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A Study of Thymidylate Synthase Expression as a Biomarker for Resectable Colon Cancer: Alliance (Cancer and Leukemia Group B) 9581 and 89803

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Disclosures of potential conflicts of interest may be found at the end of this article.

Key Words. Colon cancer • Biomarkers • Adjuvant therapy • Thymidylate synthase • Microsatellite instability • Mismatch repair deficiency

Abstract

Purpose. Tumor levels of thymidylate synthase (TS), a target of 5-fluorouracil (5-FU)-based chemotherapy for colorectal cancer, have been studied as a predictive or prognostic biomarker with mixed results.

Patients and Methods. Tumor TS levels were prospectively evaluated in two adjuvant therapy trials for patients with resected stage II or III colon cancer. TS expression was determined by standard immunohistochemistry and by automated quantitative analysis. Tumor mismatch repair deficiency (MMR-D) and *BRAF* c.1799T>A (p.V600E) mutation status were also examined. Relationships between tumor TS, MMR-D, and *BRAF* mutation status, overall survival (OS), and disease-free survival (DFS) were investigated in the subset of stage III patients.

Results. Patients whose tumors demonstrated high TS expression experienced better treatment outcomes, with DFS hazard ratio (HR) = 0.67, 95% confidence interval (CI) = 0.53, 0.84; and OS HR = 0.68, 95% CI = 0.53, 0.88, for high versus low TS expression, respectively. No significant interaction between TS expression and stage was observed (DFS: interaction HR = 0.94; OS: interaction HR = 0.94). Tumors with high TS expression were more likely to demonstrate MMR-D (22.2% vs. 12.8%; $p = .0003$). Patients whose tumors demonstrated both high TS and MMR-D had a 7-year DFS of 77%, compared with 58% for those whose tumors had low TS and were non-MMR-D (log-rank $p = .0006$). Tumor TS expression did not predict benefit of a particular therapeutic regimen.

Conclusion. This large prospective analysis showed that high tumor TS levels were associated with improved DFS and OS following adjuvant therapy for colon cancer, although tumor TS expression did not predict benefit of 5-FU-based chemotherapy. ***The Oncologist*** 2017;22:107–114

Implications for Practice: This study finds that measurement of tumor levels of thymidylate synthase is not helpful in assigning specific adjuvant treatment for colorectal cancer. It also highlights the importance of using prospective analyses within treatment clinical trials as the optimal method of determining biomarker utility.

Introduction

Adjuvant chemotherapy is an important component of treatment for resectable colon cancer. However, although postoperative adjuvant chemotherapy clearly improves survival in a subset of patients with stage III colon cancer, many patients derive no benefit, either because their disease recurs despite adjuvant treatment or because their disease is effectively

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treated by surgery alone. The benefit of adjuvant chemotherapy is even more uncertain for patients with stage II disease, as approximately 80% of these patients are cured by surgery. Unfortunately, there are no markers for stage II colon cancer that reliably define which patients will benefit from chemotherapy, and there are no markers for stage III disease that distinguish patients for whom chemotherapy can be avoided.

Since its first demonstration of clinical benefit for adjuvant therapy in 1993, 5-fluorouracil (5-FU) has been a mainstay of colon cancer treatment, both in the adjuvant and advanced disease settings [1–3]. 5-FU inhibits the activity of thymidylate synthase (TS;TYMS, HGNCID, HGNC:12441), an enzyme required for generation of thymidine monophosphate (dTMP) through a reaction converting 5,10-methylenetetrahydrofolate + deoxyuridine monophosphate (dUMP) to dihydrofolate + dTMP. Lack of TS activity produces a deficit in dTMP that alters the efficiency of DNA synthesis and repair by permitting G mispairs and misincorporation of dUMP, which in turn causes DNA/RNA strand breaks and cell death [4]. Theoretically, tumor TS levels may influence therapeutic response to 5-FU because of this relationship. However, although studies show an association between tumor TS expression and outcomes following 5-FU-based chemotherapy in a variety of patient cohorts, the available literature reports conflicting results.

Currently, the best-defined biomarkers for resectable colon cancer are DNA mismatch repair (MMR) status and the presence of a *BRAF* c.1799T>A (p.V600E) mutation. In sporadic tumors, DNA mismatch repair deficiency (MMR-D), also known as microsatellite instability, is characterized by the inability to repair single nucleotide mismatches due to loss of DNA mismatch repair proteins by transcriptional silencing [5]. MMR-D is present in approximately 25% of stage II and 16% of stage III colon cancers. Patients with colon cancers that demonstrate MMR-D have improved treatment outcomes [6–8]. *BRAF* is a part of the RAS-RAF-MAP2K signaling pathway and *BRAF* mutations are present in 10%–20% of colon cancers. Of sporadic colon cancers that are MMR-D, from 40%–50% also harbor a *BRAF* mutation. The presence of a *BRAF* mutation is associated with significantly worse patient survival in many studies [9].

Tumor TS analysis and determination of MMR status were included as prospective secondary endpoints in two randomized, phase III trials for resectable colon cancer (C9581; C89803). Study C89803, which enrolled patients with stage III, was later amended to include an investigation of *BRAF* status. The purpose of the current study was (1) to determine whether tumor TS levels were associated with survival outcome or benefit of 5-FU adjuvant therapy in patients with resectable colon cancer and (2) to explore the impact of MMR and *BRAF* status upon these relationships.

Patients and Methods

Characteristics of Study Population

In the Alliance for Clinical Trials in Oncology (Alliance)/ Cancer and Leukemia Group B (CALGB) protocol 89803 (C89803), 1,264 patients with stage III colon cancer were randomized following surgery to either adjuvant treatment with 5FU/LV or 5FU/LV and irinotecan (IFL) [10]. Alliance (CALGB) protocol 9581 (C9581) was a trial of 1,738 participants with stage II colon cancer who received edrecolomab versus observation alone [11]. The primary endpoint for both trials was overall survival (OS); disease-free survival (DFS) was a secondary endpoint. In both trials there was no difference in OS or progression-free survival (PFS) among the patients randomized to the standard versus experimental arms of each trial. These protocols were approved by the institutional review board of each treating center and all patients provided written informed consent before participating. The Alliance (CALGB) Statistical Center (Durham, NC) maintained the research database. Treatment details and primary analysis results for these trials were previously published [10, 11].

Determination of Biomarker Status

Paraffin blocks containing normal colon and tumor tissue were processed as previously described [6]. Immunohistochemistry (IHC) using the TS^{106} monoclonal antibody detected the presence of TS in primary tumor specimens. Cases were scored by TS expression on a scale from 0 to 3+, with a score of 0 or 1+ describing "low", and 2+ and 3+ representing "high," according to procedures described by Sinicrope et al. [12]. TS levels in C89803 participants were also assessed using automated quantitative analysis (AQUA), which quantified TS nuclear localization, cytoplasmic localization, the sum of the two, and the ratio as continuous measurements. Tumor MMR status was assessed using IHC to detect the presence of MLH1 and MSH2, as previously described [6]. Tumors lacking expression of either protein were categorized as MMR-D and those exhibiting expression of both proteins were determined mismatch repair intact (MMR-I). Tumor *BRAF* mutational status was determined by polymerase chain reaction and pyrosequencing spanning *BRAF* codon 600, as previously described [9].

Statistical Analysis

The original C9581 and C89803 protocols presented overarching statistical analysis plans for correlative science biomarkers including TS, MMR, and several other markers. Power estimates were provided for multiple detectable hazard ratios and proportions of available patient samples. In this study, 435 patients with stage II (25% of parent study C9581 cohort) and 463 patients with stage III (37% of parent study C89803 cohort) with data on TS are available for analysis. With these sample sizes, hazard ratios (TS high vs. low) of 0.52 and 0.58 are detectable with 80% power (2-sided $\alpha=0.01$ and 0.05, respectively) in C9581, assuming 60% of patients exhibited TS high tumor levels. Similarly, in C89803 hazard ratios of 0.61 and 0.66 were detectable with 80% power (2-sided $\alpha=0.01$ and 0.05, respectively) assuming 44% of patients exhibit TS high tumor levels. The unplanned, pooled analysis allowed detection of 0.67 and 0.72 hazard ratios with 80% power (2-sided $\alpha=0.01$ and 0.05, respectively) assuming 52% of patients with TS high tumor levels. In all cases, power is less than 67% to detect interaction DFS hazard ratios of 2.0 or less in magnitude with 307 events observed.

OS was measured from study entry until death from any cause; DFS was measured from study entry until documented progression or death from any cause. Survival curves were estimated using the Kaplan-Meier method. The log-rank test was used to test associations between TS levels, combined TS and MMR levels, and the survival outcomes (OS, DFS). Cox regression was used to analyze the following potentially prognostic variables simultaneously with TS score and the combined

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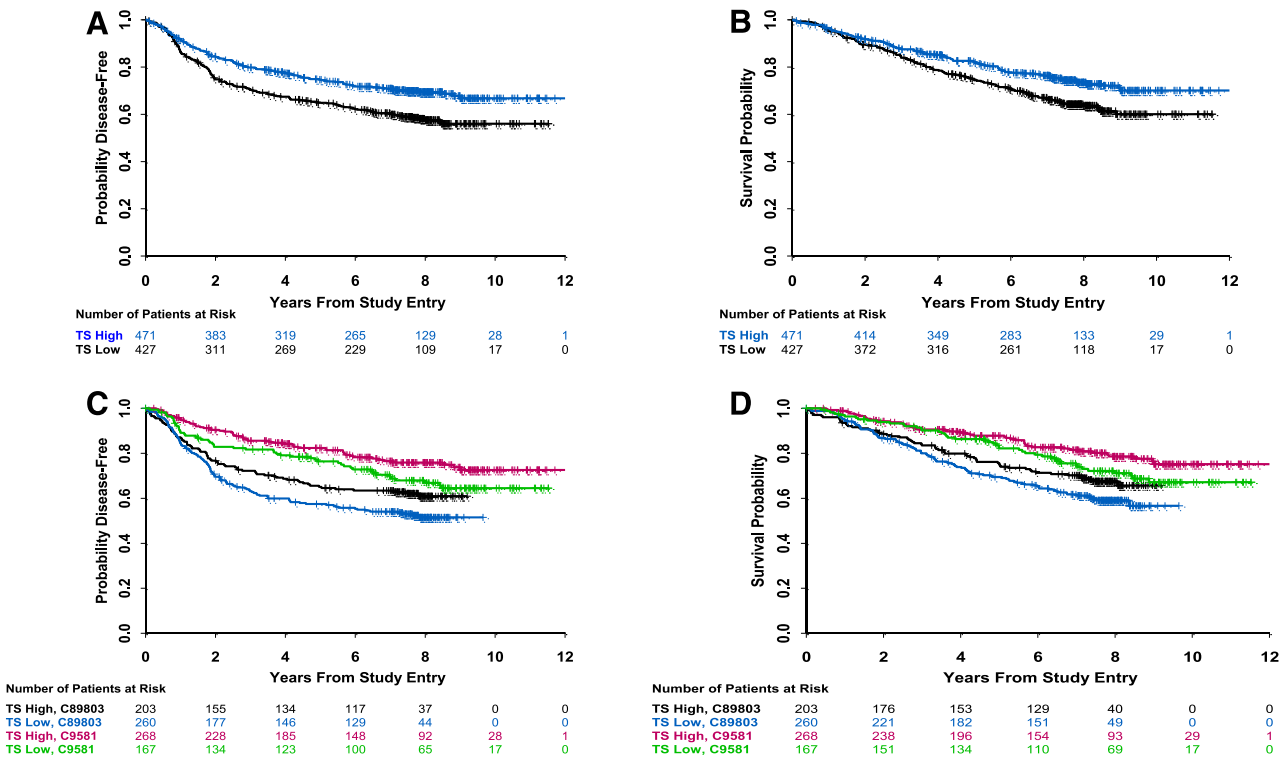

Figure 1. Relationship between tumor TS expression level and treatment outcome in the full cohort. **(A, B)**: DFS and OS, respectively, by TS (IHC) expression level for both studies combined. **(C, D)**: DFS and OS, respectively, by TS (IHC) expression level and study.

Abbreviations: DFS, disease-free survival; IHC, immunohistochemistry; OS, overall survival; TS, thymidylate synthase.

variable based on TS and MMR for DFS and OS: study (C89803 vs. C9581), age (continuous), sex (male, female), race (white, other), tumor location (left side, right side), tumor differentiation (grades I, II; grades III, IV), performance status (0, 1, 2), number of nodes sampled (continuous), T-stage (1, 2, 3, 4), and extravascular invasion (present, absent). The interactions of TS and combined TS and MMR score by study and treatment within study were also considered. In addition, the proportional hazards assumption was assessed using a time-dependent Cox model. The maximal χ^2 method was used to determine the optimal cut point for the four TS variables measured by AQUA (nuclear, cytoplasmic, total, and ratio) relative to association with DFS and OS. Fisher's exact test and the χ^2 test were used to test associations between categorical variables.

Results

High Tumor TS Expression Is Associated With Improved DFS and OS in Patients With Resectable Colon Cancer

TS levels were assessed in 898 of 3,002 patients with resectable colon cancer enrolled on either C9581 (1,738 stage II patients; 435 analyzed by IHC) or C89803 (1,264 stage III; 463 analyzed by IHC and 416 by AQUA). Reporting recommendations for tumor MARKer prognostic studies REMARK diagrams for each trial describing the distributions of cases used for marker analyses are provided in supplemental online Figure 1A, 1B. Patient and tumor characteristics were comparable between the entire study cohort and patients with without analyzed samples and between studies (Table 1). Of the 898 patients studied, 52% (n = 471) were determined to have high TS levels. This proportion of high expression cases is consistent with that of other IHC studies [12–14]. A significantly lower proportion of stage III patients exhibited high TS, 44% (203/463) versus 60% for stage II, $p < .0001$.

Patients whose tumors demonstrated high TS expression experienced better survival outcomes for both DFS (hazard ratio [HR] = 0.67; 95% confidence interval [CI] = 0.53, 0.84; log-rank $p = .0005$; Fig. 1A) and OS (HR = 0.68; 95% CI = 0.53, 0.88; log-rank $p = .002$; Fig. 1B). Seven-year DFS was 0.71 versus 0.60 for high versus low TS expression; 7-year OS was 0.76 versus 0.66, respectively. The proportional hazards assumption was not violated and no significant interaction between TS expression and stage (treatment with 5-FU) was observed (DFS: interaction HR = 0.94; 95% CI, 0.58,1.51; $p_{interaction} = 0.80$; OS: interaction HR = 0.94; 95% CI = 0.56,1.58; $p_{interaction} = 0.83$). Figure 1C and 1D illustrate DFS and OS, respectively, by TS status and study. The association between tumor TS expression and DFS remained significant upon multivariable analysis including known prognostic factors ($p = .03$; Table 2). TS status was not significant in a multivariable analysis of OS.

High Tumor TS Expression in the Subsets of Patients by Stage (With and Without 5-FU-Based Adjuvant Chemotherapy)

We examined outcomes among the patients with stage III disease (C89803) who received adjuvant chemotherapy containing 5-FU. For patients with high tumor TS expression, 7-year DFS was 0.63, compared with 0.54 for those with low TS expression (Table 3; Fig. 2A). Similarly, 7-year OS was 0.70 versus 0.61 for patients with high versus low TS expression (Fig. 2B).

For AQUA, single measurements were available for 40 patients and averages were computed when multiple measurements were available (149 patients with 2 measurements, 226 with 3 measurements, and 1 patient with 6 measurements;

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Table 1. Patient and tumor characteristics by study

Characteristic	89803 Entire cohort	89803 TS by IHC	89803 TS by AQUA	9581 Entire cohort	9581 TS by IHC
No. of patients	1,264	463	416	1,738	435
Age, years, mean (range)	59.9 (21–85)	59.8 (24–83)	60.03 (24–85)	64.1 (24–90)	64.4 (34–89)
Sex					
Male	702 (55.54%)	254 (54.86%)	228 (54.81%)	901 (51.84%)	233 (53.56%)
Female	562 (44.46%)	209 (45.14%)	188 (45.19%)	837 (48.16%)	202 (46.44%)
Treatment					
FU/LV or edrecolomab	629 (49.76%)	228 (49.24%)	218 (52.4%)	865 (49.77%)	234 (53.79%)
IFL or observation	635 (50.24%)	235 (50.76%)	198 (47.6%)	873 (50.23%)	201 (46.21%)
Tumor location					
Proximal	715 (57.85%)	254 (55.46%)	237 (57.66%)	1048 (60.97%)	259 (59.54%)
Distal	521 (42.15%)	204 (44.54%)	174 (42.34%)	671 (39.03%)	176 (40.46%)
Tumor differentiation					
Well-differentiated	71 (5.74%)	24 (5.23%)	21 (5.1%)	146 (8.49%)	36 (8.31%)
Moderately differentiated	862 (69.68%)	331 (72.11%)	295 (71.60%)	1305 (75.92%)	325 (75.06%)
Poorly or undifferentiated	304 (24.58%)	104 (22.66%)	96 (23.30%)	268 (15.59%)	72 (16.63%)
No. of positive nodes, mean (range)	3.5 (0–29)	3.5 (1–23)	3.5 (1–24)	N/A	N/A
Nodal ratio, mean (range)	0.30 (0.30–1.33)	0.29 (0.01–1.00)	0.31 (0.01–1.00)	N/A	N/A
Nodes sampled, mean (range)	14.4 (1–99)	14.7 (1–99)	14.9 (1–68)	14.4 (0–99)	13.9 (0–59)
7-year DFS (95% CI)	0.57 (0.54, 0.60)	0.58 (0.53, 0.62)	0.62 (0.57, 0.66)	0.74 (0.71, 0.76)	0.73 (0.69, 0.77)
7-year OS (95% CI)	0.65 (0.62–0.67)	0.65 (0.60, 0.69)	0.69 (0.64, 0.73)	0.79 (0.77, 0.81)	0.78 (0.74, 0.82)

Abbreviations: AQUA, automated quantitative analysis; CI, confidence interval; DFS, disease-free survival; FU/LV, 5-fluorouraciland leucovorin; IFL, FU/LV and irinotecan; IHC, immunohistochemistry; OS, overall survival; TS, thymidylate synthase.

Table 2. Multivariable analysis of disease-free survival endpoint[a]

Parameter (Cohort)	*df*	HR	95% CI	Chi-square *p* value
TS by IHC (C89803 + C9581)				
TS score (high)	1	0.77	(0.61, 0.97)	.03
Study (C9581)	1	0.48	(0.37, 0.62)	<.0001
Age (>65 years)	1	1.03	(1.01, 1.03)	.001
Performance status (1; 2)	1	1.37	(1.07, 1.75)	.01
No. of nodes sampled (more)	1	0.98	(0.96, 0.99)	.01
T stage (T4)	1	1.58	(1.19, 2.08)	.001
Extravascular invasion present	1	1.62	(1.12, 2.33)	.009

[a]Potentially prognostic variables included in the model were TS score (high vs. low); study (C9581 vs. C89803), age (continuous), sex (male, female), race (white, other), tumor location (left side, right side), tumor differentiation (grades I, II; grades III, IV), performance status (0,1,2), number of nodes sampled (continuous),T-stage (1, 2, 3, 4), and extravascular invasion (present, absent); $n = 869$ with 296 events.
Abbreviations: CI, confidence interval; HR, hazard ratio; IHC, immunohistochemistry; TS, thymidylate synthase.

total $n = 416$). Optimal cut points were obtained for each AQUA measurement and survival outcome (DFS, OS). Results using the AQUA method were consistent with those achieved by IHC, although the distribution of high- versus low-expressing cases was different (e.g., high nuclear expression in 70%; Table 3). Tumor TS expression was also examined as a predictive factor for benefit of IFL versus standard 5-FU/LV. By either IHC or AQUA methods, TS expression did not predict differential benefit to treatment with IFL. Supplemental online Figure 2a, 2b illustrates DFS and OS for TS by IHC and study treatment in C89803.

For the stage II patients on C9581, none of whom were treated in the adjuvant setting with 5-FU, 7-year DFS was 0.76 versus 0.69, and OS was 0.81 versus 0.74 for high versus low TS expression, respectively (Table 3; Fig. 2C, 2D). No significant interactions between TS expression levels and treatment (edrecolomab versus observation) were found for either DFS or OS (supplemental online Fig. 2c, 2d).

Combination of TS Expression and Tumor MMR Status May Permit Stratification of Patients Into High- and Low-Recurrence Risk and Survival Categories

Previous analyses of C89803 and C9581 showed that tumor MMR-D was a marker of favorable outcome [6, 15]. For the combined cohort, patients whose tumors demonstrated MMR-D achieved better 5-year DFS (76% vs. 67%; log-rank $p < .001$) compared with those whose tumors were MMR-I. We therefore studied TS status in relationship to MMR status in the

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Table 3. Outcome by TS expression level

		DFS					OS				
	n	7-yr DFS	95% CI	*p* value	HR	95% CI	7-yr OS	95% CI	*p* value	HR	95% CI
Patients receiving 5-FU-based chemotherapy on Study C89803: tumor TS expression measured by immunohistochemistry[a]											
High TS	203	0.63	(0.56, 0.69)	0.05[b]	0.75	(0.56, 1.01)	0.70	(0.63, 0.76)	.09[b]	0.76	(0.55, 1.01)
Low TS	260	0.54	(0.47, 0.60)				0.61	(0.55, 0.67)			
Patients receiving 5-FU-based chemotherapy on Study C89803: tumor TS expression measured by AQUA[c]											
Nuclear				.006[d]	0.55	(0.40, 0.76)			.30[d]	0.67	(0.47, 0.96)
≤6.05	126	0.50	(0.40, 0.58)				0.63	(0.54, 0.71)			
>6.05	290	0.67	(0.61, 0.72)				0.67	(0.61, 0.72)			
Cytoplasmic				.01[d]	0.58	(0.42, 0.80)			.23[d]	0.64	(0.42, 0.97)
≤4.71	117	0.50	(0.40, 0.59)				0.67	(0.61, 0.72)			
>4.71	299	0.66	(0.60, 0.71)				0.76	(0.67, 0.83)			
Total				.009[d]	0.57	(0.41, 0.78)			.11[d]	0.61	(0.41, 0.89)
≤5.16	123	0.51	(0.41, 0.59)				0.65	(0.58, 0.71)			
>5.16	293	0.66	(0.60, 0.71)				0.76	(0.69, 0.82)			
Ratio				.25[d]	1.93	(1.01, 3.66)			.23[d]	1.93	(1.01, 3.66)
≤1.04	43	0.76	(0.60, 0.86)				0.87	(0.72, 0.94)			
>1.04	373	0.60	(0.55, 0.65)				0.67	(0.62, 0.72)			
Patients not treated with 5-FU, Study 9581: tumor TS expression by immunohistochemistry[a]											
High TS	268	0.76	(0.70, 0.81)	.07[b]	0.71	(0.49, 1.04)	0.81	(0.75, 0.86)	.11[b]	0.72	(0.48, 1.08)
Low TS	167	0.69	(0.61, 0.76)				0.74	(0.66, 0.80)			

[a]High TS 5 2+, 3+; low TS = 0,1+.
[b]Log-rank *p* value.
[c]Variables except for the ratio are normalized by 1,000.
[d]Exact Gauss method.
Abbreviations: AQUA, automated quantitative analysis; CI, confidence interval; DFS, disease-free survival; 5-FU, 5-fluorouracil; HR, hazard ratio; OS, overall survival; TS, thymidylate synthase.

current analysis. Tumors with high TS expression were more likely to demonstrate MMR-D (22.2% vs. 12.8%; $p = .0003$). No significant interaction between TS and MMR status was observed (HR = 0.82; 95% CI = 0.42, 1.59, for DFS; HR = 0.62, 95% CI = 0.42, 1.35, for OS). In multivariable analyses including both TS and MMR, TS remained significantly related to DFS but not OS, whereas MMR remained significantly related to OS but not DFS (data not shown).

We further studied the combined variable defined by TS and MMR. Significant differences were found in both DFS (log-rank across four subgroups $p = .0006$) and OS (log-rank across four subgroups $p = .002$) across the four subgroups (Fig. 3A, 3B). For the combined cohort, patients whose tumors demonstrated both high TS and MMR-D had a 7-year DFS of 77%, compared with 58% for those whose tumors had low TS and were MMR-I. Seven-year OS was comparable (77%, 77%, 80%) for patients with high TS and MMR-D tumors, high TS and MMR-I tumors, and low TS and MMR-D tumors, respectively, compared with 64% for patients whose tumors had low TS and were MMR-I.

A significant interaction was found between the combined TS/MMR variable and study for both DFS and OS (DFS: $p_{interaction} = 0.002$; OS: $p_{interaction} = 0.02$). For DFS, the best and worst subgroups among stage II patients were TS high/ MMR-D and TS low/MMR-I, respectively. Intermediate responses were observed for patients whose tumors were TS high/MMR-I or TS low/MMR-D (Fig. 4). Among patients with stage III disease, a finding of MMR-D did not provide a DFS benefit regardless of TS level. These results are consistent with a potential detrimental effect of a 5-FU treatment regimen in patients with MMR-D tumors. No distinct pattern was observed for OS.

Impact of *BRAF* on TS Expression and Tumor MMR Status in Stage III Colon Cancer

Previous results from C89803 showed that *BRAF* mutation is associated with inferior survival, with a 5-year OS of 0.63 versus 0.75 ($p = .015$) for cohorts with or without tumor mutations, respectively [9]. In the current study, characterization of TS, MMR, and *BRAF* was available for 328 stage III patients, and we explored the effect of *BRAF* mutation on treatment outcomes for this subset. Although the numbers were small, the results indicate that the beneficial effect of having a tumor with high TS expression and MMR-D status was negated by the presence of a *BRAF* mutation. Among 26 cases that were high TS and MMR-D and had data on BRAF, 10 also had *BRAF* V600E-mutant tumors. In this subset, the 7-year OS = 0.875,95% CI = 0.58,0.96, versus OS = 0.50,95% CI = 0.18,0.75, for those with and without *BRAF* mutation. Because of the overall small number of cases and low mutation rate for *BRAF* mutation (16%), the contribution of *BRAF* mutation was not explored further.

Discussion

Because of its relationship to DNA synthesis and its role as a target of 5-FU activity, TS protein expression and mRNA levels

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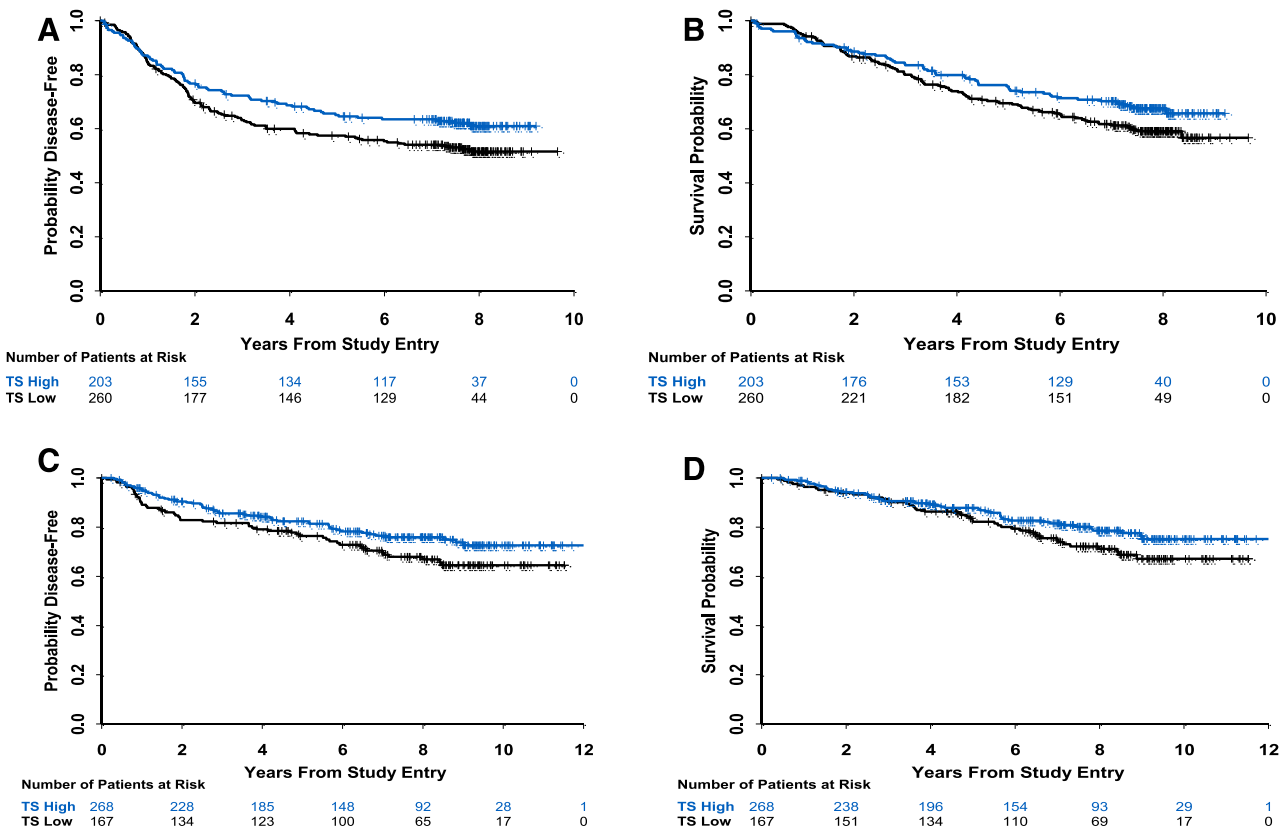

Figure 2. Relationship between tumor TS expression level and treatment outcome within study. DFS and OS, respectively, by TS expression level in C89803 **(A, B)** and C9581 **(C, D)**.

Abbreviations: DFS, disease-free survival; OS, overall survival; TS, thymidylate synthase.

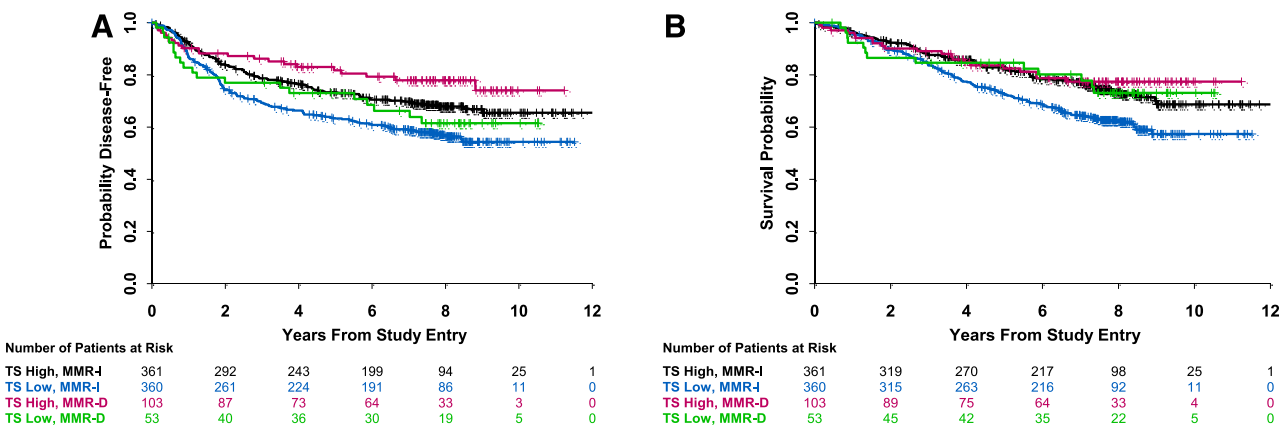

Figure 3. Relationship between TS expression level and MMR status combined and treatment outcome in the full cohort. **(A, B)**: DFS and OS, respectively, by TS expression level and MMR status measured by immunohistochemistry in the full cohort.

Abbreviations: DFS, disease-free survival; MMR, mismatch repair; MMR-D, mismatch repair deficiency; MMR-I, mismatch repair intact; OS, overall survival; TS, thymidylate synthase.

have been extensively studied in colon cancer patients treated in both metastatic and adjuvant settings. Most, but not all, studies of patients receiving 5-FU-based chemotherapy for metastatic disease showed that high TS levels were associated with inferior survival [16–19]. To date, results from the adjuvant setting have been mixed [13, 20, 21]. This prospective study from two large cooperative group trials of stage II and III colon cancers showed that TS status provided independent prognostic information, with improved outcomes for patients with high TS expression. This study also achieved the same result with both a standard IHC technique and AQUA in patients with stage III/high risk stage II disease treated with 5-FU-containing regimens. Results reported for AQUA based on the optimal cut points will need to be validated. The prospective nature and uniform treatment of patients in this study, together with a relatively large sample size, lend further validity to these results.

Prospective biomarker studies included in randomized clinical trials provide the best assessment of biomarker utility. For TS, the only large-scale, prospective data available are from an adjuvant colorectal cancer trial involving continuous intraportal vein infusion of 5-FU that was conducted in 200 hospitals throughout China [13]. This study, reported in 2006 by Popat

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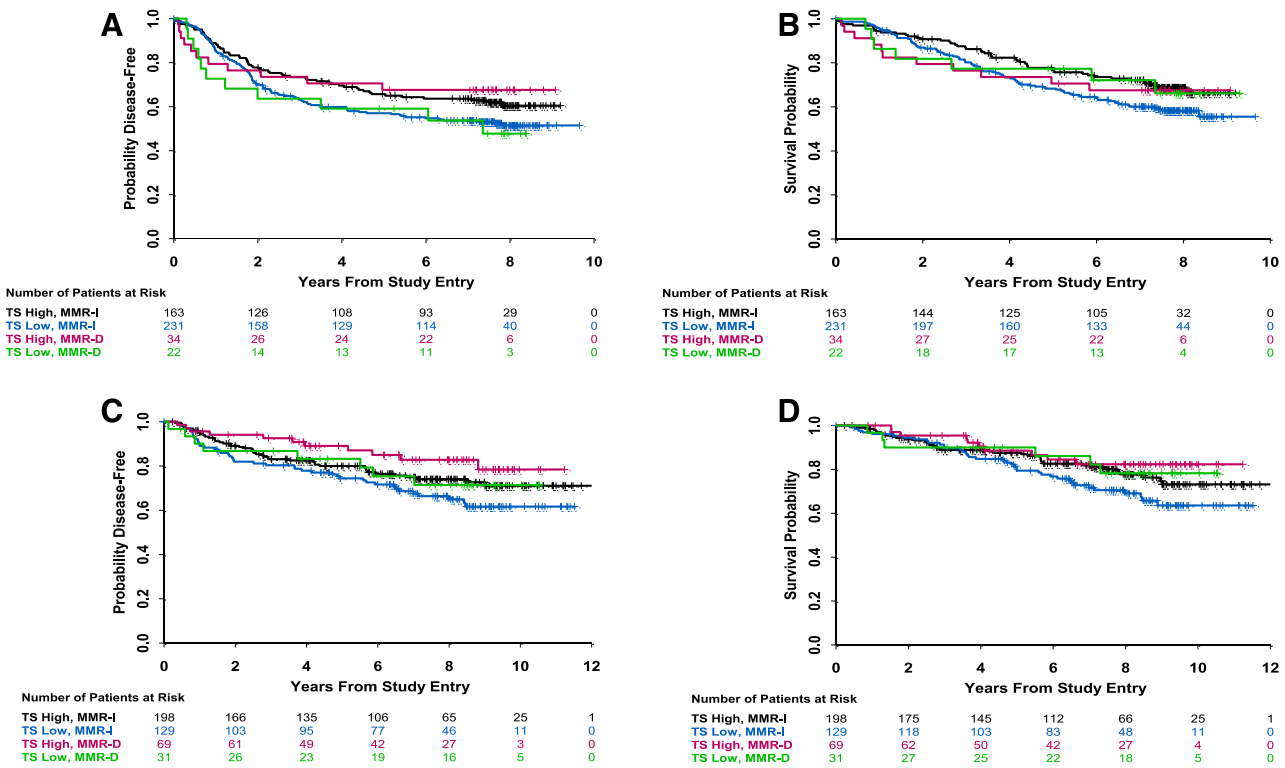

Figure 4. Relationship between TS expression level and MMR status combined and treatment outcome within study. DFS and OS, respectively, by TS Expression Level and MMR status measured by immunohistochemistry in C89803 **(A, B)** and C9581 **(C, D)**.

Abbreviations: DFS, disease-free survival; MMR, mismatch repair; MMR-D, mismatch repair deficiency; MMR-I, mismatch repair intact; OS, overall survival; TS, thymidylate synthase.

et al., differed from ours in that, of the 779 stage II and III cases studied, 59% were rectal cancers. Although the overall proportion of TS high cases was similar to ours (58%), the results failed to show prognostic significance, with a 5-year OS of 0.59 versus 0.56 for cases with high and low TS expression, respectively ($p = .6$). In another notable study, Sinicrope et al. [12] performed a retrospective analysis of TS expression in tumors from 313 patients with colon cancer treated on five randomized 5-FU-based adjuvant chemotherapy trials conducted by the North Central Cancer Treatment Group. This study also addressed the relationship between tumor TS expression and MMR status. The proportion of cases that were TS high (55%) and MMR-D (19%) were similar to those reported here. In contrast to our results, these investigators found that tumor MMR status and TS expression were unrelated and that TS failed to provide prognostic information. The methods for biomarker assessment were the same in both this study and ours. The different result presented here may be explained by the prospective nature and larger, more uniform patient cohorts from C89803 and C9581. Limitations of this study, however, include the low power to test interaction effects (e.g., TS level by stage) and the inability to distinguish between stage of disease and treatment with or without 5FU/LV.

Currently, the best-defined prognostic biomarkers for resectable colon cancer are tumor MMR status and the presence of a *BRAF* mutation. An extensive body of literature shows that tumors that are MMR-D exhibit distinct clinical and pathological features, including more common presence of poorly differentiated histology with abundant tumor-infiltrating lymphocytes, location in the proximal colon, and occurrence in female patients. Following adjuvant colon cancer treatment, MMR-D tumors clearly demonstrate improved DFS and OS [5, 6]. *BRAF* is a part of the RAS-RAF-MAP2K signaling pathway. *BRAF* mutations are present in 10%–20% of colon cancers and, like MMR-D, are associated with proximal tumor location. In contrast, however, the presence of a *BRAF* mutation was associated with significantly worse patient survival in several large studies, including C89803 [9, 22, 23]. Of sporadic colon cancers that are MMR-D, 40%–50% also harbor *BRAF* mutation. The effect of a *BRAF* mutation appears to be additive to that of MMR-D, with outcomes that are worst for patients whose tumors are both BRAF-mutant and MMR-I, intermediate for cases that are BRAF-mutant and MMR-D, and best for those that are BRAF-wild type and MMR-D [9, 22, 23].

It remains unclear whether patients whose tumors are MMR-D experience poorer response to 5-FU-based chemotherapy, although large retrospective studies suggest that this may be the case [24–26]. Although this study shows that tumor TS levels do not predict response to 5-FU-based chemotherapy, it does contribute to the mounting evidence that 5-FU treatment is ineffective or detrimental for patients with MMR-D tumors.

Conclusion

The presence of a high tumor TS expression is associated with increased survival in patients with stage II and III colon cancer. This biomarker does not predict benefit to 5-FU-based chemotherapy in the trials studied here, and therefore is of limited use in assigning specific treatment in the adjuvant setting. However, treatment with 5-FU appeared to affect the relationship between the combination of tumor TS expression and MMR-D status and survival outcomes.

CME

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Disclosures

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