Thymidylate synthase gene variations: predictive and prognostic markers

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Abstract

Since its introduction more than 50 years ago by Heidelberger et al., the fluoropyrimidine 5-fluorouracil (5-FU) has remained the mainstay of therapeutic regimens used in the treatment of colorectal cancer and other human malignancies, with single-agent response rates of 20% to 25% in advanced disease stage. Pharmacogenomics has emerged as a useful tool to address interindividual gene variations by analyzing the interplay of host and tumor genotype and drug efficacy and toxicity. Having a reliable panel of prognostic and predictive markers will be critical in selecting an individualized and tailored chemotherapy regimen based on the particular tumor and host genotype. Although conflicting results have been reported, higher thymidylate synthase (TS) protein and mRNA expression levels in tumors have generally been associated with poor clinical outcome in patients treated with 5-FU–based chemotherapy regimens. However, the cause of the variability in TS expression still remains not fully understood, although several germ-line polymorphisms seem to affect the expression of TS, some of which have been found to have an effect on prognosis and the probability of response to 5-FU–based chemotherapy. This review will provide an update on pharmacogenomic studies of TS that were aimed at elucidating their role as prognostic and predictive markers. [Mol Cancer Ther 2009;8(5):OF1–8]

Introduction

Colorectal cancer is the second leading lethal malignancy in the United States. In 2008, an estimated 148,810 new cases will be diagnosed and 49,960 people will die from this disease (1). Since its introduction more than 50 years ago by Heidelberger et al. (2), the fluoropyrimidine 5-fluorouracil (5-FU) has remained the mainstay of therapeutic regimens used in the treatment of colorectal cancer and other human malignancies. Although this traditional chemotherapeutic option has improved the median overall survival (OS) from 18 to 24 months, limitations with this treatment remain (3–6).

It remains a challenge for oncologists to understand the wide variation in response and toxicity among patients undergoing 5-FU–based chemotherapy. For example, one patient may have a significant response to a chemotherapeutic regimen, whereas another has progression of disease. The same dose and administration schedule of 5-FU may result in severe dose-limiting toxicity in one patient but not in another of the same age and sex. Pharmacogenomics has emerged as a useful tool to address these variations by evaluating the interplay between genotype and drug efficacy and toxicity (7, 8). The possibility of individualizing cancer treatment is gaining wide acceptance, and numerous germ-line polymorphisms that influence enzyme function or expression, which may predict clinical outcome and toxicity to chemotherapy, have been identified. The goal of this review is to provide an update of the most recent data on 5-FU metabolism and thymidylate synthase (TS) gene variations in colorectal cancer.

TS Gene Expression

The mechanism of action of 5-FU is through the inhibition of TS. On entry to the cell, 5-FU is converted to 5-fluoro-2-deoxyuridine monophosphate (dUMP) and forms a stable ternary complex with TS, the sole de novo source of thymidine in the cell. Inhibition of TS rapidly shuts off DNA synthesis and triggers apoptosis and other cell death processes (9, 10).

TS expression as a determinant of sensitivity to fluoropyrimidines has been shown in vitro (11, 12), and intratumoral TS expression in vivo may play an important role in determining tumor sensitivity to 5-FU. A substantial body of evidence has been accumulated over the past years, showing that TS expression varies considerably among tumors and that the response rates of tumors toward 5-FU–based chemotherapy regimens are inversely related to intratumoral TS mRNA and protein expression (13–15). Leichman et al. (15, 16) were the first to show a significant inverse relationship of intratumoral TS gene expression and response to 5-FU–based chemotherapy. In their study,
46 tissue samples of patients with colorectal cancer were analyzed for TS mRNA gene expression using quantitative real-time reverse transcription-PCR. Patients with low TS gene expression levels had a significant higher response rate and showed a superior median survival of 13.6 months compared with 8.2 months in patients whose tumors had an increased TS level (P = 0.02; ref. 16). Although conflicting results have been reported (17), higher TS protein and mRNA expression levels in tumors have generally been associated with poor clinical outcome in patients treated with 5-FU–based chemotherapy regimens (18). In a recent meta-analysis by Popat et al., 13 studies consisting of 887 patients with metastatic colorectal cancer as well as 7 studies consisting of 2,610 patients with localized colorectal cancer were analyzed. The authors showed that tumors expressing high levels of TS seemed to have a poor OS compared with tumors expressing low levels (18). However, the cause of the variability in TS expression still remains unclear, although polymorphisms within the TS gene seem to regulate the expression of TS (Fig. 1; Table 1), some of which have been found to have an effect on the probability of response to 5-FU–based chemotherapy.

**TS Gene Polymorphisms**

**TS Promoter Enhancer Region Polymorphism.** Although intracellular TS mRNA and TS protein levels seem to be crucial for 5-FU–based chemotherapy regimens, the mechanism by which TS expression is regulated has not yet been fully elucidated (14, 19). TS expression seems to be affected by a highly polymorphic tandem repeat in the TS promoter enhancer region (TSER). Horie et al. (20) were the first to describe a germ-line polymorphism upstream of the TS translational start site, containing either double (2R) or triple (3R) tandem repeats of 28-bp sequences (Fig. 1; Table 1). In addition, allelic frequencies of TS 2R/3R repeat polymorphisms are different among ethnic populations (20–23). Although allelic frequencies are similar among Caucasians and southwest Asians, homozygous triple repeat subjects are nearly twice as common in Chinese subjects (67%) compared with Caucasian subjects (38%; ref. 22). Pullarkat et al. (13) showed for the first time that the triple repeat yields 4-fold (9.42) higher TS mRNA levels in tumor tissue obtained from patients with metastatic colorectal cancer in comparison with patients who carry the 2R variant (2.60; P < 0.004). This polymorphism is of clinical significance as greater in vitro enzyme activity

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**Abbreviations:** TSER (Thymidylate Synthase Enhancer Region), 2R (two-tandem repeats) 3R (three-tandem repeats), TSER 3G (Single Nucleotide Polymorphism of TSER 3R), -6bp (TS 3'-UTR 6bp deletion polymorphism)

**Figure 1.** Regulation of TS gene expression (22). TSER polymorphism (TS 2R/3R repeat) is a tandem repeat upstream of the TS translational start site containing either double (2R) or triple (3R) repeats of 28-bp sequences. These tandem repeats regulate transcription and translation of TS. Additional functional variants of the TS gene have been identified and TS 2R/3R repeat is now studied together with a G to C SNP within the second repeat of the 3R allele. TSER 3RC/3RC genotype causes lower transcriptional activity of TS, comparable with the TS 2R/2R genotype. TS 1494del6bp is another functional variant of the TS gene and has been shown to decrease RNA stability and therefore influence TS mRNA and TS protein expression in vitro. TSER 3G, SNP of TSER 3R: -6bp, TS 3'-UTR 6-bp deletion polymorphism.
occurs with the triple repeat than observed for the double repeat (20, 24). Patients with metastatic colorectal cancer homozygous for the triple tandem repeat (TS 3R/3R) had a significant higher intratumoral TS gene expression compared with those with double tandem repeats (TS 2R/2R) within the 5′-untranslated region (UTR) region (13, 25).

**TSER 3R G to C Single Nucleotide Polymorphism.** More recently, additional functional variants within the 5′-UTR region of the TS gene have been identified. Mandola et al. (26) showed that the 28-bp TS tandem repeats contain elements that bind upstream stimulating factor (USF) and that ligand binding by USF-1 and USF-2 enhances transcriptional activity of the TS gene. Electrophoretic mobility shift analysis has shown that the presence of a G to C single nucleotide polymorphism (SNP) within the second repeat of the 3R allele leads to decreased ability of USF to bind within the repeat and therefore results in decreased transcriptional activity of the 3R TS gene variant (26). The authors showed that these polymorphisms are altering mRNA stability and therefore enzyme activity. They showed that whereas phosphorylated USF-1 bound the normal consensus sequence, the G to C substitution abolished binding (26, 27). In vitro transcription analysis showed that the TSER 3RC allele caused a lower transcription rate than the TSER 3RG variant, comparable with the TS 2R/2R genotype. Interestingly, the frequency of the 3RC allele among all 3R alleles showed a variation of 56%, 47%, 28%, and 37% for Whites, Hispanics, African-Americans, and Chinese, respectively. Although the overall frequency was similar to that reported by Mandola et al., Japanese females were noted to have lower frequency of the 3RG allele than males (28).

**TS 3′-UTR 6-bp Deletion.** A third polymorphism of the TS gene is a 6-bp deletion in the 3′-UTR region of the gene (Fig. 1). This polymorphism was identified by aligning expressed sequence tag databases (29). The deletion occurs at an allele frequency of 27% to 29% in Caucasians (+6bp/+6bp, 48%; +6bp/−6bp, 44%; −6bp/−6bp, 7%; refs. 29, 30). The authors proposed that alterations in the 3′-UTR region of the TS gene could alter RNA stability and therefore influence TS mRNA and TS protein expression (29). Preliminary data of 43 patients analyzed for TS mRNA expression and 3′-UTR 6-bp deletion suggest that patients homozygous for the 6-bp deletion allele (−6bp/−6bp) have a 3-fold lower level of steady-state TS mRNA than patients homozygous for the presence of the 6-bp insertion allele (+6bp/+6bp; P = 0.017; refs. 26, 27, 30). A recent study by Dotor et al. (31) illustrated that in series of homogenously 5-FU–treated patients, the presence of homozygous 3′-UTR 6-bp deletion (−6bp/−6bp) seems to be a strong prognostic factor that may be of benefit for at least 20% of the study population.

**Loss of Heterozygosity.** Measurements of TS mRNA expression do not account for the differences in the level of steady-state TS mRNA, which may be altered by translational efficiency and RNA stability (27, 28). In fact, Kawakami et al. (32) showed that TSER might induce high TS protein expression in the absence of increased TS mRNA levels, indicating that TS mRNA with the three-repeat sequence has greater translation efficiency than that with the two-repeat sequence both in vitro and in vivo. The association between the numbers of tandem repeats in the TS gene and TS protein expression suggests that TS genotype information may be a useful predictor of the efficacy of TS-directed chemotherapy. This expectation is based on the assumption that the genotype in the normal tissue would be identical to that in cancer tissue, thus providing valuable clinical information to predict not only response and OS but also toxicity to 5-FU–based treatment. However, recently obtained evidence shows that this assumption is not always true in the case of TS genotype (33, 34).

The TS gene is localized to the short arm of chromosome 18 at chromosome band 18p11.32 (35). Chromosome 18 is generally known to be a site of frequent deletions in colorectal cancer tissues (35). TS itself is not likely to be the target of deletions because it is an essential gene, but if TS is in proximity to the real target gene(s) of chromosome 18 deletions, it may in some cases be contained within the deleted DNA segment. In fact, a high incidence of loss of heterozygosity (LOH) has been observed at the TS locus in cancer tissues, which leads to modification of TS genotype in the tumor when it is heterozygous in normal tissue (33, 34). That is, the occurrence of LOH in individuals who have a heterozygous 2R/3R genotype in their normal tissue may result in a tumor with either a 2R/loss or the 3R/loss TSER genotype. Thus, it is possible that patients who are heterozygous and who have LOH at the TS locus in their tumor tissue might experience considerably different outcomes from chemotherapy depending on which allele became deleted during the LOH event. Zinzindohoué et al. (34) were the first to report on the idea of LOH at

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**Table 1. TS polymorphisms**

<table>
<thead>
<tr>
<th>Location</th>
<th>Frequency</th>
<th>Function</th>
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<tbody>
<tr>
<td>TSER 2R/3R repeat [5′-UTR (rs45445694)]</td>
<td>38–67%*</td>
<td>28-bp repeat (2R, 3R)</td>
</tr>
<tr>
<td>TSER 3R G/C SNP [5′-UTR (rs45445694)]</td>
<td>37–56%*</td>
<td>3R allele = 4-fold $\uparrow$ TS mRNA</td>
</tr>
<tr>
<td>TS 1494del6b [3′-UTR (rs16430)]</td>
<td>27–29%</td>
<td>C allele = $\uparrow$ USF-1 binding within the 2nd repeat of 3R</td>
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<tr>
<td></td>
<td></td>
<td>C allele = $\uparrow$ transcriptional activity of TS</td>
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<tr>
<td></td>
<td></td>
<td>−6bp deletion = $\downarrow$ stability of TS mRNA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+6bp insertion = $\uparrow$ stability of TS mRNA</td>
</tr>
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</table>

*Allelic frequency varies by ethnicity.
the TS locus. The authors showed that TS genotype from 2R/3R heterozygotes differed in ratio between 2R and the 3R bands. The observed LOH frequency at the TS locus was 63% (31 of 50). In this regard, Uchida et al. could show that TS genotype is modulated by LOH. Heterozygous 2R/3R tumor genotype had shorter OS, similar to those of 3R/3R patients, whereas deletion of the 3R allele resulted in a 2R/loss tumor genotype with high risk ratios (RR) and OS similar to homozygous 2R/2R genotypes (33).

### TS Polymorphisms as Prognostic and Predictive Factors

Most studies have consistently agreed that TS expression varies considerably among tumors and that the response rate and toxicity toward 5-FU–based chemotherapy regimens are related to TS mRNA expression levels in the tumor and normal tissue, respectively. In fact, higher expression levels of TS in tumors have generally been associated with poor prognosis and worse response to 5-FU–based chemotherapy regimens (13, 18, 36–38). Interestingly, analysis of TS germ-line polymorphisms may predict not only for response but also for toxicity to 5-FU. Pullarkat et al. showed that colorectal cancer patients with the 3R/3R genotype had significantly less toxicity when compared with the 2R/3R and 2R/2R genotype under 5-FU–based chemotherapy. The authors suggested that this might be due to TS mRNA expression levels in normal tissue, as increased TS mRNA expression in both normal and tumor tissue of patients with the 3R/3R genotype protects the cells against damage by 5-FU treatment due to the low efficacy of TS inhibition. The resulting decreased cell death rate leads to resistance (tumor tissue) and low toxicity (normal cells). On the other hand, it was shown that the lower TS mRNA level in the normal tissue of patients with the 2R/2R or 2R/3R genotype may enhance the cytotoxic effects of 5-FU, leading to more severe side effects in these patients.

**Neo)Adjuvant Colorectal Cancer.** Recent investigation by our group revealed that TS polymorphisms might be used as a surrogate for intratumoral TS levels (13) and the weight of the published literature supports the value of TS 2R/3R repeat polymorphism to be an independent prognosticator of the natural history of disease and as a predictor of responsiveness to 5-FU (13, 36). In fact, Lacopetta et al. (36) investigated the predictive value of TS in 117 patients treated with 5-FU–based adjuvant chemotherapy and 104 untreated patients. The authors showed that stage III colorectal cancer patients with the TS 3R/3R genotype (RR, 0.62; 95% confidence interval, 0.30–1.25; \( P = 0.18 \)) derive less survival benefit from 5-FU–based chemotherapy than those with either the 2R/2R or the 2R/3R genotype (RR, 0.52; 95% confidence interval, 0.32–0.82; \( P = 0.005 \); ref. 36). These findings were confirmed by Pullarkat et al. (13) in patients with metastatic colorectal cancer. In their study, TS genotype was a predictor for tumor response as well as for toxicity (13). Based on these data, TS genotype is a predictive marker (13, 14, 16, 36); further biomarker-embedded and prospective clinical trials are, however, needed to validate the predictive value of TS.

Hitre et al. (39) showed that patients with locally advanced colorectal cancer harboring the 3R variant showed better OS (\( P = 0.009 \)) and progression-free survival (PFS; \( P = 0.048 \)) compared with other genotype combinations, although haplotype analysis of all three functional TS polymorphisms was not done. The possibility of three different polymorphisms in the same gene complicates effort aimed at understanding the effect of each variant and its association with clinical outcome. To date, there are very few studies to correlate all three functional significant TS polymorphisms of the 5′-UTR and 3′-UTR region of the TS gene with clinical outcome. In a recent study by Dotor et al. (31), 129 patients with colorectal cancer were genotyped. In their study, TS 1494del6bp is a prognostic factor for patients receiving adjuvant 5-FU–based chemotherapy, and the 3R/−6bp haplotype was associated with more favorable outcome and response. The strength of this association was stronger for haplotypes harboring the −6bp allele, suggesting a prominent role of the 3′-UTR polymorphism. It should, however, be noted that their findings were based on a heterogeneous and relatively small study population. In fact, 79.1% (102 of 129) of all patients genotyped had tumors in the left colon and rectum and 65.1% (84 of 129) had either stage III or stage IV disease. In this regard, it is being increasingly recognized that colon and rectal cancers are distinct disease groups, in terms of clinical outcome and treatment, and therefore need to be considered separately (40). More recently, our group reported that patients can be separated into prognostic groups according to their TS expression status, as determined indirectly by the comprehensive assessment of all three functional TS polymorphisms. In this study, high-expression variants of TSER 3R G/C, alone or in combination with the TS 1494del6b polymorphism, independently predict tumor recurrence in patients with stage II and III colorectal cancer treated with 5-FU–based adjuvant chemotherapy (23). These findings are consistent with a recent case-control study conducted by Kawakami et al. for patients with gastric adenocarcinoma. In their study, 90 high-risk patients were treated with 5-FU–based chemotherapy after they had undergone R0 surgical resection. The clinical outcome was evaluated according to the genotype (high-expression genotype: 5′-UTR-2R/3G, -3C/3G, -3G/3G, -3UTR +6bp/+6b; low-expression genotype:5′-UTR-2R/2R, +3C/3C, -3C/3C, 3′UTR/UTR UTR −6bp/−6bp; +6bp/+6bp). Patients of the low-expression group had significantly better OS (\( P = 0.001 \)) and PFS (\( P = 0.003 \)) compared with patients of the high-expression group or patients at least carrying one high-expression allele (41).

In addition, the probability of downstaging after neo-adjuvant radiochemotherapy in patients with locally advanced rectal cancer is correlated with the TS 2R/3R repeat genotype. In fact, a study by Villafraanca et al. (42) showed that rectal tumors from patients with the 3R/3R genotype showed a lower probability of downstaging after

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preoperative chemo-(5-FU)-radiation treatment compared with 2R/2R or 2R/3R genotypes (22% versus 60%, respectively; \( P = 0.002 \)). Furthermore, patients harboring the 2R allele also showed a significantly longer PFS compared with patients of the homozygous 3R/3R group (41% versus 81%; \( P = 0.17 \)), although no significant influence on OS could be observed (42).

Interestingly, the first genotype-guided phase II clinical cancer trial is currently under way based on TS 2R/3R repeat genotype (43). Rectal cancer patients (T3 and T4) with the “low-expression” 2R allele are treated with standard therapy (radiation and 5-FU), whereas patients homozygous for the “high-expression” 3R allele are enrolled in a phase II study, in which they receive the standard radiation (45 Gy) and 5-FU (225 mg/m²/d) therapy along with irinotecan (50 mg/m² every week for 5 weeks; FOLFIRI). Preliminary data from this trial showed an improved response rate in both treatment groups, suggesting enrichment for positive response. However, a serious limitation of this trial is the lack of a true control group for both treatment arms and reports on the final results are still being awaited. A summary of these studies is presented in Table 2A.

**Metastatic Colorectal Cancer.** Etienne et al. (44) did a prospective study to examine, among other factors, the association between TS activity and TS 2R/3R repeat polymorphism status in primary tumors and metastases with clinical outcomes in 103 metastatic colorectal cancer patients receiving 5-FU–based chemotherapy. The authors showed that TS genotype does not necessarily correlate with TS activity because highest TS activity was observed with 2R/3R genotype and not as previously described with 3R/3R (13, 20, 45). Although their observation contrasts with the majority of other studies reported, it has been suggested that polymorphisms other than the 5’-UTR tandem repeat and LOH may have an additional effect on translational and posttranscriptional regulation of the TS gene (10, 44).

In this regard, Marcuello et al. (46) conducted a study in 89 patients with metastatic colorectal cancer who were uniformly treated with 5-FU–based chemotherapy. In this study, the authors did a combination analysis of both 5’-UTR TS polymorphisms (TS 2R/3R repeat and TSER 3R G/C SNP; ref. 46), which have been shown to alter TS mRNA and protein expression in vitro and in vivo (26, 28). Clinical outcome was evaluated according to the TS genotype (high-expression genotype: TSER 2R/3G, 3C/3G, and 3G/3G; low-expression genotype: 2R/2R, 2R/3C, and 3C/3C). An improved RR (\( P = 0.04 \)), PFS (\( P = 0.07 \)), and OS (\( P = 0.03 \)) were observed in the group of patients with a low-expression genotype (46), emphasizing the predictive and prognostic value of TS genotyping. A summary of these studies is presented in Table 2B.

**Conclusion**

Despite significant advances in the treatment of colorectal cancer, variation in response and toxicity among patients continues to pose an ongoing challenge for oncologists. Having a reliable panel of prognostic and predictive markers is critical in selecting optimal treatment for a patient based on his individual tumor genotype. Although some markers have been identified and investigated in colorectal cancer, none of them has been routinely used outside of clinical trials. In this review, we discussed polymorphisms within the TS gene as molecular predictive and prognostic markers in the 5-FU pathway that are important in “tailoring” individual chemotherapy.

TS gene expression is modulated by functional significant germ-line polymorphisms in the 5’-UTR (TSER 2R/3R repeat and TSER 3R G/C) and 3’-UTR (TS 1494del6b) of the gene. Further, it is becoming increasingly apparent that TS polymorphisms are not only prognosticators of disease-free survival and OS but also predictors of chemotherapeutic benefit from 5-FU–based chemotherapy.

Many factors account for the discrepant and sometimes contradictory data in the literature; those include small study sample number, lack of standardized methodologies for measuring protein and gene expression, suboptimal samples consisting of different mixtures of cells, tissue-specific differences, and study populations with different allele distributions. For example, measuring higher levels of TS mRNA or TS protein could be ascribed to the usage of laser-captured microdissection, purifying tumor cells from adjacent stroma cells, whereas mRNA analysis of nonmicrodissected tumor tissue could lead to false-negative results. In addition, it should be noted that immunohistochemistry is a semiquantitative and subjective method and is limited by the sensitivity of the monoclonal antibody and the tissue handling. Furthermore, measurements of TS mRNA expression do not account for differences in the quality of TS mRNA, which may be altered by translational efficiency and RNA stability. TS genotyping might overcome those limitations and additionally it would be of major advantage to analyze germ-line polymorphisms of the host normal tissue or peripheral blood, which offers a quick and noninvasive approach.

Despite these technical issues, TS expression and its regulation by various types of TS gene polymorphisms seem to be crucial in predicting response and overall clinical outcome. The possibility of three different polymorphisms in the same gene obviously complicates effort aimed at understanding the functional significance of each individual polymorphism. In the case of TS, there are 18 different allele combinations possible, all of which may theoretically influence clinical outcome. Thus, it is likely that the observed TS expression levels, tumor response, and toxicity may be complicated functions of multiple TS gene alterations rather than the result of one single polymorphism. In addition, the distribution of TS polymorphisms was found to differ among ethnic populations, obviously complicating efforts aimed at studying their predictive and prognostic value.

Expression of selected elements of the 5-FU metabolic pathway is predictive of response to 5-FU–based chemotherapy regimens. High levels of TS, thymidine phosphorylase, and dihydroxyamine are independent predictors of...
Table 2.  TS polymorphisms and their impact on clinical outcome in colorectal cancer

A. TS polymorphisms as predictive and prognostic markers in locally advanced colorectal cancer

<table>
<thead>
<tr>
<th>Study design</th>
<th>Results</th>
<th>Prognostic (OS, PFS)</th>
<th>Predictive (RR, PFS, toxicity)</th>
<th>Reference</th>
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<tr>
<td>221 stage III colorectal cancer patients treated with 5-FU–based adjuvant chemotherapy (117 treated and 104 untreated patients) Tumor genotyping of TS polymorphisms (TS 2R/3R repeat)</td>
<td>(a) Patients with 3R/3R genotype derive less survival benefit from chemotherapy than those with either the 2R/2R or the 2R/3R genotype</td>
<td>n/a</td>
<td>TS 3R/3R vs TS 2R/3R, 2R/2R (adjuvant chemotherapy)</td>
<td>Iacopetta et al. (36)</td>
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<tr>
<td>129 colorectal cancer patients treated with FU plus levamisole or leucovorin in the adjuvant setting were included Tumor genotyping of TS polymorphisms (TS 2R/3R repeat, G/C SNP, TS 1494del6)</td>
<td>(a) TS 3R/3R genotype showed better outcome</td>
<td>(a) TS 3R/3R: OS ↑ (P = 0.020)</td>
<td>(b) G/C SNP added no prognostic information; TS 1494del6 was protective</td>
<td>Dotor et al. (31)</td>
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<tr>
<td>166 colorectal cancer patients with Dukes B2 and Dukes C underwent radical resection and received adjuvant 5-FU–based chemotherapy Genotyping of TS polymorphisms in peripheral blood mononuclear cells (TS 2R/3R repeat, TS 1494del6)</td>
<td>(a) TS 3R/3R genotype showed significantly longer DFS and OS</td>
<td>(a) TS 3R/3R: OS ↑ (P = 0.009)</td>
<td>(b) TS 2R/3R and TS +6b/+6bp combination showed significantly longer DFS and OS</td>
<td>Hitre et al. (39)</td>
</tr>
<tr>
<td>197 colorectal cancer patients treated with FU plus levamisole or leucovorin in the adjuvant setting were included Genotyping of TS polymorphisms (TS 2R3R repeat, G/C SNP, TS 1494del6)</td>
<td>(a) High-expression variants of TSER 3R G/C, alone or in combination with TS 1494del6 polymorphism, independently predict TTR</td>
<td>(a) Low TS expression group</td>
<td>(b) Patients with the TS 3RG/+6bp haplotype are at greatest risk for the development of tumor recurrence</td>
<td>Lurje et al. (23)</td>
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decreased response, and vice versa, lower levels of TS, thymidine phosphorylase, and dihydropyrimidine correlate with higher sensitivity to 5-FU (14). High-expression levels of one of these genes, even in the presence of down-regulation of the other two, have an adverse effect on response to 5-FU. In a few years, microarray technology might be the preferred method of genotyping, with the advent of customizable chips, in addition to chips to test polymorphisms and gene expression. The introduction of new therapeutic agents and the discovery and validation of predictive and prognostic markers along with new screening tools will enable oncologist to tailor patient-specific chemotherapy regimens by maximizing drug efficacy and minimizing adverse and possibly severe side effects. Much work, however, remains to be done. Ongoing and future clinical trials hold promise for further improvements in optimizing and specifying chemotherapy individually not only in prolonging lives but also in augmenting quality of life.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**References**

3. Grothey A, Sargent D, Goldberg RM, Schmoll HJ. Survival of patients with advanced colorectal cancer improves with the availability of

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### Table 2. TS polymorphisms and their impact on clinical outcome in colorectal cancer (Cont’d)

<table>
<thead>
<tr>
<th>Study design</th>
<th>Results</th>
<th>Prognostic (OS)</th>
<th>Predictive (RR, PFS, toxicity)</th>
<th>Reference</th>
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<tbody>
<tr>
<td>89 metastatic colorectal cancer patients received 5-FU–based chemotherapy</td>
<td>(a) Patients with TS low-expression genotype (A-group) had an overall better RR, DFS, and OS than the TS high-expression genotype</td>
<td>(a) Low TS expression group (A)</td>
<td>Toxicity (P = 0.03)</td>
<td>Marcuello et al. (46)</td>
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<tr>
<td>Tumor genotyping of TS polymorphisms</td>
<td>TS genotype as independent predictor of RR, PFS, and OS</td>
<td>(b) High TS expression group (B)</td>
<td>RR (P = 0.04)</td>
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<tr>
<td>Low-expression group = TSER 2R/2R; 2R/3R; 3R/3R</td>
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<td>PFS (P = 0.07)</td>
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<tr>
<td>High-expression group = TSER 2R/3G; 3R/3RG; 3R/3RG</td>
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<td></td>
<td>Toxicity n/a</td>
<td></td>
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<tr>
<td>50 metastatic colorectal cancer patients received 5-FU–based chemotherapy</td>
<td>(a) Patients with homozygous TS 3R polymorphisms had a 3.6-fold higher mRNA expression</td>
<td>Toxicity n/a</td>
<td>RR (P = 0.041)</td>
<td>Pullarkat et al. (13)</td>
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<tr>
<td>Tumor genotyping of TS polymorphisms (TS 2R/3R repeat)</td>
<td>(b) Patients homozygous for TS 3R had worse RR than patients homozygous or heterozygous for TSER 2R</td>
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<td>Toxicity (P = 0.008)</td>
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<td></td>
<td>(c) Inverse relationship of TS 3R and toxicity</td>
<td></td>
<td>RR (P = 0.041)</td>
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<tr>
<td>103 metastatic colorectal cancer patients received 5-FU–based chemotherapy</td>
<td>(a) TS activity was found to be highest in the 2R/3R genotype group (in both the primary tumor and metastases)</td>
<td>(a) TS 2R/2R, 2R/3R, and 3R/3R genotyped tumors had the same response rate</td>
<td>RR (P = 0.047)</td>
<td>Etienne et al. (44)</td>
</tr>
<tr>
<td>Tumor genotyping of TS polymorphisms (TS 2R/3R repeat)</td>
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<td>(b) TS activity ↓</td>
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</table>

Abbreviations: n/a, not available; n.s., not significant; DFS, disease-free survival; TTR, time to tumor recurrence.
fluorouracil-leucovorin, irinotecan, and oxaliplatin in the course of treatment.
Molecular Cancer Therapeutics

Thymidylate synthase gene variations: predictive and prognostic markers

Georg Lurje, Philipp C. Manegold, Yan Ning, et al.

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