

Thymidylate Synthase Expression in Metastatic Colorectal Cancer: Correlation with the Clinical Response to Leucovorin-Modulated 5-Fluorouracil

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ABSTRACT

Purpose: The aim of the present study is to determine whether the level thymidylate synthase (TS) expression predicts the clinical response to fluorouracil (FU)-based chemotherapy in patients with recurrent or metastatic colorectal cancer.

Patients and methods: TS levels were measured on immunohistochemically specimens of colorectal cancer metastases or recurrences from 48 patients (30 males and 18 females; median age, 49 years) treated with 5FU (420 mg/m² i.v. days 1-5) and LV (20 mg/m² i.v. days 1-5); cycles were repeated every 28 days. These measurements were correlated with patient characteristics and clinical outcome.

Results: A significant correlation was found between TS expression and all the parameters of clinical outcome analyzed. In patients whose tumors had low (n=27) and high (n=21) TS levels, the overall response rates were 66.7% and 23.8%, respectively ($p = 0.003$). The percentage of tumor shrinkage after chemotherapy was linearly related to TS immunoreactivity ($r = 0.56$, $p = 0.00004$) and its mean values were 65% and 14% with low and high TS levels, respectively ($p = 0.0001$). By logistic regression analysis, low TS expression was the single best predictor of response to 5FU based-chemotherapy (relative probability, 5.0). In patients with low and high TS expression, the median time to progression was 9.6 months vs 6.2 months ($p = 0.005$) and the median survival time 18.4 months vs 15.4 months ($p = 0.02$), respectively. Two- and 3-year survival rates were 41% vs 15% and 19% vs 0% ($p = 0.02$), respectively.

Conclusion: Our results demonstrate the ability of TS protein expression to predict response to 5FUra. This could have a significant impact on the choice of drugs in treatment of advanced colorectal cancer and may change the therapeutic approach from a general to an individual treatment strategy.

Key Words: Thymidylate synthase - Metastatic colorectal cancer.

INTRODUCTION

Thymidylate synthase (TS), a rate-limiting enzyme in the DNA synthetic pathway, is a critical target for the medical treatment of colorectal cancer [1]. Fluorouracil (FU), a TS-inhibitor drug, has remained the only standard agent for the treatment of large bowel tumors for nearly four decades [8]. During this time, infusional schedules have been developed [32] and Leucovorin (LV) has been combined with the fluoropyrimidine [1] to prolong and potentiate the TS-inhibitory activity of this antimetabolite. Given the central role of this enzyme in the medical treatment of colorectal cancer, considerable efforts have been directed at identifying groups of patients unresponsive to TS inhibitors. Initially, the strategy behind these studies was to avoid useless toxicity, because the response rate to FU does not exceed 30% even with complex regimens using LV, infusional schedules and/or high FU doses [9,17,29,47]. Similarly, pure TS inhibitors under clinical testing are not more active [13,16]. More recently, non-TS-targeted antineoplastic agents with clinical activity in colorectal cancer have emerged that could be offered to patients who are unresponsive to TS inhibitors. In particular, the DNA cross-linking agent Oxaliplatin [19,31,33] and the topoisomerase I inhibitor Irinotecan [14,15,40-42] have consistently shown substantial activity in patients with advanced disease, whereas antibody therapy is being compared with FU in the adjuvant setting [39]. Therefore, the identification of patients unlikely to respond

to TS inhibitors may now also address the choice between different treatment options that were not available until a few years ago. The role of TS in determining fluoropyrimidine cytotoxicity has been clearly established in several preclinical models. High levels of TS activity have been correlated with either acquired or intrinsic resistance to FU or fluorodeoxyuridine [7,46] and exposure of tumor cells to TS inhibitors has been shown to induce an acute rise in TS levels overcoming drug-induced inhibition [11]. A series of clinical observations on small groups of patients provided further support to the general contention that TS expression may critically affect the sensitivity of human tumor cells to fluoropyrimidines [12,34,37]. However, a clear-cut correlation between intratumor TS levels and the clinical response to a single, specific FU regimen was only recently established [30]. Only partial correlations were in fact obtained in the initial clinical studies investigating the predictive role of TS expression, mainly because of the low sensitivity and other technical problems inherent to the biochemical assays that were used (assessment of the catalytic activity or quantitation of the binding capacity for fluorodeoxyuridine monophosphate) [34,37]. More sensitive methods for quantitating the levels of TS protein and TS gene expression were developed [22,26]. However, the optimal technique for measuring TS levels in patient tumor samples remains to be defined. Semiquantitative polymerase chain reaction has permitted the measurement of intratumor TS gene expression, which was found to predict response and resistance to continuous-infusion FU in a study of 42 patients with advanced colorectal cancer [30].

The development of antibodies to the TS protein has provided the opportunity to quantitate the levels of expression of this enzyme in routinely available, paraffin-embedded tumor samples, using convenient low-cost immunohistochemical assays. Further advantages of this approach are the ability to check for tumor heterogeneity and the lack of contamination of tumor samples with normal tissues. With the use of a monoclonal TS antibody, intratumor TS immunoreactivity was in fact shown to be an independent marker of survival in a retrospective study of patients with radically resected rectal cancer [24]. Moreover, TS immunostaining with the same antibody has been shown to correlate with TS mRNA expression in a series

of tumor biopsy samples [25]. A correlation between TS mRNA and TS protein levels was also found when TS expression was assessed in liver and lung colorectal cancer metastases using a rabbit polyclonal antibody to purified recombinant human TS [20,36].

In this study, we have therefore used an immunohistochemical method to analyze the level of TS expression in the recurrent or metastatic tumor in a series of patients treated by biochemically modulated FU. The main aim was to compare the levels of TS expression and to examine its relative ability to predict response to FU-based chemotherapy.

PATIENTS AND METHODS

Between August 1997 and May 1999, 48 patients with metastatic, recurrent, or locally advanced unresectable colorectal cancer were enrolled in this study.

Eligibility criteria:

- 1- A diagnosis of disseminated or recurrent colorectal cancer with a measurable lesion accessible for biopsy.
- 2- A Southwest Oncology Group performance status 1 to 2 with adequate hematological, hepatic and renal function.
- 3- No prior 5-FU.
- 4- A lesion that was bidimensionally measurable either by physical or radiological examination.

Treatment schedule:

Patients were treated with 5-FU at a dose of 420 mg/m² i.v. days 1-5 and leucovorin at the dose of 20 mg/m² i.v. days 1-5; cycles were repeated every 28 days. After two cycles measurable disease was reassessed. Response to chemotherapy was assessed according to World Health Organization criteria [1]. Briefly, complete response (CR) refers to the disappearance of all disease, partial response (PR) to a greater than 50% reduction in the size of the lesion and minor response to a 25% to 49% reduction. In addition, for all patients, the indicator lesions were measured before the start of chemotherapy and then after each cycle and a baseline tumor area (expressed in square centimeters as the sum of the products of the maximum perpendicular diameters of all the indicator lesions) was reported along with its variations at each successive cycle. Therapeutic outcome could thus

be expressed also as the maximum percentage of reduction in tumor area achieved in each patient.

Evaluation and follow-up:

Before treatment, all patients underwent evaluation including a detailed history and physical examination, tumor measurement, chest X-ray, liver imaging (CT and sonogram), complete blood count (CBC), blood chemistry and CEA. All patients visited the outpatient clinic regularly for monthly physical examination and check-up of CBC, liver and renal functions and serum CEA level during treatment. Chest X-rays were obtained every 2-3 months and sonograms of the liver and CT scans of the abdomen were obtained before each cycle. Colonoscopy was scheduled on a yearly basis. Patients with CR, PR or SD remained in the protocol until progressive disease was documented.

Immunohistochemistry:

Tissue samples: Paraffin-embedded, formalin-fixed specimens from colorectal cancer metastases or recurrences were analyzed immunohistochemically to measure TS protein expression (liver, thirty nine; lymph nodes, seven; pelvic recurrence, six; peritoneum, four; other, two). Tissue from different metastatic lesions was available from 10 (liver plus lymph nodes, n=4; liver plus peritoneal implants, n=3; liver plus pelvic masses, n=1; pelvic mass plus spleen, n=1; peritoneal implants plus lymph nodes, n=1) out of 18 patients with metastases at multiple sites (13 liver plus other sites and 5 multiple extrahepatic sites). Three patients with pulmonary and hepatic metastases and one patient with hepatic and nodal disease had TS assessed only in liver samples. One patient with liver and hilar node metastases had only the lymph nodes analyzed. TS expression was assessed only on pelvic masses in three patients with concomitant lung or peritoneal disease.

Expression of TS: The monoclonal antibody TS 106 was used to detect TS [26]. The standard avidin-biotin-peroxidase complex (Vectastain Elite ABC kit; Vector Laboratories, Inc, Burlingame, CA) technique was used [23]. Two-micrometer-thick tissue sections were cut, deparaffinized in xylene and then hydrated in decreasing concentrations of ethanol. To quench the endogenous peroxidase activity, the sections were incubated in a solution of 0.3% hydrogen peroxide for 10 min. For reduction of nonspe-

cific background staining, the sections were exposed to 20% horse serum for 30 min. Sections were incubated with TS 106 monoclonal Ab at room temperature for 90 min for TS staining. After rinsing, biotinylated horse antimouse secondary antibodies were applied for 30 min, followed by further washing and then were incubated with avidin-biotin peroxidase complexes. Immunostaining was developed by immersion in 0.05% 3,39-diaminobenzidine tetrahydrochloride and then counterstaining with a modified Harris hematoxylin.

Scoring of immunohistochemical staining:

Slides were then examined under a light microscope and scored. Only tumor cells with cytoplasmic staining were counted as positive. TS expression was quantitated using a visual grading system based on the intensity of staining and classified into five groups from 0 (undetectable staining) to 4 (very high intensity of staining). For the purpose of correlation with clinical data, intensity levels 0 to 2 were grouped together and considered low expression (Fig. 1A) and levels 3 and 4 were considered high expression (Fig. 1B). Seven out of 10 patients for whom paraffin sections from different metastases were available exhibited the same TS status (low vs. high expression) in all the metastases despite minor differences in the degree of TS immunoreactivity. For the three patients with heterogeneous TS levels in different metastases, the highest degree of TS expression was taken to define TS status. Similarly, when heterogeneous levels of TS expression were found within a tumor (9 of 15 cases), the level of TS expression of that lesion was defined according to the highest TS score that was recorded.

Statistics:

The frequency of tumors with high levels of TS expression was analyzed for each variable using the χ^2 or Fisher's exact test, as indicated. The association between TS expression and the proportion of responses was assessed using the Mantel test for trend. The baseline area of the measured tumor lesions and its maximum percentage of reduction following treatment, as well as the baseline serum levels of carcinoembryonic antigen (CEA), lactate dehydrogenase (LDH), alkaline phosphatase (AP) and WBCs, were compared between patients with low and high levels of TS expression using the Student's *t* test for unpaired samples. Survival and progression-free survival were calculated from the

onset of chemotherapy until death or progression, respectively. Patients without events were censored at the date of the last follow-up. For overall survival, death from any cause was considered as an event. Progression-free and overall survival curves were constructed using the Kaplan-Meier method and differences were assessed by the log-rank test.

To assess the relative influence of different prognostic factors on response to chemotherapy, logistic regression analysis with a backward procedure was used. Because of a limited number of events, only a limited model could be run. However, this model included all the variables that were predictive of response to chemotherapy by univariate analysis (TS, CEA, PS

and age). Data were entered, checked and analyzed using EPI-INFO (version 6.1) software package [18].

RESULTS

Patient's characteristics and response data are summarized in Tables (1 & 2). Forty-eight patients treated achieved a response rate of 48% (5 CR, 18 PR). The 95% confidence limit is 34-63%. Twenty-one (44%) of 48 patients showed high levels of TS expression, as defined by a TS score equal to 3 and 4 in 16 and five cases, respectively. Among the patients with a low level of TS expression (27 of 48, 56%), 10 exhibited negative staining for TS, nine had a staining score of 1 and eight had a score of 2.

Table (1): Patients characteristics.

| Characteristics | |
|---|-------|
| No. of patients | 48 |
| Sex: | |
| Male | 30 |
| Female | 18 |
| Age (yr): | |
| Median | 49 |
| Range | 23-61 |
| ≤ 50 | 23 |
| > 50 | 25 |
| Performance status (PS): | |
| 0 | 35 |
| 1-2 | 13 |
| Primary tumor site: | |
| Colon | 38 |
| Rectum | 10 |
| Site(s) of metastases: | |
| Liver only | 27 |
| Liver + others | 13 |
| Others | 8 |
| Baseline tumor area (cm ²): | |
| Median | 26.94 |
| ≤ 26.94 | 24 |
| > 26.94 | 24 |
| Baseline CEA (ng/dl): | |
| Median | 30 |
| ≤ 30 ng/dl | 21 |
| > 30 ng/dl | 27 |
| Baseline LDH (U/L): | |
| Median | 418 |
| ≤ 418 | 28 |
| > 418 | 20 |
| Baseline AP (U/L): | |
| Median | 231 |
| ≤ 231 | 30 |
| > 231 | 18 |
| Baseline WBC (cells/μl): | |
| Median | 6.420 |
| ≤ 6.420 | 25 |
| > 6.420 | 23 |

Table (2): Response to chemotherapy.

| Response | |
|----------|---------------|
| CR | 10.4% (5/48) |
| PR | 37.6% (18/48) |
| MR | 20.8 (10/48) |
| SD | 20.8 (10/48) |
| PD | 10.4 (5/48) |

CR: complete response PR: partial response SD: stable disease PD: progressive disease MR: minor response.

No significant differences in the level of TS expression were found with respect to age, sex, performance status (PS), site of the primary tumor, site of metastases and baseline serum LDH and WBC levels. However, TS expression seemed to be related to initial tumor bulk. The median measured baseline tumor area was 18 cm² and 63 cm² in patients with low and high levels of TS expression, respectively. Sixty-two percent (15 of 24) of the patients with a measured tumor area greater than the median value for the study (26.94 cm²) had a high TS level, compared with 25% (six of 24) of the patients with an initial tumor area of less than this value ($p = 0.009$). High levels of TS expression were also marginally associated with increased baseline CEA or AP levels ($p = 0.058$ and 0.06 , respectively). Accordingly, the median baseline serum CEA and AP levels were 14.4 ng/mL vs 47.8 ng/mL ($p = 0.04$) and 162 U/L vs 252 U/L ($p = 0.025$), respectively, in patients with low and high levels of TS expression. TS expression

was inversely correlated with response to chemotherapy (table 3; $\chi^2 = 12.60$, $p = 0.0004$, two-tailed Mantel test for linear association). The combined CR and PR rate was 66.7% (95% confidence interval, 46% to 83%) and 23.8% (95% confidence interval, 9% to 48%), respectively, in patients with low and high levels of

TS expression (Fig. 2; $p = 0.003$). All the patients ($n=5$) who achieved a CR had low levels of TS expression, whereas all the patients ($n=5$) who progressed after the first cycle had high levels of TS expression (Table 3). All but three patients with low TS levels obtained at least a minor response (Table 3).

Table (3): Outcome according to TS expression.

| TS level | N | CR | | PR | | MR | | SD | | PD | |
|----------|----|----|----|----|----|----|----|----|----|----|----|
| | | N | % | N | % | N | % | N | % | N | % |
| Low | 27 | 5 | 19 | 13 | 48 | 6 | 22 | 3 | 11 | 0 | 0 |
| High | 21 | 0 | 0 | 5 | 24 | 4 | 19 | 7 | 33 | 5 | 24 |

The relationship between TS levels and clinical response remained significant when TS expression was graded from 0 to 4 (response rate: 80%, 66%, 50%, 25% and 20% with a TS score of 0,1,2,3 and 4, respectively; $\chi^2 = 9.81$, $p = 0.0017$, two-tailed Mantel test for linear association). Also, a strong linear association was found between the level of TS immunoreactivity (TS score) and response to chemotherapy (Fig. 2; $\chi^2 = 15.73$, $p = 0.00007$, two-tailed Mantel test for linear association).

The median time to achieve an objective response was 2.0 and 3.3 months in patients with low and high levels of TS expression, respectively. Patients with low levels of TS expression also had a longer duration of response (median duration, 7.5 vs 4.6 months).

The level of TS immunoreactivity (TS score) was linearly related to the actual tumor shrinkage achieved in each patient (Fig. 3; $r = 0.56$, $p = 0.00004$). Although the actual values of the percentage of tumor reduction achieved in patients with low and high levels of TS expression overlap, the mean values for this figure were 65% and 14% in patients with low and high levels of TS expression, respectively (Fig. 4; Student's t test for unpaired samples, $p = 0.0001$). The initial PS score and the baseline CEA level are the only other variables significantly associated with response to chemotherapy (Table 4).

Logistic regression analysis of these prognostic factors indicated that intratumoral TS expression was an independent predictor of response to treatment and the relative probability of achieving an objective response among patients with low TS was 5.0 (Table 5).

Treatment administration and dose delivery seemed to be unrelated to the level of TS expression. The median number of cycles was four versus three and the mean percentage of dose reduction 13% vs 24%, in patients with low and high levels of TS expression, respectively (difference not significant). All patients in this study are now off treatment, 4 of 48 have died and 44 patients have progressed. One patient is still in CR at 41 months. The median follow-up time was 23 months and the median time to progression for all 48 patients was 8.4 months (range, 2 to 41 months). The median time to progression was 6.2 months (range, 2.0 to 15.8 months) in patients with high TS levels compared with 9.6 months (range, 3.8 to 36.4 months) in the group with low TS levels ($p = 0.005$; log-rank test).

Patients with low levels of TS expression survived longer. The 2- and 3-year survival rates were 41% vs 15% and 19% vs 0% and the median survival time was 18.4 months (range, 5.8 to 41 months) vs 15.4 months (range, 2.0 to 34 months) ($p = 0.02$; log-rank test) in patients with low and high levels of TS expression, respectively.

Table (4): Response to chemotherapy according to different patient and tumor characteristics.

| | No. of patients | Response to chemotherapy | | <i>p</i> |
|--|-----------------|--------------------------|------|----------|
| | | No. | % | |
| <i>Age, years:</i> | | | | |
| ≤ 50 | 23 | 12 | 52.2 | 0.57 |
| > 50 | 25 | 11 | 44 | |
| <i>Sex:</i> | | | | |
| Male | 23 | 10 | 43.5 | 0.55 |
| Female | 25 | 13 | 52 | |
| <i>P.S.:</i> | | | | |
| 0 | 29 | 18 | 62.1 | 0.015 |
| 1-2 | 19 | 5 | 26.3 | |
| <i>Primary tumor site:</i> | | | | |
| Colon | 38 | 17 | 44.7 | 0.62 |
| Rectum | 10 | 6 | 60 | |
| <i>Site(s) of metastases:</i> | | | | |
| Liver only | 27 | 16 | 59.3 | 0.07 |
| Liver + others | 13 | 6 | 46.2 | |
| Others | 8 | 1 | 12.5 | |
| <i>TS expression:</i> | | | | |
| Low | 27 | 18 | 66.7 | 0.003 |
| High | 21 | 5 | 23.8 | |
| <i>Baseline tumor area (cm²):</i> | | | | |
| ≤ 26.94 | 24 | 14 | 58.3 | |
| > 26.94 | 24 | 9 | 37.5 | |
| <i>Baseline CEA (ng/dl):</i> | | | | |
| ≤ 30 ng/dl | 24 | 16 | 66.6 | 0.01 |
| > 30 ng/dl | 23 | 7 | 30.4 | |
| <i>Baseline LDH (U/L):</i> | | | | |
| ≤ 418 | 28 | 14 | 50 | 0.16 |
| > 418 | 20 | 9 | 45 | |
| <i>Baseline AP (U/L):</i> | | | | |
| ≤ 231 | 30 | 19 | 63.3 | 0.23 |
| > 231 | 18 | 4 | 22.2 | |
| <i>Baseline WBC (cells/μl)</i> | | | | |
| ≤ 6.420 | 25 | 14 | 56 | 0.15 |
| > 6.420 | 23 | 9 | 39.1 | |

Table (5): Logistic regression analysis of variables predictive of response to chemotherapy.

| | Exp (coef) | 95% confidence interval | <i>p</i> |
|-----------------------------------|------------|-------------------------|----------|
| TS expression (low vs high) | 4.99 | 1.15-21.7 | 0.022 |
| PS (0 vs 1-2) | 4.51 | 1.00-20.2 | 0.035 |
| Baseline CEA (> 29 vs ≤ 29 ng.mL) | 3.63 | 0.86-15.2 | 0.064 |
| Age (> 60 vs ≤ 60 years) | 1.90 | 0.40-8.97 | 0.397 |

lated into a small, but statistically significant, survival benefit. These results compare well to those obtained by other investigators using a polymerase chain reaction-based technique to measure TS mRNA [30]. While the optimal technique to assess TS in human tumor samples remains to be defined, the potential for widespread use of the immunohistochemical assay that we have used has a major clinical relevance. Other factors have been shown to affect the clinical response to chemotherapy in this disease, including baseline CEA and AP levels, percentage of liver involvement and initial PS score [4,21,27,28,41,44]. PS was found to predict response to chemotherapy, almost to the same degree as TS expression. However, the relationship between PS and response to chemotherapy is independent from the agent that is used [41], whereas the predictive role of TS may be unique in that groups of patients could be identified who are unresponsive to a specific class of drugs and may be candidates for alternative forms of treatment. This is of major importance, especially considering the recent development of new drugs with TS-unrelated mechanisms of action that may be used in patients unlikely to respond to TS inhibitors. The existence of a linear relationship between TS immunoreactivity in tumor samples and tumor response to chemotherapy strengthens the notion that TS status is a major determinant of response to this regimen. In this study, high levels of TS expression were observed more frequently in patients with increased baseline serum CEA and AP levels. This issue needs further investigation, but may be because CEA and AP levels may reflect the actual bulk of disease [27,28] and because TS expression in this study was also associated with the measured baseline tumor area, this relationship between TS expression and clinical outcome may reflect the greater responsiveness of patients with a lower tumor burden and good clinical conditions. However, by logistic regression analysis, the predictive ability of TS expression for response to chemotherapy was independent from other important prognostic factors, including PS score and CEA levels. In addition, there were no differences in compliance with treatment or dose delivery between patients with low and high TS levels that might account for the different response rates in these two groups. Baseline pretreatment TS levels are not the only determinant of TS inhibition. The rate of fluorodeoxyu-

ridine monophosphate formation [2], as well as the rise in the competing pool of deoxyuridylate [35], the availability of 5,10 - methylenetetrahydrofolate in the more effective polyglutamylated form [3,45] and the activity of the FU catabolizing enzyme dihydropyrimidine dehydrogenase [38] have been shown to influence both the degree and the duration of TS inhibition. In addition, an acute induction of TS levels has been observed after treatment with TS inhibitors, leading to a recovery of enzyme activity [11]. Molecular factors modulating the pathways of cellular death downstream of TS inhibition [5, 6,43], as well as clinical variables generally affecting response to chemotherapy in advanced colorectal cancer [21,27,28], may also lower the predictive ability of TS expression for the clinical outcome. Considering all these factors, it is not surprising that neither TS immunoreactivity nor TS mRNA levels [30] could fully predict response to chemotherapy. In the present study, the predictive value of a low level of TS expression for a good clinical outcome seemed to be superior to that of high levels of expression for a poor outcome. Although no progressions were reported in the first group, five patients with high levels of expression obtained a partial response. In contrast, in the trial measuring TS mRNA levels, a threshold value could be identified above which no responses were obtained [30].

On the other hand, the prediction of low levels of TS expression for a good clinical outcome was stronger in the current study, as two thirds of the patients with low TS immunoreactivity achieved an objective response, all but three patients achieved at least a 25% reduction in tumor mass and no patient progressed on therapy. In contrast, an approximately 50% failure rate has been reported among patients with low TS mRNA levels [30]. This discrepancy may reflect the potential ability of immunohistochemical assays to identify high levels of TS expression in patients who seem to express low TS levels with other techniques. Although a rough correlation between TS protein and gene levels has been shown [20,25,36], translational regulation may have a substantial role in determining the levels of expression of this enzyme [10]. Immunohistochemistry may thus give a more direct and accurate estimate of TS protein content compared with mRNA quantitation. Immunohistochemical assays also allow consideration of the histologic features of the sample

analyzed, enabling detection of its contamination with normal tissues, which may be an important confounding factor.

This study demonstrates that the levels of TS protein expression, measured with a convenient, low-cost immunohistochemical technique on specimens of colorectal cancer metastases or recurrences, may predict the clinical response to FU regimens. Our data suggest that TS immunoreactivity is the single best predictor of response and may thus be used to select patients unresponsive to FU containing regimens for treatment with alternative drugs. Given the consistent results obtained in two other studies [20,30] that used completely different techniques, TS expression should also be considered before randomization in future clinical trials comparing different forms of treatment for advanced colorectal cancer. Further studies to confirm these results with a large number of patients are required. In conclusion, our results demonstrate the ability of TS protein expression to predict response to 5FU. This could have a significant impact on the choice of drugs in treatment of advanced colorectal cancer and may change the therapeutic approach from a general to an individual treatment strategy.

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