The role of 5-fluorouracil dose in the adjuvant therapy of colorectal cancer

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Summary

Background: In many centres, the use of 5-fluorouracil (5-FU) combined with levamisole has become standard therapy for the treatment of patients with Dukes' C colon cancer. However, the role of levamisole remains unclear.

Materials and methods: All of the published adjuvant studies for colorectal cancer in which 5-FU (either as a single agent or in combination with other cytotoxics or levamisole) was compared to a no-treatment control group were ranked according to the total planned dose of 5-FU (assuming a body weight of 70 kg or a body surface area of 1.7 m²) over a three-month time frame. The effect of planned total dose of adjuvant therapy on the reduction of mortality was analysed using indirect comparisons of dose on the log odds ratio of death in a linear regression analysis.

Results: Overall, this analysis demonstrated a significant reduction in the odds of death for those receiving 5-FU regimens compared to untreated controls (estimate 0.82, 95% CI 0.74 to 0.91, p < 0.001). This effect was larger in those receiving a larger planned dose; for a total dose of 5-FU in the first three months of greater than 10 grams, 8 to 10 grams, less than 8 grams or oral 5-FU, estimates were 0.71, 0.79, 0.93 and 1.04, respectively (p = 0.02 for trend). Similar results were observed when the planned total dose of 5-FU received over 12 months was analysed. The analysis was then repeated by separating those studies in which 5-FU and levamisole were compared to a no-treatment control. A larger effect was seen in the 5-FU/levamisole trials (odds ratio, 0.64) compared to the other 5-FU regimens (odds ratio 0.86, p = 0.04). However, when adjusted for dose, the effect of levamisole was no longer significant (p = 0.09).

Conclusion: These data suggest two separate hypotheses. The first is that the benefit associated with the use of 5-FU and levamisole given as adjuvant therapy in Dukes' C colon cancer is directly related to the planned total dose of 5-FU administered. Alternatively, in view of the fact that levamisole was part of the treatment regimens in two of the three studies in which the total planned dose of 5-FU exceeded 10 grams in three months (or 40 grams in 12 months), levamisole may be critical to outcome and the 5-FU total dose or dose intensity less relevant.

Key words: colorectal carcinoma, dose intensity, 5-fluorouracil, levamisole, total dose

Introduction

Until the publication by Moertel et al. [1], the benefits of adjuvant chemotherapy for colorectal cancer were uncertain. A meta-analysis by Buyse et al. [2] showed no difference in overall survival for adjuvant therapy, but in a sub-group analysis suggested a benefit for 5-fluorouracil (5-FU) containing regimens. Following the publication of the Moertel study, a second meta-analysis by Gray et al. [3] suggested a small but statistically significant survival benefit for adjuvant chemotherapy in Dukes' C colorectal cancer. The benefit of adjuvant chemotherapy seems to have been further substantiated by the introduction of the 5-FU/levamisole regimen into the adjuvant setting [1]. However, the importance of the addition of levamisole to 5-FU remains unclear and numerous investigators have noted that the absence of a 5-FU-alone control group in the Moertel trial did not enable a clear understanding of the contribution of levamisole to this drug combination [4, 5].

Levamisole, first introduced into clinical medicine as an anthelmintic [6], has been the subject of considerable investigation as a potential immunostimulant for a number of years. A wide variety of interactions with the immune system have been reported although the mechanisms by which such interactions occur are only just starting to emerge [7].

Clinically, studies which have examined the role of levamisole as a single agent have generally failed to demonstrate the utility of this drug. In either the adjuvant or advanced settings and in a variety of tumour types including colon cancer, non-small cell lung cancer and melanoma, the use of single agent levamisole has failed to demonstrate any advantage over untreated controls [1, 8–10]. Similarly, despite initially encouraging data, large prospective studies have generally failed to demonstrate any advantage to the combination of standard cytotoxic drugs and levamisole (apart from the use of 5-FU and levamisole as adjuvant therapy in colorectal cancer) in the advanced disease or adjuvant settings [11, 12].
From a theoretical point of view, it is not clear that patients with early stage colon cancer are actually immunosuppressed nor for that matter whether immunosuppression is important in the aetiology (or progression) of the common forms of gastrointestinal malignancy. Whilst there is a 2.7-fold (95% CI, 1.8–4.0) increase in the incidence of colon cancer amongst the recipients of renal allografts [13], the risk in this population is so much smaller than for diseases thought to be related to immunological dysfunction, e.g., CNS lymphoma and Kaposi's sarcoma (in which the risk is greater than 1000 fold), that the three-fold increase in the risk of colon cancer observed in these patients is difficult to interpret.

In this report, we present data suggesting that the dose of 5-FU recommended in adjuvant programs (such as the 5-FU/levamisole combination), may be important in determining the observed benefits. The importance of 5-FU dose intensity has been previously observed in metastatic colorectal cancer [14], but this report details the first attempt to consider the hypothesis that the dose of 5-FU is critical in the outcome of adjuvant therapy in colorectal cancer.

Patients and methods

Research and eligibility

To determine the effect of the total planned dose of 5-FU on the survival benefit of adjuvant 5-FU containing regimen in the treatment of Dukes' B and C colorectal cancer, all published, randomised, controlled trials (obtained from the two reviews as well as a medline search), which compared a 5-FU containing regimen to a no-chemotherapy control group were selected. Trials which used combination chemotherapy were included as well as those comparing 5-FU and immunotherapy (e.g., 5-FU and levamisole) to no systemic therapy. However, studies containing a 5-FU and folinic acid regimen in the experimental arm were excluded from this analysis as folinic acid is thought to biochemically modulate the effects of 5-FU [15, 16].

Unconfounded trials comparing treatment with 5-FU and radiotherapy versus radiation therapy alone for patients with rectal cancer were also included. All trials of patients with Dukes' B or C colon and/or rectal cancer were included.

Unpublished randomised trials were not included in this exploratory analysis. In distinction to the review by Gray [3], for the two studies using 5-FU/levamisole, comparisons were made to the no-treatment control rather than the levamisole alone arms.

Data collection and coding

For each trial, the number of deaths out of the total number of patients randomised to each treatment (intention to treat) was obtained. In addition, two subsequent studies not reported at the time of the Gray [3] publication have been added to the list of studies examined [17, 18]. Information on site, treatment duration and planned total dose of 5-FU was also recorded (Table 1).

For a typical subject (body surface area of 1.7 m² for studies where dose was given as mg/m² or weight of 70 kg in studies based on mg/kg dosing), the total dose planned for each trial was calculated for a three-month (equivalent to a comparison of dose intensity average over the same time period), as well as a 12-month period.

Statistical methods

For each study, the observed and expected number of deaths on treatment was calculated and an estimated odds ratio of mortality

### Table 1. Meta-analysis of adjuvant colorectal trials.

<table>
<thead>
<tr>
<th>Study name</th>
<th>Site</th>
<th>5-FU total planned dose (grams)</th>
<th>Treatment group</th>
<th>Control group</th>
<th>Drug regimen</th>
<th>5-FU duration</th>
<th>Method of 5-FU dosing</th>
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<tr>
<td></td>
<td></td>
<td>3 months</td>
<td>12 months</td>
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<td>45.8</td>
<td>69</td>
<td>151</td>
<td>81</td>
<td>166</td>
<td>Alone</td>
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<td>EXETER</td>
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<td>6.0</td>
<td>30</td>
<td>68</td>
<td>28</td>
<td>68</td>
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<tr>
<td>GITSIG-6175</td>
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<td>70</td>
<td>156</td>
<td>71</td>
<td>159</td>
<td>MeCCNU</td>
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<tr>
<td>GITSIG-7175</td>
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<td>32.9</td>
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<td>58</td>
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<td>2</td>
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<td>136</td>
<td>70</td>
<td>135</td>
<td>Levam</td>
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<td>32.9</td>
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<td>184</td>
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<td>14.3</td>
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<td>160</td>
<td>318</td>
<td>MeCCNU</td>
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<tr>
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<td>10.1</td>
<td>25</td>
<td>59</td>
<td>21</td>
<td>62</td>
<td>MMC, Ara-C</td>
</tr>
</tbody>
</table>

* Mortality in published trials comparing prolonged adjuvant systemic chemotherapy with no chemotherapy – treatment group.
* Mortality in published trials comparing prolonged adjuvant systemic chemotherapy with no chemotherapy – control group.
* Drug regimen indicates 5-FU administered alone (alone), or in combination with either semustine (MeCCNU), or vincristine (V), or mitomycin-C (MMC), or cytosine (Ara-C).
* Method of dosing of 5-FU based on body surface area (BSA) or body weight (wt).
obtained for combined studies using the fixed effects model [19]. For each combination, a 95% confidence interval was obtained.

Odds ratios involved the calculation of O-E where O is the number of events in the treatment group and E the number that would have been expected if the death rate in the treatment group was equal to the death rate seen in the treatment and control group together. The odds ratio was then estimated using \( \exp(\frac{O-E}{v}) \) and a 95% confidence interval using \( \exp(\frac{(O-E)\pm 1.96/\sqrt{v}}) \) where v is the estimated variance of \( (O-E) \) [19]. The odds ratios were plotted as solid circles with the area of the circle representing the size of the trial (inversely related to the variance of the estimate) and the horizontal line representing the 95% confidence interval (CI). The overall results including the 95% CI are represented as black diamonds.

To explore the relationship between the planned dose of 5-FU, tumour site and the use of levamisole to the relative odds of death in each trial, a linear regression analysis was undertaken using the log odds ratio of each study as the outcome and weighting each study inversely to its variance. After adjusting for planned 5-FU dose, the effect of levamisole on outcome was explored using a multivariate regression analysis [20].

Planned 5-FU dose was classified in two ways. The first employed the actual dose of 5-FU for intravenous regimens in the three-month period, but arbitrarily assigned oral 5-FU to zero dose (due to variable absorption). The second, used for graphically displaying the odds ratios, categorised the planned dose of 5-FU into four categories: >10 grams, between 8 and 10 grams, <8 grams or oral chemotherapy. A second regression analysis using the categories for planned dose, resulted in similar conclusions.

Results

A previous meta-analysis of all studies comparing 5-FU to a non-5-FU control group in patients with Dukes' B and C colorectal cancer, demonstrated a survival benefit in favour of the use of adjuvant chemotherapy [3]. We regrouped each of these studies [1, 21–40] according to the total planned dose of 5-FU over a three-month time frame (Fig. 1) and added two trials which had not been published at the time [17, 18].

Whilst individual studies themselves did not usually demonstrate a significant reduction in mortality for adjuvant therapy, the combined results (Fig. 1) as reported by Gray et al. [3] clearly demonstrate a survival benefit for adjuvant therapy (odds ratio 0.82, 95% CI 0.74–0.91, p < 0.001).

These studies were then combined according to the total dose of 5-FU received over a three month period. The planned total dose of 5-FU received after three months of therapy was assigned into four separate categories as described previously. The magnitude of the odds ratio favouring an improvement in survival was greatest in those studies in which the largest dose of 5-FU was planned; the odds ratios were 0.71, 0.79, 0.93 and 1.04 for group 1, group 2, group 3 and group 4, respectively (Fig. 2). The trend to a greater effect for the categories with a larger planned dose was statistically significant (p < 0.02).

When the various studies were compared within each category, according to whether total planned dose was based on a body surface area of 1.7 m² or an average body weight of 70 kg, the effect of planned dose in the first three months remained significant.

The analysis was then repeated by separating those studies in which 5-FU and levamisole were compared to a no-treatment control (Fig. 3). Once again, the largest effect was seen in the 5-FU/levamisole trials (odds ratio 0.64, 95% CI 0.49–0.85) compared to the other 5-FU regimens. The latter were also associated with a significant improvement in survival (p = 0.01). However, as the 5-FU/levamisole studies were also the ones that involved the largest dose of 5-FU, the importance

![Fig 1. Mortality in published trials comparing 5-FU containing regimens to a no-treatment control.](image)

![Fig 2. Mortality in published trials comparing 5-FU containing regimens to a no-treatment control, based on the planned total dose of 5-FU over three months, doses are ranked into four categories: >10 grams, between 8 and 10 grams, <8 grams and oral chemotherapy.](image)
of levamisole remains uncertain. Using linear regression analysis, the most important parameters in determining overall outcome were determined to be planned three-month dose and the effect of levamisole. (A similar but not significant effect (p = 0.09) was also seen for the planned 12 month dose). However, the effect of levamisole was not significant (p = 0.09) when the results were adjusted for the planned three-month dose (Table 2).

Table 2. Regression analysis of adjuvant colorectal cancer studies.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Univariate p-value</th>
<th>Multivariate p-value</th>
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</thead>
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<tr>
<td>Planned 3-month dose</td>
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<td>0.08</td>
</tr>
<tr>
<td>Planned 12-month dose</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>Levamisole</td>
<td>0.04</td>
<td>0.09</td>
</tr>
<tr>
<td>Site</td>
<td>0.32</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

In retrospective analyses, response rates in trials involving patients with metastatic disease have been shown to correlate with the dose intensity of cytotoxic agents for a variety of tumour types, including breast cancer, small cell lung cancer, lymphoma and ovarian carcinoma [39]. Response rates in advanced colorectal cancer have also been correlated with the dose intensity of intravenous 5-FU [14, 41] in a retrospective analysis of several randomised trials. However, such a relationship has not been previously shown in the adjuvant therapy of colorectal carcinoma.

In this study, we have attempted to examine the role of the planned, total dose of 5-FU in the outcome of adjuvant chemotherapy for Dukes’ B and C colorectal cancer. We found the greatest improvement in survival was in studies in which the largest dose of 5-FU was planned, particularly over the first three month but also that the largest effect was seen in the 5-FU/levamisole regimens compared to other 5-FU regimens. Based on a regression analysis, the relative importance of adding levamisole to 5-FU versus a larger, planned dose of 5-FU was not clear.

This exploratory analysis suggests that two hypotheses are plausible. The first possibility is that the magnitude of the benefits associated with the use of 5-FU and levamisole as adjuvant therapy in Dukes’ B and C colorectal cancer is directly related to the planned, total dose of 5-FU administered. Thus, the results of two recent trials [17, 18] that represent the basis for the use of 5-FU and levamisole as standard therapy in this disease, may depend on the fact that both of these studies involved the highest, planned doses of 5-FU. Compared to the cohort of previous studies in this disease, the contribution of levamisole may not have been significant in this scenario. Alternatively, in view of the fact that levamisole was part of the treatment regimen in two of the three studies in which the total planned dose of 5-FU exceeded 10 grams in three months (or 40 grams in 12 months), levamisole may be critical to outcome and the total, planned dose of 5-FU less relevant.

It is not possible to establish which of these two hypotheses is correct given the current data set. It should also be pointed out that the relative effects of 5-FU dose and of levamisole are based on indirect, non-randomised comparisons in this analysis, so that confounding by the type of patients being studied in each trial is a possibility. However, this report does raise a reasonable hypothesis which can only be confirmed in a randomised clinical trial of 5-FU/levamisole compared to 5-FU alone.

This meta-analysis has excluded trials involving 5-FU and folinic acid. The recent publication of the IMPACT study [16] suggests that 5-FU and folinic acid may be as effective as 5-FU and levamisole in the adjuvant therapy of Dukes’ C colon cancer, consistent with the role of folinic acid, a biochemical modulator of 5-FU, effectively increasing the relative total dose of 5-FU. These results further suggest that the effective dose of 5-FU may be critical in determining outcome. Once again, this can be only clarified through prospective randomised trials. For the moment, existing data do not justify a change in clinical practice for those clinicians who consider 5-FU and levamisole as standard therapy for patients not involved in clinical studies.

The current meta-analysis has a number of limitations. Firstly, individual data sets from each of the reported trials has not been obtained, nor has the quality of data been reviewed. Secondly, no attempt has been made to identify unpublished trials or follow-up results. Thirdly, data on the actual dose administered were not available so that it has not been possible to classify these trials according to the actual doses given in each study.

The current Oxford collaboration looking at individual data has the opportunity to explore these questions in greater detail. However, even with this systematic overview it may still not be possible to determine which of these hypotheses are correct, an issue which can only be clarified by a randomised controlled trial comparing 5-FU/levamisole to 5-FU.
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References


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