REPORTS

Thymidylate Synthase Expression and Response to Neoadjuvant Chemotherapy in Patients With Advanced Head and Neck Cancer

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Background: **Thymidylate synthase (TS), an essential enzyme in DNA synthesis, is a target for the fluoropyrimidines, an important group of antineoplastic agents used widely in the treatment of head and neck cancer.** ***Purpose:*** **We evaluated relationships between the level and/or pattern of tumor TS expression and response to fluorouracil (5-FU)-based neoadjuvant chemotherapy in patients with advanced head and neck cancer.** ***Methods:*** **Tumor specimens from 86 patients were available for this retrospective analysis. The patients were enrolled in four consecutive phase II studies that tested combinations of 5-FU, leucovorin, and cisplatin with or without added methotrexate plus piritrexim or interferon alfa-2b (IFN α-2b). TS protein expression in the tumors was assessed by use of the TS 106 monoclonal antibody and standard immunohistochemical staining techniques. TS immunostaining was classified according to its level of intensity (TS 0-1 = low, TS 2 = intermediate, and TS 3 = high) and according to its extent (focal pattern = less than 25% of tumor cells positive; diffuse pattern = greater than or equal to 25% of tumor cells positive). Data from 79 patients were available for an analysis of tumor TS expression and patient/tumor characteristics; 70 patients were assessable for their response to neoadjuvant chemotherapy.** ***Results:*** **There was a statistically significant association between the level of tumor TS expression and the degree of tumor differentiation; a higher proportion of patients whose tumors exhibited TS 0-1 immunostaining had undifferentiated or poorly differentiated tumors than patients whose tumors exhibited TS 2 or TS 3 immunostaining (*P* = .04, Jonckheere–Terpstra trend test). Among the 70 patients who were assessable for response to neoadjuvant chemotherapy, TS 3 tumor immunostaining was associated with a lower rate of complete response (i.e., complete disappearance of clinically detectable disease for a minimum of 4 weeks from time of initial determination) than was TS 2 or TS 0-1 immunostaining, but this association was not statistically significant (*P* = .09, exact trend test); among the 39 patients who were treated with regimens that included 5-FU, leucovorin, cisplatin, and IFN α-2b, this inverse association between TS immunostaining intensity and response was statistically significant (*P* = .02, exact trend test). Tumor TS immunostaining intensity and overall survival were not found to be associated. Patients with tumors exhibiting a focal pattern of TS immunostaining have experienced significantly longer survival than patients with tumors exhibiting a diffuse pattern; for the 53 patients with diffuse tumor TS immunostaining, the median survival was 24.7 months, whereas the median survival has not yet been reached for the 22 patients with focal tumor TS immunostaining (*P* = .04, two-tailed logrank test). However, the survival advantage for the focal versus diffuse TS immunostaining pattern was limited to patients whose tumors also exhibited a TS 3 level of immunostaining intensity.** ***Conclusions and Implications:*** **Characterization of tumor TS expression may be of value in identifying patients with advanced head and neck cancer who would benefit from fluoropyrimidine-based neoadjuvant chemotherapy.** **[J Natl Cancer Inst 1997;89:308-13]**

Thymidylate synthase (TS)—EC 2.1.1.45—catalyzes the methylation of deoxyuridine monophosphate to deoxythymidine monophosphate, an essential step in DNA biosynthesis (*1,2*). TS is also a critical target for the fluoropyrimidines, an important group of antineoplastic drugs that are widely used in the treatment of head and neck cancers (*3,4*).

The clinical importance of TS in the development of tumor resistance to fluoropyrimidines has been suggested by studies (*5-8*) demonstrating that increased levels of TS protein as well as stable amplification of TS-specific genes may be associated with fluorouracil (5-FU) resistance. Other studies (*8-10*) have demonstrated in vivo as well as in vitro that TS enzyme levels in neoplastic cells rise acutely when the cells are exposed to cytotoxic agents such as 5-FU. This increase in TS after exposure to 5-FU results in the maintenance of free TS enzyme. These studies also suggest that the degree of fluorodeoxyuridine monophosphate binding and the ability of a tumor to acutely overexpress TS in response to cytotoxic agents may play a role in the development of 5-FU resistance. Thus, the quantitation of TS is critical in understanding mecha-

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See ''Notes'' following ''References.''

nisms that may be involved in the development of antimetabolite resistance in patients.

In view of the potential for using TS as a determinant of response to fluoropyrimidine-based chemotherapy, immunologic assays have been developed that employ monoclonal antibodies directed against TS to quantitate TS enzyme levels in the tumor tissue of individual patients (*7,11*).

In patients with head and neck cancer, the combination of cisplatin and 5-FU has shown considerable activity, with overall response rates ranging from 70% to 100% (*3,4*). Use of this combination in the neoadjuvant setting has led to increased rates of response, reduced incidence of distant metastases, and improved chances for organ preservation. Attempts to increase the activity of neoadjuvant combination chemotherapy with cisplatin and 5-FU have focused on the modulation of 5-FU by the addition of leucovorin and interferon alfa (IFN α). A series of phase I and phase II investigations (*12-15*) has established the feasibility of adding leucovorin to the cisplatin and 5-FU combination as part of induction chemotherapy in patients with advanced disease. More recently, investigators at the University of Chicago (*16-18*) have shown that the cisplatin, 5-FU, leucovorin, and IFN α-2b combination (PFL-IFN) is highly active and produces clinical complete remissions in more than 50% of patients with advanced head and neck cancers, as well as high survival rates if followed by concomitant chemotherapy and radiotherapy. Thus, in this retrospective study, our aim was to determine whether the level or pattern of TS expression in tissues derived from patients with advanced head and neck cancer might allow the prediction of responses to induction chemotherapy with 5-FU-based regimens.

Patients and Methods

Treatment

The patients included in this study were each treated in one of four consecutive phase II studies testing the biochemical modulation of 5-FU in the induction chemotherapy setting. The four phase II studies have been described previously (*13-18*). In brief, the first study utilized cisplatin at a dose of 100 mg/m^2 on day 1, followed by a 5-day continuous infusion of 5-FU at 800 mg/m^2 per day, with 100 mg of *d/l*-leucovorin administered orally every 4 hours during the 5-FU infusion (PFL regimen) (*13,14*). Patients were treated with two cycles of this combination. The second study maintained the PFL regimen and added methotrexate at a dose of 50 mg/m^2 on day 15 and the lipid-soluble methotrexate analogue piritrexim at 100 mg/m^2 given orally twice each day on days 22-26. Two cycles were administered at 28-day intervals (*15*). Patients in the third and fourth studies were treated with three cycles of neoadjuvant chemotherapy. Study 3 included cisplatin at a dose of 100 mg/m^2 on day 1, followed by a 5-day infusion of 5-FU at 640 mg/m^2 per day. During administration of the chemotherapy, *d/l*-leucovorin was given orally every 4 hours and 2 million units/m^2 doses of IFN α-2b were given daily for 6 days (*16,17*). Three cycles of induction chemotherapy were administered at 21-day intervals. Study 4 included identical chemotherapy; however, instead of oral leucovorin, a continuous 132-hour intravenous infusion of the pure, biochemically active L stereoisomer of leucovorin was administered at 300 mg/m^2 per day (*18*). In all four studies, induction chemotherapy was followed by optional surgery and concurrent adjuvant chemotherapy (5-FU and hydroxyurea) and radiation therapy.

The primary end points for the induction chemotherapy regimens consisted of determinations of complete and overall response rates and toxic effects, as reported previously. Response was assessed by at least two observers after the induction chemotherapy had been completed and after the surgery. Response assessments included repeat clinical and endoscopic examinations. A complete response (CR) was defined as the complete disappearance of all clinically detectable disease for a minimum of 4 weeks from the time of initial determination. A partial response was defined as the reduction of all measurable lesions by at least 50%, and stable disease was defined as an average decrease of all measurable disease by less than 50%.

TS Study Cohort

The TS study samples consisted of pretreatment formalin-fixed, paraffin-embedded tissue sections available from 86 patients. The clinical and pathologic data available for the patients were kept separately and were provided by investigators at the University of Chicago. Data from a total of 79 cases were available for final statistical analysis. The data from seven cases were not eligible for final analysis for the following reasons: One patient had tissue specimens that were incorrectly labeled, another patient did not receive induction chemotherapy, and five patients were found to have lymphoepithelioma. The 79 patients were treated in clinical phase II trials testing the biochemical modulation of cisplatin and 5-FU combinations by the addition of leucovorin and/or IFN α-2b as outlined above. Seventeen of the patients were treated in the first study outlined above (*13,14*), 19 patients were treated in the second study (*15*), and the final group of 43 patients were treated in the studies testing PFL-IFN (*16-18*). Nine of the 79 patients were not assessable for response to chemotherapy for the following reasons: Five patients received only one cycle of therapy, and four patients died early during the first or second cycles of therapy.

Immunohistochemical Staining

Paraffin-embedded tissue sections (6 μm thick) were deparaffinized in xylene, rehydrated through graded alcohols, and washed in phosphate-buffered saline. The sections were then probed with the TS 106 monoclonal antibody by use of the avidin–biotin complex (ABC) immunohistochemical technique (*19*). The TS 106 monoclonal antibody was produced at the National Cancer Institute, and its use in TS immunoquantitation and immunocytochemistry has been validated in previous studies (*7,11*).

Tissue Evaluation

The slides were examined and scored independently by two observers (P. G. Johnston and K. A. Behan) who were blinded to both the clinical and the pathologic data. TS expression was quantified by use of a visual grading system based on the intensity of staining (0-3) as well as on its extent, i.e., pattern (focal = less than 25% of tumor cells staining positive, or diffuse = greater than or equal to 25% of tumor cells staining positive) (*see* Fig. 1). There was close agreement (>90%) in the TS evaluation between both investigators. In cases of disagreement, consensus was determined by joint review. Duplicate samples were included in the study as internal controls for TS immunostaining and interpretation, as were positive and negative tissue controls. The TS staining intensity was similar from one batch of slides to the next.

Determination of Dihydropyrimidine Dehydrogenase Activity

Approximately 20 mL of whole blood was collected in heparinized tubes. Peripheral blood lymphocytes were isolated, and dihydropyrimidine dehydrogenase (DPD) activity was determined as described previously (*18*). DPD is the first and rate-limiting enzyme that is involved in the catabolism of 5-FU, and expression levels of this enzyme have been associated with the response to 5-FU therapy (*1*).

Plasma 5-FU Measurement

Plasma 5-FU levels were measured in patients treated with 640 mg/m^2 5-FU per day as part of a 5-day infusion during PFL-IFN induction therapy. Samples were collected on day 1 at 8 AM (control prior to chemotherapy) and on day 3 both at 8 AM and at 5 PM (for 5-FU plasma measurements). Blood was collected in EDTA-containing tubes. Immediately after the samples were collected, *l*-ascorbic acid was added to achieve a final concentration in the range of 1-2 mg/mL of whole blood. The tubes were centrifuged immediately at 1000*g* for 10 minutes at 4 °C, and the resulting plasma supernatants were transferred to individual 5-mL polypropylene tubes and stored at −80 °C. 5-FU plasma concentrations were analyzed by means of high-performance liquid chromatography as described previously (*16*).

Statistical Analysis

The results of the TS immunohistochemical analysis were submitted to the University of Chicago for statistical evaluation using a coded identification system to maintain adequate blinding of the investigators. The TS staining pattern (focal, diffuse, or negative) was treated as a nominal variable, and the TS staining intensity (0, 1, 2, or 3) was treated as an ordinal variable. The association between TS staining pattern (focal or diffuse) and TS level (1, 2, or 3)

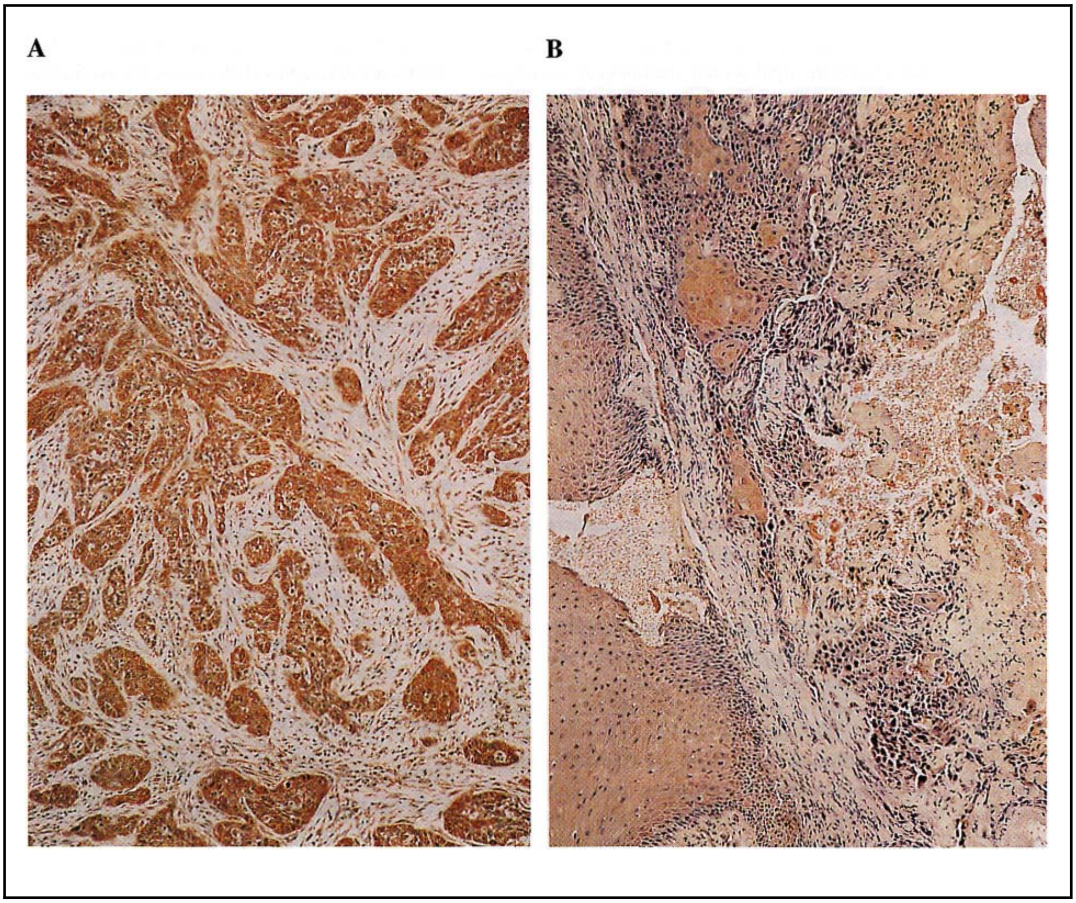

Fig. 1. Thymidylate synthase (TS) immunohistochemical staining in squamous cell carcinomas of the head and neck. The tumor sections demonstrate diffuse, high-intensity TS staining (TS 3) **(A)** and focal, low-intensity TS staining (TS 1) **(B).**

was assessed by use of Fisher's exact test. Associations of TS staining pattern (focal, diffuse, or negative) with patient demographic variables or induction chemotherapy responses (CR versus non-CR) were evaluated by use of Fisher's exact test for categorical variables or the Kruskal–Wallis test for ordinal variables [such as T or N stage; *see* (*20*) for details on tumor staging]. Comparisons of DPD activity and 5-FU concentration according to TS staining pattern (focal or diffuse) were performed by use of Student's *t* test. Associations of TS level (0-1, 2, or 3) and patient demographic variables or induction responses were evaluated by the chi-squared test, the Cochran–Armitage trend test (*21*), or the Jonckheere–Terpstra test for trend (*22*). Comparison of DPD activity or 5-FU concentration with TS level was performed by a one-way analysis of variance. Overall survival was measured from the date of treatment in the relevant study until death from any cause or until the date of last patient contact. Survival was estimated by the method of Kaplan and Meier (*23*) and compared by use of the logrank test (*24*). All tests were two-sided, with the exception of the exact trend tests. All analyses were performed using SPSS6.1 for Windows (SPSS, Chicago, IL) or Stat Xact 3 software (Cytel Software Corporation, Cambridge, MA).

Results

Association of TS Expression With Patient and Tumor Characteristics

Tumor samples from 79 patients who were treated with biomodulated cisplatin and 5-FU-based regimens at the University of Chicago were analyzed for TS expression. The TS immunostaining pattern was predominantly in the form of granular cytoplasmic staining in the tumor cells. TS staining was also observed in lymphocytes adjacent to the tumors, and minor antibody cross-reactivity was observed with fibroblasts, smooth muscle, and other connective tissue elements. Heterogeneity of TS staining was apparent both within individual tumors and between different tumors. Within the study group, 48% of the tumors had a high TS level (TS 3), 30% stained at a moderate level (TS 2), 17% had low staining (TS 1), and 5% had no staining (TS 0). Twenty-eight percent of the tumors displayed a focal TS staining pattern, whereas 67% had a diffuse staining pattern. No association was observed between TS expression level, sex, regional lymph node status, tumor stage, or site of the primary tumor. TS expression was significantly associated with the degree of tumor differentiation. A higher proportion of patients with low TS scores (0-1) had undifferentiated or poorly differentiated tumors ($P = .04$) (Table 1).

Clinical Associations

Among the 70 patients who were assessable for response, an inverse association was observed between TS expression level and the response to induction chemotherapy. Approximately 35% of the patients with high TS levels (TS 3) had a complete remission compared with approximately 62% and 53% of the patients whose tumors had intermediate (TS 2) or low (TS 0-1) TS levels, respectively ($P = .09$) (Table 2, A). In the subgroup of 39 patients who were treated with three cycles of induction PFL-IFN and were assessable for response, the patients with tumors that had high levels of TS (TS 3) had a significantly lower rate of complete remission than the patients with tumors that had intermediate or low TS levels ($P = .02$) (Table 2, B). Only 42.9% of the patients with tumors that had a high TS level achieved a complete remission compared with 70.0% and approximately 88% of the patients whose tumors had moderate and low TS levels, respectively. An inverse association between tumor TS expression level and response to induction chemotherapy was observed for patients within each individual study; however,

Table 1. Demographic and histopathologic characteristics of patients (total n = 79) according to tumor thymidylate synthase (TS) immunohistochemical staining score*

	TS 0-1 (n = 17)		TS 2 (n = 24)		TS 3 (n = 38)		
	No.	%	No.	%	No.	%	P†
Sex							
Male	11	64.7	15	62.5	28	73.7	.26
Female	6	35.3	9	37.5	10	26.3	
Tumor stage‡							
2	1	5.9	3	12.5	2	5.3	.40
3	4	23.5	9	37.5	11	28.9	
4	12	70.6	12	50.0	25	65.8	
Lymph node stage‡							
0	3	17.6	8	33.3	4	10.5	.16
1	5	29.4	2	8.3	10	26.3	
2	6	35.3	11	45.8	16	42.1	
3	3	17.6	3	12.5	8	21.1	
Tumor differentiation							
Well differentiated	2§	13.3	4	16.7	7	18.4	.04
Moderately differentiated	6	40.0	17	70.8	26	68.4	
Poorly differentiated	5	33.3	2	8.3	3	7.9	
Undifferentiated	2	13.3	1	4.2	2	5.3	
Tumor site							
Oral	1	5.9	3	12.5	5	13.2	.20
Nasopharynx	2	11.8	1	4.2	1	2.6	
Oropharynx	5	29.4	6	25.0	19	50.0	
Hypoglottis	3	17.6	3	12.5	3	7.9	
Supraglottis	2	11.8	8	33.3	4	10.5	
Glottis	1	5.9	0	—	0	—	
Other	3	17.6	3	12.5	6	15.8	

*TS 0-1 = low staining intensity, TS 2 = intermediate staining intensity, and TS 3 = high staining intensity. *See* ''Patients and Methods'' section for details.

†*P* value for sex from Cochran–Armitage trend test; *P* values for tumor stage, lymph node stage, and tumor differentiation from Jonckheere–Terpstra trend test; *P* value for tumor site from two-tailed chi-squared test.

‡*See* (*20*) for details on tumor staging.

§The numbers of patients whose tumors had TS 0-1 immunostaining scores in this category do not add to 17 because information on tumor differentiation was not available for two patients.

Table 2. Association of tumor thymidylate synthase (TS) expression level and response to leucovorin-modulated fluorouracil (5-FU)-based induction chemotherapy for all assessable patients **(A)** and for the subset treated with regimens containing interferon alfa-2b **(B)**

Response	No. of patients (%) by TS level*			
	TS 0-1	TS 2	TS 3	P†
A. All assessable patients (n = 70)				
Complete‡	8 (53.3)	13 (61.9)	12 (35.3)	
Partial‡	7 (46.7)	8 (38.1)	22 (64.7)	
Total No. of patients	15	21	34	.09
B. Subset of patients (n = 39)				
Complete‡	7 (87.5)	7 (70.0)	9 (42.9)	
Partial‡	1 (12.5)	3 (30.0)	12 (57.1)	
Total No. of patients	8	10	21	.02

*TS 0-1 = low staining intensity, TS 2 = intermediate staining intensity, and TS 3 = high staining intensity. *See* ''Patients and Methods'' section for details.

†Exact trend test; TS 0-1 versus TS 2 versus TS 3.

‡*See* ''Patients and Methods'' section for details.

this inverse association did not reach statistical significance in the first two studies because of the small numbers of patients involved. No association between tumor TS level and overall survival was detected in either the entire group (P = .9) or the subset of patients treated with PFL-IFN (P = .23).

No association was noted between the TS staining pattern (focal versus diffuse), patient demographics, histologic parameters, or response; however, patients whose tumors exhibited a focal pattern of TS staining had statistically significantly longer survival than patients whose tumors had diffuse staining. The 53 patients with tumors exhibiting a diffuse TS staining pattern had a median survival of 24.7 months; however, the median survival has not been reached for the 22 patients whose tumors had a focal TS staining pattern, with 14 of 22 patients alive at 55 months (P = .04) (Fig. 2). The survival difference between focal and diffuse TS staining patterns was observed only for patients whose tumors had high TS levels (P = .01). No survival difference was noted for patients whose tumors had moderate (TS 2) or low (TS 0-1) TS levels. In the subgroup treated with PFL-IFN, the median survival was 46.5 months for the 28 patients whose tumors had a diffuse staining pattern, and the median survival for the 15 patients whose tumors had focal staining has not been reached, with a median follow-up of 52 months (P = .02). The TS staining pattern was not associated with TS staining intensity.

The distributions of the mean and maximal plasma 5-FU concentrations for the 43 patients treated uniformly with three cycles of PFL-IFN induction therapy were not significantly different among the TS groups, and the mean level of DPD activity measured in the peripheral blood mononuclear cells of a subset of 21 of these patients was not associated with TS staining levels (Table 3).

Discussion

In this study, we have analyzed the expression of TS protein in tumors from patients with advanced head and neck cancer. We have demonstrated that patients whose tumors have high TS expression are less likely to achieve a CR to fluoropyrimidine-based therapy than patients whose tumors have low levels of TS enzyme. Moreover, patients whose tumors demonstrated a more focal pattern of TS staining, in particular those with high TS levels, appear to have better survival.

Recent clinical trials for advanced head and neck cancer have investigated the use of neoadjuvant combination chemotherapy and subsequent intensive concomitant chemotherapy and radiotherapy. This treatment approach has resulted in improved disease-free survival and overall survival in several randomized studies [reviewed in (*3*)]. Induction chemotherapy with the combination of cisplatin and 5-FU has resulted in increased re-

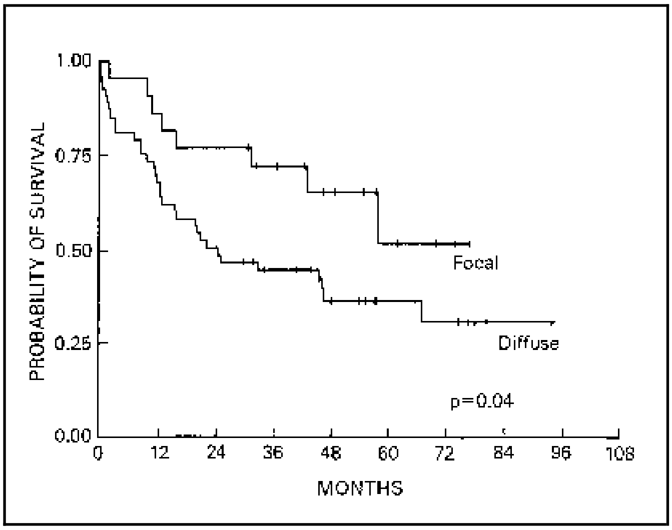

Fig. 2. Association of the pattern of thymidylate synthase (TS) immunostaining with survival in the TS study group. Survival differences were assessed between 53 patients whose tumors had diffuse TS staining and 22 patients whose tumors had focal TS staining. Survival was estimated by use of the Kaplan–Meier method. Survival was 72% (95% confidence interval [CI] = 52%-92%) at 36 months and 53% (95% CI = 23%-83%) at 72 months for focal staining and 45% (95% CI = 31%-59%) at 36 months and 32% (95% CI = 16%-48%) at 72 months for diffuse staining. The *P* value (two-sided) is based on the logrank test.

Table 3. Association between tumor thymidylate synthase (TS) expression level* and mean and maximal levels of serum fluorouracil (5-FU) and mean level of peripheral blood mononuclear cell dihydropyrimidine dehydrogenase (DPD) for patients receiving leucovorin-modulated 5-FU-based induction chemotherapy containing interferon alfa-2b (n = 43)

	TS 0-1†		TS 2†		TS 3†		
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	*P*‡
DPD, mean level	5	0.21 ± 0.14	3	0.16 ± 0.14	13	0.25 ± 0.19	.72
5-FU							
Mean level	9	261.1 ± 76.0	12	262.3 ± 122.9	22	133.4 ± 28.4	.71
Maximal level	9	289.1 ± 100.6	12	300.0 ± 135.6	22	336.0 ± 187.6	.70

*TS 0-1 = low staining intensity, TS 2 = intermediate staining intensity, and TS 3 = high staining intensity. *See* ‘‘Patients and Methods’’ section for details.

†N = number of patients; SD = standard deviation.

‡One-way analysis of variance (three-group comparison).

sponse rates and the ability to decrease local tumor burdens, with enhanced rates of success in organ-preserving surgery. Several clinical studies (*11,15-18*) suggest that induction therapy with the cisplatin, 5-FU, and leucovorin combination and the cisplatin, 5-FU, leucovorin, and IFN α-2b combination results in further improvements in local control and fewer distant failures, with a median survival of greater than 3 years. Leucovorin increases 5-FU cytotoxicity by increasing the formation and the stability of the TS ternary complex and, thus, the degree of TS inhibition (*2*). Several mechanisms have been described to account for the interaction between 5-FU and IFN α-2b, including the inhibition of TS protein induction by 5-FU therapy. Chu et al. (*25*) have shown that induction of TS protein synthesis is controlled in a post-transcriptional autoregulatory manner and may be an important protective mechanism by which malignant cells rapidly become resistant to cytotoxic stress, such as that induced by 5-FU. Thus, understanding the role of TS as a determinant of chemotherapeutic responsiveness in patients with head and neck cancer may lead to improved therapeutic strategies for this disease.

The importance of TS expression levels has been established in a number of clinical and preclinical studies. Increased levels of TS protein, overexpression of TS messenger RNA (mRNA), and amplification of TS-specific genes have been demonstrated in cell lines selected for resistance to 5-FU, and acute induction of TS protein has been shown to occur in response to 5-FU both in vivo and in vitro (*5-10*). Recent data from several studies (*26-30*) measuring intratumoral TS levels in patients with a variety of solid tumors also suggest that high levels of TS expression are associated with unresponsive disease, indicating that TS expression may be an independent prognostic factor for disease recurrence and clinical outcome in patients. Our group (*26*) has demonstrated that TS is an independent prognostic marker in rectal cancer and may help identify subgroups of patients who benefit from chemotherapy in the adjuvant disease setting. A collaborative effort by our group and investigators at the University of Southern California (*27*) has shown an inverse association between the level of TS protein, TS mRNA, and clinical responsiveness to fluoropyrimidine-based therapy in patients with advanced colorectal or gastric cancer. More recently, Lenz et al. (*28*) have confirmed that high levels of TS mRNA expression not only predict for response but also are associated with decreased survival in a larger cohort of patients with gastric carcinoma. Peters et al. (*29*) have also confirmed that colon cancers with elevated TS levels are resistant to 5-FU. Two other studies (*10,30*) have examined the importance of the percentage of total TS found in the form of the ternary complex. Both studies have shown that the amount of TS-ternary complex also predicts for responsiveness to 5-FU-based regimens in both breast and gastric carcinomas. Etienne et al. (*31*) measured TS catalytic activity and DPD activity in 60 patients with head and neck cancer treated with cisplatin and 5-FU with or without leucovorin. For the 52 patients assessable for response, Etienne et al. (*31*) observed a trend toward significance between low DPD levels and response to 5-FU-based chemotherapy; however, they found no association between TS activity and response.

The preliminary data from this retrospective study suggest that, for patients with advanced head and neck cancer, high tumor TS levels are associated with a lack of response to 5-FU-based chemotherapy and, for patients with high TS levels, a focal pattern of TS immunostaining is associated with significantly improved survival. The degree of focal versus diffuse TS immunostaining within a tumor may reflect differences in total TS content, which may account for the survival differences observed. Further studies examining TS protein levels using several assays will be needed to address this question. The ability to relate response and outcome on the basis of TS protein expression in patients with head and neck cancer before, or early in the course of, treatment may ultimately help target therapy to selected patients with this disease.

In conclusion, the level of tumor TS protein expression predicts for response to 5-FU-based chemotherapy in patients with locally advanced head and neck cancer. Further studies will be necessary to determine the role of TS as an indicator of therapeutic benefit and survival for this disease.

References

(*1*) Grem JL. Fluorinated pyrimidines. In: Chabner BA, Collins JM, editors. Cancer chemotherapy, principles and practice. Philadelphia: Lippincott, 1990:180-224.

(*2*) Grem JL. 5-Fluorouracil plus leucovorin in cancer therapy. In: DeVita VT Jr, Hellman S, Rosenberg SA, editors. Principles and practice of oncology update series, 2(7) July. Philadelphia: Lippincott, 1988.

(*3*) Vokes EE, Weichselbaum RR, Lippman SM, Hong WK. Head and neck cancer. N Engl J Med 1993;328:184-94.

(*4*) Dreyfuss AI, Clark JR, Wright JE, Norris CM Jr, Busse PM, Lucarini JW, et al. Continuous infusion high-dose leucovorin with 5-fluorouracil and cisplatin for untreated stage IV carcinoma of the head and neck. Ann Intern Med 1990;112:167-72.

(*5*) Washtien WL. Increased levels of thymidylate synthetase in cells exposed to 5-fluorouracil. Mol Pharmacol 1984;25:171-7.

(*6*) Clark JL, Berger SL, Mittelman A, Berger FG. Thymidylate synthase gene amplification in a colon tumor resistant to fluoropyrimidine chemotherapy. Cancer Treat Rep 1987;71:261-5.

(*7*) Johnston PG, Drake JC, Trepel J, Allegra CJ. Immunological quantitation of thymidylate synthase using the monoclonal antibody TS 106 in 5-fluorouracil-sensitive and -resistant human cancer cell lines. Cancer Res 1992;52:4306-12.

(*8*) Chu E, Koeller DM, Johnston PG, Zinn S, Allegra CJ. Regulation of thymidylate synthase in human colon cancer cells treated with 5-fluorouracil and interferon-gamma. Mol Pharmacol 1993;43:527-33.

(*9*) Chu E, Zinn S, Boarman D, Allegra CJ. The interaction of γ interferon and 5-fluorouracil in the H630 human colon carcinoma cell line. Cancer Res 1990;50:5834-40.

(*10*) Swain SM, Lippman ME, Egan EF, Drake JC, Steinberg SM, Allegra CJ. Fluorouracil and high-dose leucovorin in previously treated patients with metastatic breast cancer. J Clin Oncol 1989;7:890-9.

(*11*) Johnston PG, Liang CM, Henry S, Chabner BA, Allegra CJ. Production and characterization of monoclonal antibodies that localize human thymidylate synthase in the cytoplasm of human cells and tissue. Cancer Res 1991;51:6668-76.

(*12*) Clark J, Busse P, Norris CJ, Dreyfress A, Rossi R, Poulin M, et al. Long term results of induction chemotherapy with cisplatin, 5-FU and high dose leucovorin (PFL) for squamous cell carcinoma of the head and neck. Proc ASCO 1996;897:317.

(*13*) Vokes EE, Weichselbaum RR, Mick R, McEvilly JM, Haraf D, Panje WR. Favorable long-term survival following induction chemotherapy with cisplatin, fluorouracil, and leucovorin and concomitant chemoradiotherapy for locally advanced head and neck cancer. J Natl Cancer Inst 1992;84:877-82.

(*14*) Vokes EE, Schilsky RL, Weichselbaum RR, Kozloff MF, Panje WR. Induction chemotherapy with cisplatin, fluorouracil, and high-dose leucovorin for locally advanced head and neck cancer: a clinical and pharmacologic analysis. J Clin Oncol 1990;8:241-7.

(*15*) Vokes EE, Haraf DJ, McEvilly JM, Mick R, Kozloff MF, Goldman MD, et al. Neoadjuvant PFL augmented by methotrexate and piritrexim followed by concomitant chemoradiotherapy for advanced head and neck cancer: a feasible and active approach. Ann Oncol 1992;3:79-81.

(*16*) Vokes EE, Ratain MJ, Mick R, McEvilly JM, Haraf D, Kozloff M, et al. Cisplatin, fluorouracil, and leucovorin augmented by interferon alfa-2b in head and neck cancer: a clinical and pharmacologic analysis [published erratum appears in J Clin Oncol 1994;12:643]. J Clin Oncol 1993;11:360-8.

(*17*) Vokes EE, Kies M, Haraf DJ, Mick R, Moran WJ, Kozloff M, et al. Induction chemotherapy followed by concomitant chemoradiotherapy for advanced head and neck cancer: impact on the natural history of the disease. J Clin Oncol 1995;13:876-83.

(*18*) Vokes EE, Mick R, Kies MS, Dolan E, Malone D, Athanasiades I, et al. Pharmacodynamics of fluorouracil based induction chemotherapy in advanced head and neck cancer. J Clin Oncol. In press.

(*19*) Hsu SM, Raine L, Fanger H. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 1981;29:577-80.

(*20*) American Joint Committee on Cancer. Head and Neck Cancer. In: Beahrs OH, Henson DE, Hutter RV, Myers MH, editors. Manual for staging of cancer. 3rd ed. Philadelphia: Lippincott, 1988:51-6.

(*21*) Armitage P. Test for linear trend in proportions and frequencies. Biometrics 1955;11:375-86.

(*22*) Holland M, Wolfe DA. Nonparametric statistical methods. New York: Wiley, 1958.

(*23*) Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 1958;53:457-81.

(*24*) Mantel N. Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer Chemother Rep 1966;50:163-70.

(*25*) Chu E, Koeller DM, Casey JL, Drake JC, Chabner BA, Elwood PC, et al. Autoregulation of human thymidylate synthase messenger RNA translation by thymidylate synthase. Proc Natl Acad Sci U S A 1991;88:8977-81.

(*26*) Johnston PG, Fisher ER, Rockette HE, Fisher B, Wolmark N, Drake JC, et al. The role of thymidylate synthase expression in prognosis and outcome of adjuvant chemotherapy in patients with rectal cancer. J Clin Oncol 1994;12:2640-7.

(*27*) Johnston PG, Lenz HJ, Leichman CG, Danenberg KD, Allegra CJ, Danenberg PV, et al. Thymidylate synthase gene and protein expression correlate and are associated with response to 5-fluorouracil in human colorectal and gastric tumors. Cancer Res 1995;55:1407-12.

(*28*) Lenz HJ, Leichman CG, Danenberg KD, Danenberg PV, Groshen S, Cohen H, et al. Thymidylate synthase mRNA level in adenocarcinoma of the stomach: a predictor for primary tumor response and overall survival. J Clin Oncol 1995;14:176-82.

(*29*) Peters GJ, van der Wilt CL, van Groeningen CJ, Smid K, Meijer S, Pinedo HM. Thymidylate synthase inhibition after administration of fluorouracil with or without leucovorin in colon cancer patients: implications for treatment with fluorouracil. J Clin Oncol 1995;12:2035-42.

(*30*) Alexander HR, Grem JL, Hamilton JM, Pass HI, Hong M, McAtee N, et al. Thymidylate synthase protein expression is associated with response following neoadjuvant chemotherapy and resection for locally advanced gastric cancer. Cancer J 1995;1:49-54.

(*31*) Etienne MC, Cheradame S, Fischel P, Formento P, Dassonville O, Renee N, et al. Response to fluorouracil therapy in cancer patients: the role of tumoral dihydropyrimidine dehydrogenase activity. J Clin Oncol 1995;13:1663-70.

Notes

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