Predictive value of thymidylate synthase and dihydropyrimidine dehydrogenase protein expression on survival in adjuvantly treated stage III colon cancer patients


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Background: The predictive value of thymidylate synthase (TS) and dihydropyrimidine dehydrogenase (DPD) expression on long-term survival by influencing 5-fluorouracil (5-FU) effect were determined in primary tumours and node metastases of stage III colon cancer patients treated adjuvantly with 5-FU regimens (n = 391). The effect of TP53 mutation status, which is thought to be functionally linked to TS inhibition, was also examined.

Patients and methods: TS and DPD protein expression was determined by immunohistochemical analysis using tissue microarrays of these colon tumours. Two hundred and twenty tumours had already been screened in a previous study for TP53 mutations.

Results: Low TS protein levels in primary stage III colon tumours appeared to be associated with mucinous histology and low DPD protein levels with young age at time of randomisation. Concordance between TS and DPD expression in primary and metastatic tumours was low. No associations were found between disease-free survival (DFS) and TS or DPD protein levels. When stratified by TP53 mutation status DFS did not differ with TS expression.

Conclusions: Expression of TS and DPD proteins is not predictive for survival in patients with stage III colon cancer treated adjuvantly with 5-FU regimens. TS protein levels did not alter the effect of TP53 mutation status.

Key words: colon cancer, dihydropyrimidine dehydrogenase, prediction 5-fluorouracil regimen, thymidylate synthase, TP53

Introduction

5-Fluorouracil (5-FU) has proven to be effective in the adjuvant treatment of patients with stage III colon cancer, increasing the survival rate by 15% [1, 2]. The action of 5-FU mainly depends on intracellular conversion to its active metabolite, 5-fluoro-2’-deoxyuridine-5’-monophosphate (FdUMP), which inhibits DNA synthesis by forming a stable complex with thymidylate synthase (TS) in the presence of folates [3]. Inhibition of FdUMP by TS leads to depletion of 2’-deoxythymidine-5’-monophosphate (dTMP) and 2’-deoxythymidine-5’-triphosphate (dTTP), inhibiting DNA synthesis and initiating cell cycle arrest or cell death, a process in which p53 also plays an important role [4].

Dihydropyrimidine dehydrogenase (DPD), the first and rate-limiting enzyme in the three-step pathway of uracil and thymine catabolism, is also important in the degradation and inactivation of 5-FU. DPD converts over 85% of clinically administered 5-FU into the inactive metabolite dihydrofluorouracil, a process that mainly occurs in the liver [5].

Different therapeutic strategies, including additional administration of leucovorin (5-formyltetrahydrofolate), have been explored to enhance the anticancer activity of 5-FU. Leucovorin increases the intracellular levels of reduced folate, which is necessary for optimal binding of FdUMP to TS, resulting in a prolonged inhibition of TS and subsequently of DNA synthesis [6]. Therefore, adding leucovorin to 5-FU is generally thought to improve the response to 5-FU and to prolong survival. TS protein also functions as an RNA-binding protein. It can bind to its own mRNA, that of c-myc and that of p53, thereby repressing translation [7–9]. If FdUMP is bound to TS, no binding is possible to mRNA, resulting in an increase of TS, c-myc and p53 expression.

Literature is confusing regarding the prognostic or predictive value of TS and or DPD. In the past, TS has been evaluated in patients who did not receive subsequent adjuvant chemotherapy.

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In such studies TS can be considered as prognostic and low TS values have been related to a longer survival [10]. However, when patients were adjuvantly treated with 5-FU-containing chemotherapy, high TS levels appeared to be predictive of a longer survival [10–13], while others found no survival difference [14–16]. The predictive value of TS in treatment of patients with advanced disease is more straightforward, since in most studies a low TS level was associated with a longer survival and/or a better response rate to 5-FU-containing therapy [17–19]. However, this relationship appeared to be limited to TS levels determined in metastases, rather than in primary tumours [18, 19]. From these studies it can be concluded that the role of TS as a predictive factor is dependent on the type of disease to be treated with a 5-FU-containing regimen. Regarding the prognostic value of TS, data are relatively limited because of the low number of patients available for evaluation after receiving surgery alone. Regarding the role of DPD as a prognostic factor, the data are even more limited. Most studies focused on the role of DPD as a predictive marker for patients treated with a 5-FU-containing regimen. A low DPD level was generally related to either a longer survival or a better response rate [20–23] to a 5-FU containing regimen. In combination, the predictive value of DPD was even better [12, 23]. In some studies DPD was not predictive or prognostic [24, 25], but in none of the reported studies was DPD a negative predictive parameter.

Since TP53 plays a crucial role in the cellular response to 5-FU-induced DNA damage and TP53 is found mutated in ~50% of colorectal cancers, loss of functional p53 might reduce the response to 5-FU [26, 27]. Therefore, TS might play a pivotal role in regulating cell cycle checkpoints and apoptosis through regulation of p53 expression and other cell cycle-related proteins in response to 5-FU treatment. Suppression of p53 by TS may abrogate G1 phase arrest and subsequently DNA repair. It is still not clear whether this response to 5-FU regulated by TS is TP53 status dependent. Chu et al. [7, 8] showed in a series of studies that TS not only showed autoregulation of its translation, but also that p53 plays an important role in the regulation of translation of TS protein. This effect was dependent on the TP53 status, mutated or not. In a previous study, we found that presence of TP53 mutation in colon tumours was associated with a shorter disease-free survival (DFS) \( (P = 0.009) \). Therefore, we included the TP53 mutation status in our analysis.

In this study we wanted to (semi-)quantitatively investigate TS and DPD protein levels by immunohistochemical analysis of primary tumours and lymph node metastases from stage III colon cancer patients adjuvantly treated with 5-FU-based chemotherapy, on tissue microarrays. We analysed a homogenous group of patients treated with either 5-FU–levamisole or 5-FU–levamisole–leucovorin. Since leucovorin did not add to the outcome of treatment, these groups of patients can be considered as equivalent and form a large homogenous group suitable for evaluation of predictive parameters [28]. In addition we determined whether the use of leucovorin and TP53 mutation status of the primary tumour, both thought to be functionally linked to TS activity, altered the effect of TS protein level.

**Materials and methods**

**Patient material**

Formalin-fixed, paraffin-embedded primary tumour specimens from 391 patients with stage III colon cancer from a nationwide randomised colon trial (CKVO 90–11) were included in this study. All patients underwent curative resection and were randomly assigned to one of two adjuvant 5-FU-based chemotherapy schedules (5-FU–levamisole or 5-FU–levamisole–leucovorin) for a period of 1 year, as previously described in more detail [28].

For all patients, clinical and tumour characteristics were derived from the clinical database maintained at the Comprehensive Cancer Centre North Netherlands. The mean age of all patients at the time of randomisation was 58.7 years (range 26.2–75.8). Two hundred and thirteen patients (54.5%) were males. One hundred and eighty-two tumours (46.5%) were right-sided, i.e. proximal of the splenic flexure. With regard to histological grade, 17.5% of the tumours were well differentiated, 57.3% moderately differentiated and 25.2% were poorly differentiated. According to the tumour–node–metastasis (TNM) staging system, 13.6% of tumours were in stages T1 and T2 and 86.4% in stages T3 or T4. Furthermore, 74.2% of the resection specimens belonged to stage N1, while 101 (25.8%) were staged N2 [29]. Two hundred and twenty tumours had previously been screened for TP53 mutations using denaturing gradient gel electrophoresis (exons 4–8) and DNA sequencing [30]. Of these, 116 showed presence of a TP53 mutation (Westra JL, Schaapveld M, Hollema H et al. J Clin Oncol 2005: 23; in press).

**Tissue microarrays**

Representative tumour tissue from all included patients were selected using haematoxylin and eosin-stained slides and arrayed into five tissue microarrays, constructed according to standard protocols [31]. Briefly, three 0.6-mm tissue cores were taken from representative regions of each tumour, using the manual tissue arrayer (MTA-I) from Beecher Instruments (Sun Prairie, WI, USA) and these were put into a standard-size recipient paraffin block, the tissue microarray block. Each array contained 322 tissue cores, representing 94 tumour samples in triplicate; 10 internal controls (i.e. the same on all five arrays) in duplicate; and 10 normal tissues in duplicate. In total, five arrays were made: four with primary tumour samples and one with matching lymph node metastases. Four-micrometre sections were cut from each array block, using the paraffin tape-transfer system with adhesive-coated slides from Instrumedics (Hackensack, NJ, USA).

**Immunohistochemical analysis of TS and DPD**

TS and DPD staining was performed as reported by Van Triest et al. [32] and Huang et al. [33], respectively. Briefly, the sections were deparaffinised and rehydrated. Antigen retrieval was performed by heating the slides in a microwave oven at 750 W for 10 min in a 10 mM sodium citrate buffer (pH 6.0), followed by cooling to room temperature for 20 min. Subsequently the sections were washed, blocked and incubated for 1 h with the primary polyclonal TS (diluted at 1:100) or DPD (rabbit IgG, diluted at 1:500) antibody. They were then incubated for 1 h with the secondary antibody (biotinylated goat anti-rabbit IgG, diluted 1:300), followed by incubation with the horse-radish peroxidase-labelled avidin–biotin complex for 30 min. The antibody binding was visualised with 3-amino-9-ethylcarbazole (AEC). Lastly, the sections were counterstained with haematoxylin.

The immunostaining sections of TS and DPD were scored using a visual grading system of four categories based on intensity (0 = no expression, 1 = low expression, 2 = moderate expression, 3 = strong expression). The score was determined by the highest degree of staining seen in the tumour tissue cores. Lymph node metastases were also put in triplicate on the tissue microarray to keep tissue loss as low as possible in view of intratumoural variation.
as well. Scoring was done the same way for primary tumour as for nodal metastases, using the average of the three samples. Scoring was performed by two experienced observers without knowledge of the clinical outcome for the patients. For discordant cases the two observers reached consensus. Categories 0 and 1 were considered to represent low expression, and categories 2 and 3 to represent high expression (Table 1). Statistical analyses

For comparison of distributions of continuous variables, the $t$-test or analysis of variance was used. The $\chi^2$-test was used for analysis of categorical variables. DFS was used to assess the prognostic value of the various molecular markers, as it is the least biased indicator of treatment failure, given the fact that disease-specific survival might be influenced by selective use of second-line treatments and overall survival is biased due to deaths attributable to causes unrelated to colon cancer. DFS was defined as the period from the date of randomisation to the date of the first documented relapse or to the date of death, without documented relapse due to colon cancer, or to the date of last contact, whichever occurred first. Censoring occurred when a patient was alive without relapse at last contact or when the patient died of a cause other than colon cancer, not attributable to colon cancer. Time to event curves were estimated using the product-limit method and the survival distributions were compared with the log-rank test and the Breslow test. For testing of concordance, the kappa coefficient and the McNemar–Bowker test were used. All reported $P$ values are two-sided, unless stated otherwise.

Results

TS and DPD protein levels in primary tumours and clinicopathological features

The rate of tissue loss of the tissue microarrays was <15%, which is low compared with reported rates of 15–33% of tissue damage [31]. The TS immunohistochemical staining was as expected with a cytoplasmic location. The interpretation of TS staining between the observers showed a concordance of ~80%. The interpretation of the DPD staining was more difficult, as illustrated by a concordance of 65% between the independent observers. Interpretable results for TS could be obtained in 90% (352 of 391) of the tumour samples. For DPD analysis, 87% (341 of 391) of the tumour samples were informative. Fourteen per cent (48 of 352) of the included samples showed low TS expression (combined categories 0 and 1) and 86% (304 of 352) of the primary tumour samples showed high TS expression (combined categories 2 and 3). For DPD, 26% (88 of 341) of the tumour samples presented with low expression, and 74% showed high expression. Results of immunohistochemical analyses for TS and DPD in combination with clinicopathological features are shown in Table 1. Low levels of TS protein were associated

<table>
<thead>
<tr>
<th></th>
<th>Low TS protein expression</th>
<th>High TS protein expression</th>
<th>$P$ value</th>
<th>Low DPD protein expression</th>
<th>High DPD protein expression</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>48 100</td>
<td>304 100</td>
<td>0.746</td>
<td>88 100</td>
<td>253 100</td>
<td>0.010</td>
</tr>
<tr>
<td>Mean age (at time of randomisation), years</td>
<td>59.0 58.5</td>
<td>0.746</td>
<td>56.2 59.6</td>
<td>0.010</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>32 67</td>
<td>160 53</td>
<td>0.070</td>
<td>49 56</td>
<td>136 54</td>
<td>0.755</td>
</tr>
<tr>
<td>Female</td>
<td>16 33</td>
<td>144 47</td>
<td></td>
<td>39 44</td>
<td>117 46</td>
<td></td>
</tr>
<tr>
<td>Site</td>
<td></td>
<td></td>
<td>0.995</td>
<td></td>
<td></td>
<td>0.218</td>
</tr>
<tr>
<td>Proximal</td>
<td>22 46</td>
<td>138 45</td>
<td>0.043</td>
<td>46 52</td>
<td>113 45</td>
<td>0.535</td>
</tr>
<tr>
<td>Distal</td>
<td>26 54</td>
<td>166 55</td>
<td></td>
<td>42 48</td>
<td>140 55</td>
<td></td>
</tr>
<tr>
<td>Histology</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-mucinous</td>
<td>27 50</td>
<td>218 72</td>
<td>0.010</td>
<td>57 65</td>
<td>179 71</td>
<td>0.069</td>
</tr>
<tr>
<td>Mucinous</td>
<td>20 42</td>
<td>73 24</td>
<td></td>
<td>25 28</td>
<td>66 26</td>
<td></td>
</tr>
<tr>
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<td>1 8</td>
<td>8 3</td>
<td></td>
<td>6 7</td>
<td>6 2</td>
<td></td>
</tr>
<tr>
<td>Differentiation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well/moderate</td>
<td>27 56</td>
<td>221 73</td>
<td></td>
<td>53 60</td>
<td>187 74</td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>14 29</td>
<td>64 21</td>
<td></td>
<td>27 31</td>
<td>51 20</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>7 15</td>
<td>18 6</td>
<td></td>
<td>8 9</td>
<td>15 6</td>
<td></td>
</tr>
<tr>
<td>Tumour invasion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscularis propria</td>
<td>3 6</td>
<td>43 14</td>
<td>0.132</td>
<td>13 15</td>
<td>28 11</td>
<td>0.357</td>
</tr>
<tr>
<td>Subserosa and beyond</td>
<td>45 94</td>
<td>261 86</td>
<td></td>
<td>75 85</td>
<td>225 89</td>
<td></td>
</tr>
<tr>
<td>Tumour-positive lymph nodes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;4</td>
<td>34 71</td>
<td>225 74</td>
<td></td>
<td>66 75</td>
<td>186 74</td>
<td></td>
</tr>
<tr>
<td>≥4</td>
<td>14 29</td>
<td>79 26</td>
<td></td>
<td>22 25</td>
<td>67 26</td>
<td></td>
</tr>
</tbody>
</table>
with mucinous histology \( (P = 0.04) \) and low levels of DPD protein were associated with younger age at time of randomisation \( (P = 0.01; \) low versus high DPD expression, 56.2 and 59.6 years, respectively). No associations with TS or DPD were found for any of the other clinicopathological characteristics.

**Survival related to TS or DPD protein levels in primary tumours**

No significant associations were found between TS or DPD expression levels and DFS \( (P = 0.17, \) Figure 1A; and \( P = 0.09, \) Figure 1B, respectively). Furthermore, we did not observe any combined effect of TS and DPD in relation to DFS \( (P = 0.19) \).

**Concordance between TS and DPD protein levels in primary tumours and lymph node metastases**

The levels of TS and DPD expression in the primary tumours were not concordant with their expression levels in the lymph node metastases (Table 2A and B). The kappa coefficients of the results of immunohistochemical staining of TS and DPD were 0.16 and 0.06, respectively. Percentages of agreement were 59\% and 53\%, respectively. These agreements were considered as poor when kappa was <0.20 and as good when kappa was >0.60. Lymph node metastases showed loss rather than gain of TS expression compared with matching primary tumours.

**Survival related to TS or DPD expression in nodal metastases**

Patients whose tumours had a high expression of TS or DPD seem to show a slightly longer survival than those whose tumours had a low expression. The log-rank test showed that this difference in DFS, when comparing low versus high TS or DPD expression levels in the tumour-positive lymph nodes, was not significant (Table 3A and B; \( P = 0.298 \) and \( P = 0.200 \), respectively).

**Survival related to the TP53 mutation status stratified by TS protein expression**

Mutant TP53 status of the primary tumour, stratified by TS protein levels, was associated with a worse DFS \( (P = 0.0019; \) Figure 2). Although TS expression did not change the prognostic effect of TP53 status significantly, the effect of TP53 status appeared to be stronger in the group with high expression of TS protein \( (P = 0.0001) \). In fact, in univariate analysis no effect of TP53 mutation status was seen in the low TS expression group \( (P = 0.30) \), probably because the number of low TS expressing tumours was small.

![Figure 1.](image-url)
whether the suggested association of low DPD expression with younger than those with a high intratumoural DPD expression/C24 with the mean age at time of randomisation. Patients with a low and DPD protein levels, an association between TS and mucin-

Therefore, TS and DPD protein levels may predict the DFS in patients treated with 5-FU regimens and antifolates. 5-FU likely depend on the expression levels of TS and DPD.

Resistance of 5-FU in relation to TS usually depends on altered kinetics of TS, increased dUMP level, decreased FdUMP concentration, reduced stability of the ternary complex and intra-

kine
tics of TS, increased dUMP level, decreased FdUMP concentra-
tion, reduced stability of the ternary complex and intra-

clu
tcellular depletion of folates [3, 4]. Moreover, 80–90% of the administered dose of 5-FU is catabolis
ted by DPD in the liver, and consequently patients with reduced DPD activity have a high risk of severe 5-FU toxicity [5]. Since TS is the target enzyme for 5-FU and DPD is the rate-limiting enzyme in the catabolism of 5-FU, differences in chemosensitivity of colon tumours on 5-FU likely depend on the expression levels of TS and DPD. Therefore, TS and DPD protein levels may predict the DFS in patients treated with 5-FU regimens and antifolates.

In searching associations between clinical parameters and TS and DPD protein levels, an association between TS and mucin-

ous histology was noted. For DPD, an association was found with the mean age at time of randomisation. Patients with a low intratumoural DPD protein expression were in general ~3 years younger than those with a high intratumoural DPD expression (56.2 and 59.6 years, respectively). This raises the question of whether the suggested association of low DPD expression with longer survival [21, 22] is due to the low DPD level proper or to other factors associated with younger age.

We did not find any prognostic significance of TS expression. This is in agreement with several adjuvant studies [14–16]. Our study also showed no prognostic relevance of intratumoural DPD expression in adjuvantly treated patients with stage III colon cancer. This is in agreement with results published by Kornmann et al. [24] and Ikeguchi et al. [25], but is in contradiction to results published by others [21–23]. A combined TS and DPD effect was not found in our study, probably due to many subgroups and the relatively large group of patients with high TS expression. We might exclude the possible existence of a strong combined effect, since it would have been detected even with small numbers.

Why are the results of the studies mentioned so different? The effect of adjuvant chemotherapy in stage III colon cancer is limited to ~10% of the treated patients and only in these 10% is a predictive parameter of value, because the other patients would already have been cured by surgery alone. This may lead to a lack of statistical power to determine whether TS or DPD are predictive for survival of patients receiving adjuvant therapy. For treatment of patients with advanced disease the group to be evaluated is different, because all patients have cancer that is sensitive or resistant to chemotherapy. A partial explanation may be found in the way TS and DPD protein levels were determined. Another technical explanation might be the difference in antibodies used in the different reported studies, monoclonal (TS106 or RTSMA2) or polyclonal, as in our study, to recognise different epitopes [32]. A clinical explanation might be the selection of patients that are very heterogeneous in the different studies, with respect to both tumour stage and tumour location (in colon or rectum), and with respect to treatment regimens (5-FU-based or other; bolus or infusional administration), in adjuvant settings or related to advanced disease. Comparison of results of the reported studies is therefore very difficult.

As mentioned, TP53 plays a central role in cell cycle control and apoptosis, and also seems to be important in the response to 5-FU-induced DNA damage under TS inhibition [26, 27]. As 220 of our tumours had already been screened for TP53 mutations, we were able to investigate a possible combined effect of TP53 and TS. TS expression levels did not change the adverse prognostic effect of TP53 that we found earlier (Westra et al.). This absence of a TS effect does not suggest an interaction between TS and TP53, which is in line with results of others [34, 35], who found TS inhibition in response to 5-FU to be independent of TP53 status. It should be noted, however, that the group with low intratumoural TS expression is small and that analysis therefore lacks the power to confirm the hypothesis that the highest response to 5-FU, implying a longer survival, would be seen in the p53 wild-type cases in the low TS expressing group.

The trial in which our patients participated did not show a survival benefit when leucovorin was added to the combination of 5-FU and levamisole [28]. We found no effect of leucovorin on DFS of patients stratified by TS expression in their primary tumours.

When comparing expression levels of TS in primary tumours and lymph nodes, a low concordance of 59% was found, almost

**Table 3A.** Disease-free survival (DFS) of 79 patients according to thymidylate synthase expression in tumour-positive nodes

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Observed events</th>
<th>Expected events</th>
<th>Log-rank test</th>
<th>3-year DFS (%)</th>
<th>5-year DFS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None/low</td>
<td>38</td>
<td>21</td>
<td>17.8</td>
<td>0.298</td>
<td>48.1</td>
<td>41.6</td>
</tr>
<tr>
<td>High/intermediate</td>
<td>41</td>
<td>16</td>
<td>19.2</td>
<td></td>
<td>65.4</td>
<td>58.5</td>
</tr>
</tbody>
</table>

**Table 3B.** Disease-free survival (DFS) of 80 patients according to dihydropyrimidine dehydrogenase expression in tumour-positive nodes

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Observed events</th>
<th>Expected events</th>
<th>Log-rank test</th>
<th>3-year DFS (%)</th>
<th>5-year DFS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None/low</td>
<td>38</td>
<td>20</td>
<td>16.2</td>
<td>0.200</td>
<td>50.2</td>
<td>46.6</td>
</tr>
<tr>
<td>High/intermediate</td>
<td>42</td>
<td>16</td>
<td>19.8</td>
<td></td>
<td>65.7</td>
<td>59.8</td>
</tr>
</tbody>
</table>

**Effect of leucovorin on DFS of patients with low and high TS expression in primary tumours**

No survival differences were observed when we compared the low and high TS protein levels in primary tumours of patients receiving leucovorin with 5-FU + levamisole and of those who received 5-FU + levamisole only (log rank, \( P = 0.20 \)). There was also no survival difference between patients receiving chemo-
therapy for <6 months or for ≥6 months, stratified by TS protein levels (log rank \( P = 0.44 \) and \( P = 0.55 \), respectively).

**Discussion**

Resistance of 5-FU in relation to TS usually depends on altered kinetics of TS, increased dUMP level, decreased FdUMP concentra-
tion, reduced stability of the ternary complex and intra-
cellular depletion of folates [3, 4]. Moreover, 80–90% of the administered dose of 5-FU is catabolised by DPD in the liver, and consequently patients with reduced DPD activity have a high risk of severe 5-FU toxicity [5]. Since TS is the target enzyme for 5-FU and DPD is the rate-limiting enzyme in the catabolism of 5-FU, differences in chemosensitivity of colon tumours on 5-FU likely depend on the expression levels of TS and DPD. Therefore, TS and DPD protein levels may predict the DFS in patients treated with 5-FU regimens and antifolates.

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We did not find any prognostic significance of TS expression. This is in agreement with several adjuvant studies [14–16]. Our study also showed no prognostic relevance of intratumoural DPD expression in adjuvantly treated patients with stage III colon cancer. This is in agreement with results published by Kornmann et al. [24] and Ikeguchi et al. [25], but is in contradiction to results published by others [21–23]. A combined TS and DPD effect was not found in our study, probably due to many subgroups and the relatively large group of patients with high TS expression. We might exclude the possible existence of a strong combined effect, since it would have been detected even with small numbers.

Why are the results of the studies mentioned so different? The effect of adjuvant chemotherapy in stage III colon cancer is limited to ~10% of the treated patients and only in these 10% is a predictive parameter of value, because the other patients would already have been cured by surgery alone. This may lead to a lack of statistical power to determine whether TS or DPD are predictive for survival of patients receiving adjuvant therapy. For treatment of patients with advanced disease the group to be evaluated is different, because all patients have cancer that is sensitive or resistant to chemotherapy. A partial explanation may be found in the way TS and DPD protein levels were determined. Another technical explanation might be the difference in antibodies used in the different reported studies, monoclonal (TS106 or RTSMA2) or polyclonal, as in our study, to recognise different epitopes [32]. A clinical explanation might be the selection of patients that are very heterogeneous in the different studies, with respect to both tumour stage and tumour location (in colon or rectum), and with respect to treatment regimens (5-FU-based or other; bolus or infusional administration), in adjuvant settings or related to advanced disease. Comparison of results of the reported studies is therefore very difficult.

As mentioned, TP53 plays a central role in cell cycle control and apoptosis, and also seems to be important in the response to 5-FU-induced DNA damage under TS inhibition [26, 27]. As 220 of our tumours had already been screened for TP53 mutations, we were able to investigate a possible combined effect of TP53 and TS. TS expression levels did not change the adverse prognostic effect of TP53 that we found earlier (Westra et al.). This absence of a TS effect does not suggest an interaction between TS and TP53, which is in line with results of others [34, 35], who found TS inhibition in response to 5-FU to be independent of TP53 status. It should be noted, however, that the group with low intratumoural TS expression is small and that analysis therefore lacks the power to confirm the hypothesis that the highest response to 5-FU, implying a longer survival, would be seen in the p53 wild-type cases in the low TS expressing group.

The trial in which our patients participated did not show a survival benefit when leucovorin was added to the combination of 5-FU and levamisole [28]. We found no effect of leucovorin on DFS of patients stratified by TS expression in their primary tumours.

When comparing expression levels of TS in primary tumours and lymph nodes, a low concordance of 59% was found, almost
equal to the value expected by chance. This agrees with results reported by others [18, 36]. We could not confirm that high TS levels in metastases predict for non-responsiveness to 5-FU, which normally leads to shorter survival [18, 20].

In conclusion, in this group of stage III colon cancer patients treated adjuvantly with a 5-FU-containing regimen, expression of TS and DPD proteins are not predictive for survival of patients. Concordance between TS and DPD protein levels determined in primary tumours and in the matching lymph node metastases was low. TS protein levels did not alter the effect of either adding leucovorin to the adjuvant treatment or the effect of TP53 mutation status.

![Survival analysis according to TP53 mutation status stratified by thymidylate synthase (TS) expression. DFS, disease-free survival.](image)

Figure 2. Survival analysis according to TP53 mutation status stratified by thymidylate synthase (TS) expression. DFS, disease-free survival.
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References


