Glimelius et al., 1998). Thus, I prefer true boluses even in adjuvant treatment. Other potential factors that affected the tolerability in the IMPACT substudies are the thoroughness of reporting the toxicity and frequency of toxicity assessment. The NCI-CTC substudy, with higher toxicity in the Mayo regimen, had weekly full blood cell counts and weekly toxicity assessment done by nurses or study personnel (Labianca et al., 1995).

In our study that compared 5-FU and LV (Mayo regimen) with continuous infusion 5-FU and LV (simplified de Gramont schedule) the toxicity level was higher than in some other studies. An
overall Gr 3-4 toxicity of 87% was found in the bolus and 45% in the continuous arm (Study III). The study with 150 patients was powered to detect a clinically relevant 20 to 25% absolute reduction in Gr 3-4 toxicity between treatment arms. For example, the GERCOR co-ordinated study patients had an overall toxicity of 26% vs. 11%, when FULV (5-FU, lower dose and short infusion, and LV) was compared with LV5FU2 (48h bolus and infusion of 5-FU/LV) (Andre et al., 2001). Thus true bolus administration, with Mayo doses, exaggerates toxicity. Secondly, patients’ self-reporting during chemotherapy may increase doctor monitored toxicity. Thirdly, the common toxicity criteria used also have an impact on the toxicity reported. For example stomatitis grade 3 is defined differently. In all classifications commonly used, patients have painful erythema or ulcers, but the consequences of these vary, in the NCI-C CTC system (used in the present studies) patients “cannot eat”, in the WHO criteria “needs liquid diet”, and in the NCI CTC “requiring iv hydration”. Fourthly, 54 patients of the 148 studied had radiotherapy combined with chemotherapy, which lead to a non-significantly increased overall toxicity and more diarrhoea (Study III). Finally, the present patients had full blood cell counts taken once a week or fortnight, or even more often when clinically relevant. In many studies the overall worst toxicity recorded per patient is not denoted and for example would probably be equivalent in the Mayo arms in the IMPACT sub-study and in our study (Labianca et al., 1995; Study III).

In study III continuous infusion was better tolerated than bolus injection modality. The toxicity profile of the continuous regimen, with significantly less diarrhoea, stomatitis and neutropenia, was anticipated based on earlier findings (de Gramont et al., 1997a; Andre et al., 2003; Kohn et al., 2003; Saini et al., 2003). PPE was not of concern in this study and was easily manageable, as in other continuous regimens (de Gramont et al., 1997a). The Gr 3-4 diarrhoea frequency during chemoradiation was higher in the bolus arm than previously reported (48% vs. 14-39%) (O'Connell et al., 1994; Tveit et al., 1997; Wolmark et al., 2000), and similar in the protracted arm (22% vs. 24%) (O'Connell et al., 1994). This subgroup analysis was predefined but due to small sample size (n=54), no conclusive evidence can be drawn from this material. The possible interaction was accounted for in choice of logistic regression as statistic method with added treatment modality, whether radiotherapy was given or not and nutritional supplement status added in the model.

The compliance rate in study III was 89%, as compared with 58-83% (Fisher et al., 1988; Laurie et al., 1989; Wolmark et al., 1993; Andre et al., 2003). In the continuous arm the median dose
intensity was high (93%, which compares well with studies using prolonged 5-FU administration; ≥90% (Andre et al., 2001)) and in the bolus arm significantly lower (80%, ≥70% reported in earlier studies using 5-FU bolus or short infusions (Labianca et al., 1995; O'Connell et al., 1998; Wolmark et al., 2000; Saini et al., 2003)).

In comparison with published protracted venous infusion and capecitabine safety data, continuous infusion administration compares well (Table 11). All three modalities appear to be better tolerated than the bolus 5-FU and LV comparison arm. Grade 3-4 diarrhoea is found in 4-13%, stomatitis in 2-4% and neutropenia in 1-26% (Table 11). The survival with 5-FU with or without LV as a bolus regimen vs. protracted or continuous infusion is equivocal (Andre et al., 2003; Saini et al., 2003) and first capecitabine survival data are awaited in 2004. The survival data from the present study will be analyzed autumn 2004 when median follow up is 5 years and minimum follow up 3 years, but this study is not powered to detect any significant differences in treatment efficacy. The combination of oxaliplatin and 5-FU and LV given as continuous infusion has been investigated in the adjuvant setting and exaggerates toxicity, especially diarrhoea, neutropenia and neurotoxicity, compared with continuous infusion, but gives an absolute 5% 3-year disease free survival benefit (Andre et al., 2004).

Taking together, based on data available about efficacy and tolerability of adjuvant chemotherapy, I would suggest 6 months of chemotherapy with 5-FU and LV for a patient with Dukes’ C colon cancer. I would also discuss that the combination with oxaliplatin could be of additional benefit, but with higher toxicity, especially neurologic sequelae. The choice of 5-FU and LV regimen (Mayo vs. continuous de Gramont) would be thoroughly discussed. I think the continuous infusion regimens can replace the Mayo regimen, due to the favourable toxicity profile seen throughout. The confirmatory efficacy results of the PETACC, and other ongoing studies are awaited with interest. Capecitabine results are awaited and probably bolus 5-FU and LV can be replaced by oral therapy. The forthcoming studies with irinotecan and oxaliplatin in combinations and EGFR, VEGF or VEGFR inhibitors will probably give additional survival benefit in Dukes’ B and C colorectal cancer, possibly with exaggerated toxicity compared with 5-FU and LV as continuous infusion.
9.1.2. Quality of life and resource utilization

In the present study a surrogate endpoint for quality of life was used. Patient well being during therapy was assessed using a diary, where patients recorded subjective number of good quality of life days during each chemotherapy cycle. These days were defined as days with normal pretreatment level of activity and without impairment of quality of life due to treatment. In comparison 61% vs. 78% were classified as “good days” in the bolus and continuous arms, respectively. The number of “good days” correlated well with the worst overall toxicity, suggesting that this measure was a clinically relevant endpoint for wellbeing. In the study with protracted venous infusion compared with monthly 5-FU and LV bolus(?), the quality of life was assessed, and it showed a significant difference at 1 and 2 weeks when toxicity was bothering patients in the bolus arm, but further on did not show a difference when measured at toxicity resolution prior to each bolus cycle (Saini et al., 2003).

The complication frequency of VADs and tunnelled exteriorized catheters is of concern in continuous infusion. In large patient series the replacement frequency of VADs has been 2.9% and the complication frequency 17-31% in VADs and 58% with tunnelled exteriorized catheters (Carde et al., 1989; Gleeson et al., 1993; Kock et al., 1998). In study III any VAD complication was seen in 10%, of which 4% were serious and leading to catheter replacement in 3%, figures lower than reported by Saini in a subgroup of Hickman line patients (Saini et al., 2003). The low complication rate may be attributed to experienced anaesthesiologists, who inserted the VADs and skilful use of the port.

Number of outpatient visits to the clinic (median 13 vs. 36), was significantly lower in the continuous infusion arm than in the bolus arm. Only 8% of patients returned to clinics to discontinue continuous infusion, showing that patient education is feasible with low VAD complication rates. Any in-bed hospitalizations were needed only in 24% of patients. More patients needed hospitalization in the bolus arm and for longer periods of time (total 205 days in bolus vs. 78 days in continuous). Most of the patients suffered from infections (56%).

The more favourable toxicity profile, fewer outpatient and inpatient hospital visits, a greater number of good quality of life days and a low frequency of VAD complications suggest that continuous infusion are preferable to bolus injection administration to the average patient, as efficacy is not compromised. In combination with oxaliplatin the continuous infusion schedule
would be chosen. A better assessment of quality of life, cost-benefit in non-monthly-bolus regimens and VAD complications prospectively, is needed.

9.1.3. Lactose intolerance during chemotherapy

Prior to chemotherapy I would question the patient about hypolactasia and lactose intolerance. For a Finnish male the risk of hypolactasia is 17-18% (Sahi, 1974; Sahi, 1994). This figure varies worldwide from 5% in the Scandinavian countries to 100% (Scrimshaw & Murray, 1988; Sahi, 1994). Symptoms of lactose intolerance include loose stools, abdominal bloating, pain, flatulence, nausea, diarrhoea and borborygmia. These symptoms may be somewhat milder in a male. The down regulation of the lactase enzyme is genetically determined, and primary hypolactasia can be diagnosed with a genetic test (Kuokkanen et al., 2003).

The frequency and clinical significance of chemotherapy-induced secondary lactose intolerance have been infrequently studied. Hypolactasia can be easily diagnosed using simple lactose tolerance tests, and it can be efficiently treated with dietary counselling and avoidance of lactose or with oral lactase supplements. Although many commonly used anti-cancer agents, notably 5-FU and its derivatives, commonly cause diarrhoea and other gastrointestinal complaints as an adverse effect, chemotherapy-induced lactose intolerance often remains undiagnosed and untreated. This may result in chemotherapy dose reductions, treatment interruptions, or in the worst scenario in discontinuation of potentially life-saving therapy. Prospective intervention studies where avoidance of lactose or the use of lactase supplements are investigated, as a strategy to improve treatment tolerance, are thus warranted.

5-FU has been linked with small bowel toxicity both in humans (Fata et al., 1999; Sartori et al., 2000) and in animals (Hirata & Horie, 1999b; Hirata & Horie, 1999a), and 5-FU-based chemotherapy may result in a low villus height to crypt depth ratio (Decker-Baumann et al., 1999). Lactose malabsorption is the functional consequence of enzymatic derangement in the microvilli of the small intestine (Welsh & Porter, 1967; Bayless et al., 1968; Villako & Maaroos, 1994). Reversibility of secondary hypolactasia is related to the repair of mucosal injury in the microvilli, and production of new mucosa-linked enzymes, including lactase (Welsh & Porter, 1967; Villako & Maaroos, 1994).
In study IV the frequency of hypolactasia before adjuvant 5-FU based treatment was 24%. The frequency of hypolactasia during treatment found by us (35%) is similar to that reported by others in adults receiving various chemotherapy regimens for different histological types of cancer (30%) (Parnes et al., 1994) or in children receiving chemotherapy (33% to 55%) (Hyams et al., 1982; Halton et al., 1993; Pettoello-Mantovani et al., 1995). Importantly, abnormal lactose absorption test results were closely associated with clinical lactose intolerance, poor treatment tolerability and with an impaired nutritional status during chemotherapy. Although the simplified de Gramont regimen was generally associated with less gastrointestinal symptoms than the Mayo regimen, chemotherapy-associated hypolactasia was more commonly detected in patients treated with continuous 5-FU infusions, than among those who received 5-FU as boluses only, 45% vs. 25%, respectively). Chemotherapy-associated hypolactasia and lactose intolerance were transient and fully reversible in the great majority of patients.

The timing of the lactose absorption test in relation to chemotherapy infusions may influence the imbalance in frequency of hypolactasia detected. In one small study on 27 adult patients, who were treated with various cancer chemotherapy regimens, lactose intolerance was found to be reversible in a few patients within the first few weeks following chemotherapy (Parnes et al., 1994). In the present series the oral lactose absorption test was done approximately 12 days after the last day of chemotherapy in the continuous group and 23 days after the last bolus administration. A rapid reversal of lactose malabsorption coupled with regeneration of the intestinal epithelium following chemotherapy may account for the apparent discrepancy between a better general gastrointestinal tolerability of the simplified de Gramont regimen as compared with the Mayo regimen (de Gramont et al., 1997a; de Gramont et al., 1997b; Andre et al., 2001) despite the higher frequency of hypolactasia detected in patients treated with the de Gramont regimen.

Hence, the hypolactasia frequency figures reported by us probably need to be viewed as minimum frequencies due to the study design, and transient hypolactasia may be more common than detected by us especially in patients treated with the Mayo regimen.

My normolactic patient is at risk (at least 6-16% absolutely) of developing hypolactasia during adjuvant 5-FU based chemotherapy. Secondary hypolactasia during therapy is probably symptomatic (94%) in this patient, possibly leading to nutritional impairment, abdominal discomfort with flatulence, borborygma and bloating, and to diarrhoea following bolus 5-FU administration. The impact of dietary lactose restriction or lactase supplements on the frequency
and severity of gastrointestinal toxicity and decreased nutritional status has not been formally studied, but might improve tolerability of treatment. A lactose absorption test should be carried out in my patient, treated with 5-FU-based chemotherapy, when symptoms compatible with lactose intolerance occur.

9.1.4. Probiotic Lactobacillus GG and guargum fibre supplement

The present patient has been subjected to major abdominal surgery and administration of prophylactic intravenous antibiotics. His intestinal microbial flora is thus possibly disturbed. Chemotherapeutic agents may further affect the intestinal microbial balance. The effect of chemotherapy on the microbial flora has been insufficiently studied. The association between the flora and treatment tolerability in humans has not been adequately assessed and should be addressed in future studies. In a study on rats, intravenous 5-FU administration increased the number of facultative anaerobes and gram-negative rods in the oral cavity and in the large intestine and also bacterial translocation to draining lymph nodes. These microbial changes were in part counteracted by Lactobacillus plantarum probiotics administration. Lactobacillus improved food intake and maintained body weight gain (Von Bultzingslowen et al., 2003).

The effect of nutritional supplements on treatment tolerability has not been extensively studied in conjunction with chemo- or radiotherapy. Probiotics, especially Lactobacillus rhamnosus GG, have been thoroughly evaluated in a variety of intestinal disorders. They alleviate viral, Clostridium difficile, antibiotic related and travellers diarrhoea, milk allergy etc by multiple mechanisms (Ouwehand et al., 2002; Vaarala, 2003). Chemo- or radiotherapy induced mucosal damage leads to gastrointestinal symptoms, of which diarrhoea is most prominent. In three studies with probiotics during radiotherapy a significant reduction in diarrhoea frequency has been noted (Salminen et al., 1988; Urbancsek et al., 2001; Delia et al., 2002b). In the present study patients who were randomized to receive LGG supplements had significantly less diarrhoea and abdominal discomfort including borborygmia, abdominal distension and flatulence (Study III). The factorial design of this study makes individual treatment allocation groups quite small. The assumption of no interactions was made in power calculations and n=150 was found sufficient for LGG and treatment arm comparisons. The statistics were performed with multiple logistic regression, where interaction is taken into account and bolus vs. continuous, chemotherapy vs. chemoradiation, and LGG or not were added to the model.
The combination of hydrolyzed guargum fibre and lactobacilli have been suggested to be effective in controlling diarrhoea (Meier et al., 2003). One third of patients in study III received this combination, but no further reduction in the gastrointestinal toxicity was noted when compared to LGG or no supplements. The daily dose of 11g hydrolyzed guargum fibre during 8 days a month was probably too low, timing of guargum dosage on non-chemotherapy days (due to risk of taste aversion) may have been suboptimal, and administration was not feasible in 18%. Nutritional supplement containing sufficiently hydrolyzed guargum intended for oral and not enteral use might be better accepted by the patients, but it was not available at the time of the study. The increased flatulence caused by guargum may also limit its clinical use in this setting (Homann et al., 1994; Patrick et al., 1998). The lack of supplementary efficacy in combination with LGG makes me await further studies before recommending its routine use.

I would discuss administration of prophylactic Lactobacillus GG during chemotherapy, because our study showed a significant reduction in diarrhoea and abdominal discomfort frequency. The evidence so far has shown very little risk for LGG induced infections in constantly monitored bacteraemia series in Finland (Saxelin, 1996; Saxelin et al., 1996; Salminen et al., 2002; Study III). LGG is available in many countries worldwide in a variety of economical administration modes and appears to be safe when administered to patients who receive 5-FU and LV chemotherapy. However, further studies that verify its regarding efficacy and safety are still needed.

9.1.5. Combination chemotherapy in metastatic colorectal cancer

My patient had been assessed prior to surgery with a chest X-ray and an abdominal ultrasound. Postoperatively, markers did not normalize fully (CEA 8) and I decided to ask for a whole body computer tomography, which showed multiple, bilobar, and small liver metastases. Fine needle biopsy verified adenocarcinoma metastases in the liver. Physical examination, the full blood cell count and serum biochemistry were within normal limits. The WHO PS was 0 and he had no weight loss. I was now challenged with first-line treatment for a previously healthy 60-year male with metastatic colon cancer.

Several studies have shown a 6-month survival benefit from first-line 5-FU based chemotherapy as opposed to best supportive care (Glimelius et al., 1992; Scheithauer et al., 1993; Glimelius et al., 1995) (Figure 7). Combination chemotherapy consisting of irinotecan or oxaliplatin and 5-FU and
LV have shown survival benefit over 5-FU and LV alone (Chapter 5.1.4). In these studies prognostic factors associated with a poor outcome have been WHO PS >0, synchronous metastases, ≥2-3 metastatic sites, elevated serum alkaline phosphatase, bilirubin or lactate dehydrogenase, age <58-65 years, leukocytosis, anaemia, weight loss >5% and no irinotecan/oxaliplatin in the treatment regimen (de Gramont et al., 2000; Douillard et al., 2000; Saltz et al., 2000). Thus, my patient has few adverse prognostic features and most probably nonresectable metastases, and 5-FU based chemotherapy without irinotecan or oxaliplatin can be discussed as the first-line therapy.

![Graph showing overall survival in randomized trials on metastatic colorectal cancer. BSC= best supportive care, FULV= 5-FU and LV, CDDP= cisplatin, MTX= methotrexate, LV5FU2= bolus plus continuous 5-FU and LV given over 48 hours, ILF= irinotecan, FULV bolus given weekly, FOLFIRI= irinotecan plus FULV given over 48 hours, chrono= chronomodulated chemotherapy, MMC= mitomycin C.](image)

*Figure 7  Overall survival in randomized trials on metastatic colorectal cancer. BSC= best supportive care, FULV= 5-FU and LV, CDDP= cisplatin, MTX= methotrexate, LV5FU2= bolus plus continuous 5-FU and LV given over 48 hours, ILF= irinotecan, FULV bolus given weekly, FOLFIRI= irinotecan plus FULV given over 48 hours, chrono= chronomodulated chemotherapy, MMC= mitomycin C.*

Median overall survival has been the traditional endpoint in studies of metastatic colorectal cancer (Figure 7). At present efficient second- and third-line treatments have an impact on OS, and TTP
in conjunction with RR might also be a valid primary end-point (Cunningham et al., 2003; Di Leo et al., 2003; Johnson et al., 2003; Tournigand et al., 2004)

The choice of the 5-fluoropyrimidine is mostly based on tolerability. Continuous, chronomodulated and protracted infusion modalities have generally been better tolerated than intravenous bolus administrations, and have been associated with some more responses, but rarely with TTP or OS benefit (Chapter 5.1.1.1; 5.1.1.2; 5.2.1.1). Oral fluoropyrimidines often have a favourable toxicity profile. For example, carmofur has very mild overall toxicity, but it has no significant OS benefit compared with bolus 5-FU and LV (Chapter 5.1.2; 5.2.1.2). Raltitrexed as a single agent has a feasible toxicity profile, with less neutropenia and stomatitis, and similar survival (in all but one study) compared with 5-FU and LV (Mayo regimen) (Chapter 5.1.3; 5.2.1.3).

The reason to use raltitrexed and 5-FU based combinations lie in preclinical studies that suggest schedule-dependent synergy (Jackman et al., 1999; Kano et al., 2000). In the present phase I study with raltitrexed combined with carmofur the MTD was reached at a dose-level of raltitrexed 3.0 mg/m² and carmofur 400 mg/m², where 6 patients out of 9 had DLT (Study I). The recommended doses to be used in combination are raltitrexed 3.0 mg/m² given as a 15-minute infusion on day 1, and carmofur 300 mg/m² as divided into 3 daily doses and given on days 2 to 14, repeated every 3 weeks. When used as single agent and in most combinations the recommended dose of raltitrexed has been 3.0 mg/m² (Sobrero, 1997b; Caponigro et al., 1999; Schwartz et al., 1999; Fizazi et al., 2000; Scheithauer et al., 2001b; Cunningham et al., 2002) and of carmofur 300-600mg/m² (Koyama, 1981; Sawada et al., 1983; Sipila et al., 1989; Grohn et al., 1994; Ito et al., 1996b; Ono et al., 1997).

The most common adverse events reported earlier with raltitrexed monotherapy include diarrhoea, nausea/vomiting, reversible transaminase rise, fatigue (asthenia), leukopenia and anaemia (Table 8) (Cunningham, 1998), whereas the most common major adverse effects of single-agent carmofur are increased urinary frequency and hot flashes (Table 7) (Grohn et al., 1990). In the present study, DLTs for the combination of raltitrexed and carmofur were Gr 3-4 fatigue, diarrhoea, anorexia, anaemia, mucositis, thrombocytopenia, and neutropenia (Table 21) (Study I).
Table 21  Efficacy and safety of single-agent raltitrexed, single-agent 5-FU/LV bolus, irinotecan combinations, and oxaliplatin combinations in treatment of metastatic colorectal cancer.

<table>
<thead>
<tr>
<th>Study</th>
<th>(Cunningham et al., 1996; Pazdur &amp; Vincent, 1997; Cocconi et al., 1998)</th>
<th>(Koyama, 1981; Grohn et al., 1990; Ito et al., 1996a; Ito et al., 1996b)</th>
<th>(Study I)</th>
<th>(de Gramont et al., 2000)</th>
<th>(Saltz et al., 2000) n=225+219</th>
</tr>
</thead>
<tbody>
<tr>
<td>Event Gr 3-4%</td>
<td>3 weekly raltitrexed Monthly 5FU+LV bolus 300-600 mg/m² all grades</td>
<td>3 weekly raltitrexed + oral carmustine 2 weeks</td>
<td>2 weekly 5FU bolus+ cont inf +LV</td>
<td>2 weekly 5FU bolus+ cont inf +LV + oxaliplatin</td>
<td>5FU+LV short infusion Weekly 5FU bolus +LV high dose+ irinotecan</td>
</tr>
<tr>
<td>Mucositis</td>
<td>2-3 11-22</td>
<td>4</td>
<td>2</td>
<td>17</td>
<td>2</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>10-14 13-19</td>
<td>21</td>
<td>5 12</td>
<td>13</td>
<td>23</td>
</tr>
<tr>
<td>Nausea</td>
<td>9-13 8-9</td>
<td>14</td>
<td>2 6</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Leukopenia/ Infection</td>
<td>6-18 13-41</td>
<td>7</td>
<td>5 42</td>
<td>66</td>
<td>54</td>
</tr>
<tr>
<td>Neurotoxicity</td>
<td>Rare</td>
<td>11</td>
<td>2 2</td>
<td>15</td>
<td>7</td>
</tr>
<tr>
<td>Fatality</td>
<td>3-5 1-3</td>
<td>4</td>
<td>1 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First line</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RR %</td>
<td>1.6 1.2</td>
<td>0.1</td>
<td>4</td>
<td>0.9</td>
<td>1.4</td>
</tr>
<tr>
<td>TTP months</td>
<td>14-26 15-18</td>
<td>17-50</td>
<td>50</td>
<td>22 51</td>
<td>31 49</td>
</tr>
<tr>
<td>OS months</td>
<td>3.1-4.8 3.6-5.3</td>
<td>4.7-5.5</td>
<td>5.2</td>
<td>6.2 9.0</td>
<td>4.4 6.7</td>
</tr>
<tr>
<td>9.7-11.2 10-11.8</td>
<td>9.0</td>
<td>9.1</td>
<td>14.7 16.2</td>
<td>14.1 17.4</td>
<td></td>
</tr>
</tbody>
</table>

In comparison with reported Gr 3-4 adverse events, in single raltitrexed and 5-FU and LV bolus, the combination has a similar toxicity profile, though with slightly less neutropenia and stomatitis than 5-FU and LV combinations (Table 21). In comparison with the ILF regimen (irinotecan+ 5FU/LV) and FOLFOX (oxaliplatin+ 5FU/LV), raltitrexed plus carmustine causes less neutropenia and neurotoxicity. The combination of fatigue with non-neutopenic fever, not responsive to antibiotics, flu-like symptoms and anorexia was unexpected, and has not been thoroughly reported earlier. Fever has been reported to be present in 0-3%, pain in 5-14% and fatigue in 5-18% of the patients treated with single-agent raltitrexed treatment (Cunningham et al., 1996; Pazdur & Vincent, 1997; Cocconi et al., 1998).

When raltitrexed has been given as a single agent RR has been 14-26%, median TTP 3.1-4.8 months and median OS 9.7-11.2 months (Cunningham et al., 1995; Zalcberg et al., 1996; Pazdur & Vincent, 1997; Cocconi et al., 1998). Response rates in two phase II studies using carmustine were 16.9% and 36.4% (Koyama, 1982; Grohn et al., 1990). Although it is difficult to compare
treatment results between nonrandomized studies due to case selection, the combination of raltitrexed and carmofur may result in an improved RR as compared with either drug used as a single agent, and the RR does not appear to be much lower than those obtained with the most effective combinations of irinotecan and oxaliplatin (de Gramont et al., 2000; Douillard et al., 2000; Saltz et al., 2000). In the present study on raltitrexed and carmofur the patient series was not heavily selected, and many patients had multiple metastatic sites and extensive bulky metastases, such as metastases affecting more than 30% of the liver volume. Since patients with bulky, extensive disease generally have a short life expectancy, this may explain why the high RR observed in the study did not translate into a long median survival.

The response rate with raltitrexed when combined with bolus 5-FU and LV in the first line therapy has been 24% and 25%, respectively (Comella et al., 2000b; Caponigro et al., 2001a). When raltitrexed was combined with infusional 5-FU a high preliminary RR of 53% was found in first-line treatment (Schwartz et al., 1999). In second- and third-line treatments some disease stabilizations were obtained (Schwartz et al., 1999; Tsavaris et al., 2002a) and when raltitrexed was combined with bolus 5-FU (in most patients as a second-line treatment) a RR of 12.5% was reported (Dragnev et al., 1998). The combination of raltitrexed with a longer 5-FU administration may thus be slightly more efficient than combinations with bolus 5-FU.

Would I recommend the raltitrexed and carmofur combination to the patient as the first-line treatment? The efficacy of the combination appears promising, with a unique toxicity profile consisting of fatigue, non-neutropenic fever and flu-like symptoms. The use of this combination is still limited by missing the phase II (under analysis) and III confirmatory data, by the availability of carmofur, and by the toxicity profile, with inflammatory symptoms that need further attention. Thus further studies are required before accepting this combination for routine use.

### 9.1.6. Chemotherapy triggered systemic inflammatory reaction

The rapid onset of spiking fever, fatigue, flu-like symptoms, and the increases in the serum CRP and inflammatory cytokine levels suggest that the patients treated with the combination of raltitrexed and carmofur or with single-agent raltitrexed developed a systemic inflammatory reaction a few days following chemotherapy infusions (Study II). Several lines of evidence suggest that the symptoms were not triggered by undiagnosed microbial infections, but that the major cause of symptoms was drug-related systemic inflammation. First, we failed to find
evidence of microbial infection in the majority of patients despite extensive and repeated clinical examinations. Second, treatment with antibiotics appeared to have little effect on the clinical symptoms or the CRP levels. Third, the patients did not have neutropenia, an important risk factor for acute infection, on the days preceding the symptoms. The leukocyte nadir occurred on cycle days 10 to 14 after the raltitrexed infusion, whereas fever was usually present earlier, starting on the cycle days 2 to 4 and resolving in all cases by cycle day 9, whereas most of the verified infectious episodes developed after the day 7 of the cycle. Fourth, in most patients fever, fatigue, and the serum CRP elevations recurred following every chemotherapy cycle, which is not suggestive of infection-related fever, and the symptoms and CRP elevations were limited to patients who received raltitrexed. Taken together, although the presence of infection cannot be excluded with certainty, the present findings suggest that the symptoms and the related serum chemistry changes were caused by a treatment-related systemic inflammatory reaction.

The severity of the systemic inflammation, as defined by the systemic inflammatory composite score, appeared to increase with the duration of raltitrexed treatment, and in general the combination of raltitrexed and carmofur caused more severe symptoms than raltitrexed alone. The symptoms and the concomitant elevations in the serum CRP and cytokine levels did not occur in patients treated with the 5-FU based regimens. Systemic inflammation that is not triggered by infection is apparently unusual and has not been reported in colorectal cancer patients treated with chemotherapy regimens consisting of 5-FU, oxaliplatin (with reservation), or irinotecan (de Gramont et al., 1997a; Bleiberg & de Gramont, 1998; de Gramont et al., 2000; Douillard et al., 2000; Saltz et al., 2000; Hoff et al., 2001).

However, similar symptoms have been observed in up to one half of the patients receiving cytarabine or bleomycin, or more rarely in those treated with paclitaxel or oxaliplatin, which may induce inflammatory cytokine production (Chiche et al., 1993; Sleijfer et al., 1998; Ulrich-Pur et al., 2000; Ek et al., 2001; Tonini et al., 2002; Ek & Abrahamsson, 2004; Pusztai et al., 2004). The toxicities found in the present raltitrexed-based therapies, and those described with the use of cytarabine, bleomycin, paclitaxel and oxaliplatin, resemble the effects linked to proinflammatory cytokines, such as IL-6 and TNFα. Typical symptoms are fever within 72 hours of administration and fatigue, which increasingly bothers patients over repeated cycles of chemotherapy. Patients may also suffer from pain, have reversible elevations of hepatic enzymes, rash, pulmonary reactions and even anaphylaxis. At present, the underlying mechanism(s) by which
chemotherapeutics alone or in combinations trigger systemic inflammatory reactions remain unknown.

Recurring fever, fatigue, and elevations of serum CRP could be fully inhibited with prolonged dexamethasone administration in all patients tested in the raltitrexed plus carmofur study. Similar findings have been made with cytarabine and oxaliplatin administration (Ulrich-Pur et al., 2000; Tonini et al., 2002). When paclitaxel is used, dexamethasone premedication at the time of infusion probably diminishes the reaction. Hypothetically, a systemic inflammatory response triggered by chemotherapeutics might be beneficial by enhancing the immune defence against cancer. TNF-α and IL-6, which are pleiotropic cytokines, may have anti-tumour properties (May et al., 1988; Gluckman et al., 1997; Tanaka et al., 1997). They have been used as chemotherapy agents in experimental cancer and even in the clinical treatment series of colorectal cancer (de Vries et al., 1998; Tomita et al., 1998; de Vries et al., 1999; Lindner et al., 1999) and might act in synergy with conventional chemotherapeutics such as 5-FU (Watanabe et al., 1988; Oka et al., 1997). Increased anti-tumour activity associated with systemic inflammation might in part explain the high RR of 50% obtained by us with the combination of raltitrexed and carmofur as the first-line treatment of metastatic colorectal cancer (Study I). However, the effect of the cytokines and that of dexamethasone on the treatment efficacy remains unknown and requires further study.

The severity of systemic inflammation is regulated at least partly by genetic factors (Louis et al., 1998). This has also been evident with bleomycin triggered pulmonary toxicity (Huang et al., 2004). Systemic inflammation may lead to inflammation-mediated organ failure (Mira et al., 1999), and it may also trigger a counter-reaction characterized by the development of immune suppression (Bone, 1996a), which is associated with poor outcome in patients with sepsis (Volk et al., 1996). Although raltitrexed has a tolerable toxicity profile in general, the raltitrexed treatment-related mortality rate was recently found to be unexpectedly high, 1.9-6% (Anonymous, 1999a; Maughan et al., 2002). Some of the patients with a fatal outcome had symptoms suggestive of repeated systemic inflammation on prior chemotherapy cycles, and ultimately developed multiorgan failure, at neutropenia and gastrointestinal toxicity inducing infection. Many of the patients with severe toxicities did not have a proper dose reduction based on treatment-related neutropenia occurring in conjunction with diarrhoea or a deteriorating kidney function, which may have led to accumulation of raltitrexed toxicity (Anonymous, 1999a; Maughan et al., 2002).
Hence, although a systemic inflammatory reaction might have theoretical potential benefits by increasing tumour cell destruction, it might also have detrimental effects by eliciting toxicity, possibly leading to immune suppression and organ dysfunction. Therefore, I would be reluctant to use the raltitrexed and carmofur combination until its efficacy is better proven, and the nature of the systemic inflammatory reaction is better understood.

In the treatment decision 5-fluoropyrimidines, with continuous simplified de Gramont or oral regimens, would be the main alternatives, due to good tolerability, easy administration and reasonable efficacy. An early response evaluation at 7-8 weeks would be performed. At the slightest hint of progression I would give the patient combination chemotherapy with irinotecan or oxaliplatin combined with the familiar 5-FU used in first line. The EGFR and VEGF inhibitors are not currently available and will not be further discussed here, but by the time he is progressing, addition of cetuximab for example could be an alternative.

Mr Median would survive about 21 months receiving 3 lines of chemotherapy. The cost of 5-FU based therapy for 6 months would be about 7,000€ (Wiklund & Pekurinen, 2003). Based on Helsinki university hospital prices and estimates from above the cost for 6 months of second-line oxaliplatin combined with 5-FU based therapy would be approximately 16,000€ and for 3 months of third-line irinotecan combined with 5-FU based therapy 8,000€ (if combined with cetuximab for 3 months 22,000€), in total 31,000€ (or 53,000€ with fourth-line cetuximab based giving him 24 months??). He would mostly be quite happy with reasonable toxicity from his outpatient treatment, giving him “more days with more”.
10. CONCLUSIONS

Study I
The combination of raltitrexed 3.0 mg/m² given intravenously every 3-weeks and carmofur 300 mg/m² given orally for 13 days is feasible. This combination has a unique but manageable toxicity profile consisting of fatigue, diarrhoea and fever, which usually is not associated with neutropenia. The anticancer efficacy of the combination appears promising, since 44% of the patients treated for metastatic colorectal cancer achieved a partial response in this dose-escalation study.

Study II
Colorectal carcinoma patients treated with single-agent raltitrexed or raltitrexed plus carmofur often develop fever, fatigue and elevations in the serum CRP and proinflammatory cytokine levels, which peak a few days after raltitrexed infusion and may recur after subsequent cycles. These features are suggestive of a drug-related systemic inflammation, which may be difficult to differentiate from those caused by microbial infections. Corticosteroids may be effective in prevention of the symptoms, but they may mask underlying microbial infections, and their influence on chemotherapy efficacy is unknown.

Study III
The simplified de Gramont regimen containing continuous 5-FU infusion is generally better tolerated than the Mayo bolus regimen, even when the VAD related adverse events are taken into account. Milder stomatitis, diarrhoea and neutropenia and less hospitalisation, but slightly more hand-foot syndrome is found in patients treated with continuous infusions as compared with bolus injections. Chemotherapy and –radiation related diarrhoea and abdominal discomfort might safely be alleviated by oral probiotic supplement with Lactobacillus rhamnosus GG during treatment.

Study IV
5-FU-based chemotherapy is not infrequently associated with transient secondary hypolactasia, likely caused by 5-FU-related mucosal bowel injury. Hypolactasians generally have more severe gastrointestinal symptoms and a poorer nutritional status than those with normolactasia. The impact of dietary lactose restriction or lactase supplements has not been formally studied, but might improve tolerability of treatment. A lactose tolerance test should be considered whenever symptoms compatible with lactose intolerance occur during 5-FU-based treatment.
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When times were hard, I dedicated my efforts to God Almighty and his earthly angels. None of you angels had wings, although some of you were dressed in white e.g. Ville, Asko, Päivi and Markku, but most of you, family and friends, were just there for me when I needed you most.

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*Pia Österlund*
12. REFERENCES


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