5-Fluorouracil plus leucovorin is an effective adjuvant chemotherapy in curatively resected stage III colon cancer: long-term follow-up results of the adjCCA-01 trial

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Background: Adjuvant postoperative treatment with 5-fluorouracil (5-FU) and leucovorin in curatively resected stage III colon cancer significantly reduces the risk of cancer recurrences and improves survival. The impact of 5-FU plus leucovorin on survival and tumor recurrence was analyzed in a long-term follow-up study in comparison with the effects of 5-FU plus levamisole in the prospective multicenter trial adjCCA-01.

Patients and methods: Patients with a curatively resected stage III (International Union Against Cancer) colon cancer were stratified according to tumor, node and grading category and randomly assigned to receive one of the two adjuvant treatment schemes: 5-FU 400 mg/m² body surface area intravenously in the first chemotherapy course, then 450 mg/m² × 5 days, plus leucovorin 100 mg/m², 12 cycles (arm A), or 5-FU plus levamisole (Moertel scheme; arm B).

Results: Six hundred and eighty (96.9%) of 702 patients enrolled into this study were eligible. To date, 261 patients have died, 117 on arm A and 144 on arm B (P = 0.007). After a median follow-up time of 82 months, the 5-FU plus leucovorin combination significantly improved disease-free survival [79.8 months in arm A versus 69.3 months in arm B (P = 0.012)] and significantly increased median overall survival (88.9 months in arm A versus 78.6 months in arm B; P = 0.003). Adjuvant treatment with 5-FU plus levamisole as well as 5-FU plus leucovorin was generally well tolerated; only a minority of patients experienced grade 3 and 4 toxicities.

Conclusions: After curative resection of a stage III colon cancer, adjuvant treatment with 5-FU plus leucovorin is generally well tolerated. This long-term follow-up study demonstrates that adjuvant treatment with 5-FU plus leucovorin given for 12 cycles is significantly more effective than 5-FU plus levamisole (Moertel scheme) in reducing tumor relapse and improving survival.

Key words: adjuvant chemotherapy, colon cancer, 5-fluorouracil, leucovorin, levamisole

Introduction

Colorectal cancer is one of the leading cancers in the western world [1]. In Germany >50000 new cases of colorectal cancer are diagnosed each year [2]. Approximately 50% of these patients diagnosed with colorectal cancer die of their disease. In patients with curatively resected colon carcinoma the prognosis depends on the pathological staging. Invasion of regional lymph nodes, as well as high tumor (T) category, decreases the 5-year survival significantly in these patients [3].

There is clear evidence that adjuvant chemotherapy has a beneficial effect on tumor relapse and overall survival in patients with a curatively resected stage III colon carcinoma. Adjuvant therapy with 5-fluorouracil (5-FU)/levamisole for 12 months (Moertel scheme) reduced the risk of cancer recurrence by 41% and the overall death rate by 33% after more than 5 years of follow-up [4]. The combined adjuvant chemotherapy of 5-FU/leucovorin showed also significant improvement in tumor relapse and overall survival [5, 6]. Results of the adjCCA-01 trial demonstrated that adjuvant treatment with 5-FU plus leucovorin was significantly more effective than 5-FU/levamisole (Moertel scheme) in reducing tumor relapse and improving survival after a median follow-up of 46.5 months [7]. The present paper shows the final long-term follow-up results of the adjCCA-01 trial.

Patients and methods

This trial was initiated by the Arbeitsgemeinschaft Gastrointestinale Onkologie (AGO) and approved by the ethics committee of the faculty of medicine of the Heinrich-Heine-University of Düsseldorf. The cut-off date for analysis was 31 July 2001.
Enrollment of patients began in December 1991 and was completed in December 1994. To be eligible, patients had to fulfill the following inclusion criteria: potentially curative en bloc resection of an adenocarcinoma of the colon without gross or microscopic evidence of residual disease, presence of lymph node metastasis, a leukocyte count of at least 4000/µl, a platelet count of at least 130 000/µl, performance status (Eastern Cooperative Oncology group) of zero or one, and written informed consent.

Chemotherapy administration schedules

**Arm A.** Chemotherapy consisted of leucovorin 100 mg/m2 and 5-FU 400 mg/m2 (increased to 450 mg/m2 after the first course) intravenously (i.v.) on days 1–5 every 4 weeks for 12 cycles. 5-FU was administered as a short infusion over 30 min.

**Arm B.** Levamisole 50 mg was given orally three times a day for 3 days and repeated biweekly. Patients received 5-FU 450 mg/m2 by short infusion daily for 5 consecutive days. On day 29, 5-FU was given once weekly and continued for 44 weeks.

Toxicity

Toxicity was evaluated according to WHO standard criteria. In cases of severe toxicity the 5-FU and/or the levamisole dosage was reduced depending on the grade of toxicity.

Follow-up

Follow-up of each patient was done in the first 2 years on a quarterly basis, then the next 3 years on a semi-annual basis, and yearly thereafter. Follow-up consisted of medical examinations, which included blood counts and blood chemistries (carcinoembryonic antigen assays were optional), ultrasounds, computed tomography scans, chest radiographs and colonoscopy.

Statistical analysis

The aim of this long-term follow-up was to determine the overall survival rate. In addition, the time to tumor relapse and the toxicity profile were evaluated in both study arms.

Survival time was defined as the interval between the date of surgery and the date of death or the date of diagnosis of the first recurrence. The cut-off date for analysis was 31 July 2001. Survival curves were generated by the Kaplan–Meier method [8] and compared by means of the one-sided log-rank test. The multivariate Cox proportional hazard model was applied to identify factors significantly related to recurrence and survival using the backward regression procedure. Statistical significance was P <0.05. All analyses were performed on an intention-to-treat basis.

Results

Allocation of patients, age, gender and tumor site to treatment arm is given in Table 1. At the time of writing the median follow-up time is 82 months. To date, 277 recurrences have occurred. In the leucovorin group the recurrence rate was 37.2% (130 of 349) and in the levamisole group 44.4% (147 of 331). Locoregional relapses as the site of the first relapse were seen in 14 patients (arm A) and 20 patients (arm B), distant metastases in 88 and 98 patients, respectively, and a combination of locoregional recurrence plus distant metastases in 28 and 29 patients, respectively. Relapses were seen after a median observation time of 79.8 months [95% confidence interval (CI) 75–85 months] in arm A and of 69.3 months (95% CI 64–75) in arm B (P = 0.012) (Figure 1). These curves had no tendency to converge during long-term follow-up. Cox regression analysis with age, sex, interval between surgery and beginning chemotherapy, tumor location, tumor obstruction, tumor perforation, T and node (N) category, and histological differentiation as predictors revealed that nodal status (P <0.0001), interval between surgery and beginning of chemotherapy (P <0.01), tumor invasion (P <0.02) and obstruction (P <0.02) were significant contributors to tumor relapse (Table 2). The Cox regression also revealed that 5-FU/leucovorin is superior to 5-FU/levamisole treatment in terms of tumor relapse (P <0.002).

During this study, 29 second primary cancers were documented: 16 (4.6%) of 349 in arm A and 13 (3.9%) of 331 in arm B (P = NS). Two cases of non-Hodgkin’s lymphoma were observed in arm B. The large bowel was the site of a second primary cancer in six cases.

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**Table 1. Allocation of patients, age, gender and tumor site to treatment arm**

<table>
<thead>
<tr>
<th></th>
<th>Arm A</th>
<th>Arm B</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>349</td>
<td>331</td>
</tr>
<tr>
<td>Median age, years</td>
<td>62</td>
<td>63</td>
</tr>
<tr>
<td>Sex (%)</td>
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<tr>
<td>Male</td>
<td>43</td>
<td>46</td>
</tr>
<tr>
<td>Female</td>
<td>57</td>
<td>54</td>
</tr>
<tr>
<td>Primary site (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cecum and right colon</td>
<td>33</td>
<td>34</td>
</tr>
<tr>
<td>Flexures and transverse colon</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>Left colon</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Sigmoid and rectosigmoid</td>
<td>43</td>
<td>44</td>
</tr>
</tbody>
</table>

**Figure 1.** Curves showing significant improvement in overall survival in arm A [open diamonds, 5-fluorouracil (5-FU)/leucovorin] compared with arm B (filled squares, 5-FU/levamisole) (P = 0.0035).
To date, 261 patients have died; 117 (33.5%) on the 5-FU/leucovorin arm and 144 (43.5%) on the 5-FU/levamisole arm ($P = 0.007$). The univariate overall survival analysis demonstrated that therapy with 5-FU/leucovorin was significantly superior to treatment with 5-FU/levamisole [median survival in arm A 88.9 months (95% CI 85–93 months) versus 78.5 months (95% CI 74–83 months) in arm B ($P = 0.0035$)] (Figure 2). Again, the curves had no tendency to converge. The 5-year survival in arm A is 70% and in arm B 60.8%. In 40 patients, death was caused by non-cancer related diseases (arm A: 19 cases; arm B: 21 cases). In 20 of these patients, the cause of death was cardiovascular ($P = \text{NS}$). A multivariate Cox analysis demonstrated that chemotherapy, age, tumor obstruction, T and N category were independent prognostic factors for overall survival (Table 2). The Cox proportional hazards model also confirmed a significant survival advantage for the 5-FU/leucovorin arm ($P = 0.01$).

**Discussion**

Our adjuvant chemotherapy study, starting in 1991, was based on the results reported by Moertel and co-workers with the combination of 5-FU/leucovorin and the promising findings of the AGO trial (5-FU/leucovorin) in patients with advanced colon carcinoma [9]. Therefore, we compared both chemotherapy regimens for 12 months in a multicenter prospective trial. Our adjuvant study demonstrated for the first time a significant improvement of tumor relapse ($P = 0.0037$) and a significantly increased overall survival ($P = 0.0089$) after a median follow-up of 46.5 months for patients treated with 5-FU/leucovorin in comparison with 5-FU/levamisole [7].

Today, after a median follow-up time of 82 months, the previous data are confirmed. The 5-FU/leucovorin treatment over 12 cycles was superior and significantly more effective than the 5-FU/levamisole combination.

In the 1990s the combination of 5-FU/levamisole was recommended as standard adjuvant treatment of Dukes’ C colon cancer. Large trials showed activity of 5-FU/levamisole chemotherapy in colorectal cancers versus surgery alone. The Intergroup Study 0035 showed a significant survival advantage after a median follow-up time of 6.5 years. The mortality rate of patients who received 5-FU/levamisole was reduced by 33% ($P = 0.0007$) in comparison with those patients who had surgery alone. No benefit could be demonstrated in Dukes’ B carcinoma [10, 11].

Numerous attempts have been performed to modulate the activity of 5-FU. In advanced colorectal cancer the remission rates were increased by additional leucovorin [12, 13]. Consequently studies were undertaken to show the efficacy of combined 5-FU and leucovorin in adjuvant treatment of colon cancer [14–16].

A study with 239 enrolled stage B and C colon carcinoma patients reported that 5-FU/leucovorin (5-FU 400 mg/m$^2$ i.v. and leucovorin 200 mg/m$^2$ i.v., days 1–5, every 4 weeks for 12 cycles) was a good and well-tolerated adjuvant therapy regimen in contrast to surgery alone. In patients with stage C colon carcinoma the recurrence rate was reduced with prolongation of disease-free survival ($P = 0.0016$) and overall survival ($P = 0.0025$). Patients with stage B colon carcinoma did not profit from adjuvant chemotherapy [14].

The International Multicenter Pooled Analysis of Colon Cancer Trials (IMPACT) investigators showed that adjuvant treatment with 5-FU/leucovorin (5-FU 370–400 mg/m$^2$ i.v. and leucovorin 200 mg/m$^2$ daily for 5 days, every 4 weeks for six cycles) in patients with Dukes’ B and C colon cancer was effective. The

| Table 2. Prognostic factors for recurrence and survival (Cox regression analysis) |
|-----------------------------|-----------------------------|-----------------------------|
| **Cox regression analysis**                  | **P value**     |               |
| **Recurrence**       |               |               |
| Chemotherapy; 5-FU/leucovorin versus 5-FU/levamisole | 0.002         | 0.01          |
| Age; <61 versus ≥61 years | NS            | 0.004         |
| Days since surgery; <27 versus ≥27 | 0.01         | NS            |
| T category; T1 + T2 versus T3 + T4 | 0.02         | 0.04          |
| N category; N1 versus N2 + N3 | 0.0001      | 0.0001        |
| Obstruction; yes versus no | 0.02         | 0.005         |

5-FU, 5-fluorouracil; N category, node category; T category, tumor category.

![Figure 2](https://academic.oup.com/annonc/article-abstract/14/3/395/210677/fig2)

**Figure 2.** Curves showing significant improvement in recurrence-free survival in arm A [open diamonds, 5-fluorouracil (5-FU)/leucovorin] compared with arm B (filled squares, 5-FU/levamisole) ($P = 0.012$).
combination of 5-FU/leucovorin significantly reduced mortality by 22% and reduced the risk of treatment failure by 35% in comparison with surgery only. The 3-year recurrence-free survival rate for patients with Dukes’ B and C colon cancer was increased from 62% to 71% and also the overall survival rate from 78% to 83%. A subgroup analysis for patients with Dukes’ B carcinoma showed borderline significance for event-free survival but not for overall survival [15].

Furthermore, the NCCTG (National Cancer Treatment Group/ Mayo Clinic) study demonstrated, in patients with stage II and III colon carcinoma, that adjuvant treatment with 5-FU/low-dose leucovorin (425 mg/m² i.v. plus leucovorin 20 mg/m² by rapid i.v. injection for 5 days, every 4 weeks for six cycles) had similar effects to the previous study. In comparison with surgery alone the patients who received 5-FU/leucovorin had a significant improvement in time of survival and time of tumor relapse [16].

These studies demonstrated clearly that combined adjuvant chemotherapy with 5-FU/leucovorin was effective and well-tolerated in comparison with surgery alone. It still remains unclear whether patients with Dukes’ B colon carcinoma profit from adjuvant chemotherapy.

Nevertheless, these studies did not feature the optimal duration of adjuvant treatment and also the optimal dosage of leucovorin. Further, no study compared the 5-FU/leucovorin schedule with the so-called Moertel scheme, which was recommended as standard therapy in patients with Dukes’ C colon carcinoma in the early 1990s. Therefore, subsequent studies focused on identifying the optimal chemotherapy combination for adjuvant treatment including the addition of levamisole to 5-FU/leucovorin, the optimal duration of therapy (6 versus 12 months) and high-versus low-dose leucovorin.

The NSABP (National Surgical Adjuvant Breast and Bowel Project) group implemented a trial (C-04) comparing 5-FU/leucovorin with 5-FU/levamisole versus 5-FU/leucovorin plus additional levamisole. The results of this trial did not show any significant difference in overall and disease-free survival for patients with Dukes’ B and C carcinoma, but pair-wise statistical comparisons indicated an advantage in survival for patients who received 5-FU/leucovorin [17].

The INT-0089 study group investigated 3759 patients with high-risk stage II/III colon cancer. This study compared 12 months of 5-FU/levamisole with two different 5-FU/leucovorin schedules [high dose (8 months) and low dose (6 months)] and with a triplet combination of 5-FU/leucovorin/levamisole (6 months). The results after a median follow-up of 5 years demonstrated that a 6-month treatment with both 5-FU/leucovorin schedules (high and low dose) was as effective as the standard 12-month therapy with 5-FU/levamisole. The 6-month triple regimen was not superior to 5-FU/leucovorin treatment [18].

A further study compared 5-FU/levamisole versus 5-FU/leucovorin and additional levamisole in 915 patients with Dukes’ B and C colon carcinoma. Patients were also assigned to receive either 6 months or 12 months of chemotherapy. After a median follow-up time of 5.1 years the combined chemotherapy of 5-FU/leucovorin and levamisole given for 6 months was superior to 6 months of 5-FU/levamisole (5-year survival rate 70% versus 60%; \( P < 0.01 \)). There was no difference in survival when chemotherapy was given for 12 months compared with 6 months [19].

These studies demonstrated that 5-FU/leucovorin for 6 months was as effective as 12 months of 5-FU/levamisole. There were no better results seen when levamisole was added to 5-FU/leucovorin chemotherapy.

The Quasar (Quick And Simple And Reliable) study group studied two different leucovorin schedules (high-dose: 175 mg/m² and low-dose: 25 mg/m²) with combined 5-FU (370 mg/m², days 1–5, every 4 weeks for six cycles) in a 2 × 2 design. Patients were also randomly assigned to receive either levamisole or placebo. In the high- and low-dose leucovorin group, the survival and the recurrence rates after 3 years were similar (70.1% versus 71%, \( P = 0.43 \); 36.0% versus 35.8%, \( P = 0.94 \), respectively). The survival in patients with additional levamisole was worse than in the placebo group (69.4% versus 71.5% at 3 years; \( P = 0.06 \)) and also the recurrence rate was increased (37.0% versus 34.9% at 3 years; \( P = 0.16 \)) [20].

The results of the Quasar study showed that high-dose leucovorin was not superior to low-dose leucovorin application. Like the studies above, the authors considered that levamisole had no benefit on survival and recurrence rates when given for 6 months.

To summarize the results of the trials mentioned above, together with our own significant results, the standard for patients with curatively resected stage III colon cancer should be a 5-FU-based regimen combined with leucovorin.

An analysis of adjuvant chemotherapy trials showed that reduction in the odds of death was 5-FU dose-dependent. Patients who received larger planned 5-FU doses for the first 3 months had a significantly reduced odds of death (>10 g, 8–10 g and <8 g; odds ratio: 0.71, 0.93 and 1.04, respectively; \( P = 0.02 \)) [21]. In comparison with the NSABP-C04 study (8.5 g) and NCCTG study (9.4 g) the total planned dose of 5-FU for the first 3 months was 11.05 g in the adjCCA-01 trial. Whether the 5-FU dose was responsible for the significant survival difference in our trial seems speculative. Nevertheless, these findings necessitate investigation of whether 5-FU dose intensity or total 5-FU dose might be responsible for the increase in disease-free survival and overall survival. Therefore, we are performing a study (adjCCA-02) comparing the five-times-weekly 5-FU/leucovorin regimen with the weekly high-dose 5-FU/leucovorin and with high-dose 5-FU monotherapy.

References

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