Research Article

Measurement of Nucleic Acid Metabolizing Enzymes in Stage III Colorectal Cancer Adds Precision to Adjuvant Fluorouracil and Leucovorin Therapy

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Abstract

Background: Recurrence of colorectal cancer has been shown to be inhibited by adjuvant chemotherapy. To achieve effective adjuvant chemotherapy, a target molecule is necessary. Therefore, we performed a prospective clinical trial to determine the relationship between adjuvant chemotherapy outcomes and prognostic factors in colorectal cancer based on nucleic acid metabolizing enzyme levels.

Methods: A total of 147 patients with stage IIIA, IIIB, and IIIC cancer were selected for adjuvant chemotherapy. We analyzed Disease-Free Survival (DFS) as the primary endpoint and Overall Survival (OS) as the secondary endpoint in relation to the Thymidylate Synthase (TS) and Dihydropyrimidine Dehydrogenase (DPD) levels as measured by enzyme-linked immunosorbent assay. According to the levels of the mean preliminary TS of 21.0 ng/mg protein and DPD of 115 ng/mg protein, the patients were divided into 4 groups. They were then administered postoperative adjuvant chemotherapy that primarily included fluorouracil and Leucovorin (FL) or Uracil/Tegafur (UFT) and Leucovorin (UZEL) for 6 months.

Results: The 5-year DFS in advanced stage III cancer was 63.9%. A significant difference was observed between the LL (low TS and low DPD levels) and HL (high TS and low DPD levels) groups and the LH (low TS and high DPD levels) and HH (high TS and high DPD levels) groups. Multivariate analysis revealed a significant difference in stage, TS level, and TS and DPD levels. The OS was 79.6% for all the groups. A significant difference was observed between the LL, LH, and HL groups and the HH groups. Multivariate analysis revealed a significant difference in the stage, age, TS level, and TS and DPD levels.

Conclusions: Measurement of the TS and DPD levels of a primary colorectal cancer lesion as target molecules may be helpful for achieving an effective postoperative adjuvant chemotherapy. TS and DPD can potentially serve as more accurate predictors of OS than age and cancer stage.

Keywords: Adjuvant Chemotherapy; Colorectal Cancer; Metabolizing Enzyme; Precision Medicine

Introduction

In colorectal cancer with lymph node metastasis, recurrence has been shown to be inhibited by postoperative adjuvant chemotherapy, which involves the administration of anticancer agents (mainly fluorouracil) for 6 months as a standard treatment

[1]. Fluorouracil and Leucovorin (FL) therapy has been shown to inhibit recurrence by 18% compared with the untreated group in the previous IMPACT study [2]. Moreover, comparable results have been found with the administration of Uracil/Tegafur (UFT)/Leucovorin (UZEL) [3] and capecitabine [4]. In patients in whom FL therapy is inadequate as adjuvant chemotherapy and who have a high risk of recurrence, as well as in patients in whom FL therapy is adequate but to further ensure the inhibition of recurrence,

oxaliplatin-based combination adjuvant chemotherapy is typically administered. A 24% decrease in the risk of recurrence and further inhibitory effect on recurrence were observed in a previous multicenter study by Andre, et al. [5]. However, such effects have not been shown in combination therapy with target molecules [6,7]. There have also been no reported clinical trials to date showing ‘folinic acid, 5-fluorouracil, and irinotecan’ (FOLFIRI) therapy as an effective adjuvant chemotherapy [8]. In unresectable, advanced, and recurrent colorectal cancer, the effects of ‘oxaliplatin, 5-fluorouracil, and folinic acid’ (FOLFOX) and FOLFIRI [9] therapies have been found to be comparable, whereas combination with other molecular targeting agents has been shown to effectively prolong patient survival [10,11]. When oxaliplatin alone is used for treatment, it does not elicit a response for colon cancer. However, when oxaliplatin is combined with FL therapy, a high response is elicited. From this background, fluorinated pyrimidines have been extensively used in colorectal cancer for adjuvant and intensive chemotherapy [12,13]. In terms of fluorinated pyrimidine anticancer drug metabolism, this is strongly affected by Thymidylate Synthase (TS) and Dihydropyrimidine Dehydrogenase (DPD) as nucleic acid metabolizing enzymes. In FL therapy, it is expected that the effects will enhance the TS inhibition rate. In the present study, we measured the TS and DPD levels by Enzyme-Linked Immunosorbent Assay (ELISA) and examined the potential clinical application of these 2 enzymes in postoperative adjuvant chemotherapy in the form of FL therapy.

Materials and Methods

This study was approved by the Ethical Review Board of Tokyo Medical University and Tokyo Medical University Hachioji Medical Center. TS and DPD levels were measured by ELISA in the primary colorectal cancer lesions of 147 patients with advanced stage III cancer between February 2004 and May 2007. Patients with advanced stage III cancer had a mean age of 65.1 ± 11.5 years, with 80 males and 67 female patients. The cancer staging was IIIA and IIIB in 96 patients and IIIC in 51 patients (Table 1).

1. Adjuvant chemotherapy (n = 147)	
Mean age:	65.1 ± 11.5 years
Male patients:	80 patients
Female patients:	67 patients
Stage IIIA and Stage IIIB:	96 patients
Stage IIIC:	51 patients
FL therapy:	34 patients
UFT/UZEL:	113 patients

Table 1: Patient characteristics.

Based on the results of a previous study indicating a mean TS level of 21.0 ng/mg protein and a mean DPD level of 115 ng/mg protein [14], all 147 patients were divided into the LL (low TS and low DPD levels), HL (high TS and low DPD levels), LH

(low TS and high DPD levels), and HH (high TS and high DPD levels) groups. The LL group consisted of 36 cases, HL group 35 cases, LH group 36 cases, and HH group 40 cases. Postoperative chemotherapy was administered to the patients for 6 months with FL therapy or UFT and UZEL. The primary endpoint was Disease-Free Survival (DFS) and the secondary endpoint was Overall Survival (OS). During the period of the present clinical trial, the use of FOLFOX has not yet been approved for adjuvant chemotherapy in Japan.

Enzyme Activity Measurement

The protein expression levels of TS and DPD in the tissue samples excised fresh from operative specimens were measured. The excised tissue measured approximately 5 mm³ which corresponded to a wet weight of 100-200 mg. The tissue was immediately frozen and stored at -80°C until further processing. The TS and DPD protein levels were quantified by ELISA in collaboration with Taiho Pharmaceutical Co., Ltd. (Tokyo, Japan) using a two-step sandwich method. Tissue samples for ELISA were homogenized with 4 volumes of 20 mM TBS buffer (pH 7.5) containing 0.1% (v/v) Tween-20 and were centrifuged at 105,000 g for 60 min. The supernatants were then used for the TS and DPD level measurements by ELISA. Crude enzyme extracts were diluted with 20 mM TBS buffer (pH 7.5) containing 0.1% Tween-20 to determine the amount of TS, or with 20 mM PBS buffer (pH 7.0) containing 2% (w/v) bovine serum albumin and 0.1% (v/v) Triton X-100 to determine the amounts of DPD. Crude enzyme extracts and standard proteins were added to the wells of the TS and DPD ELISA plates and colorimetric measurement at an optical density of 490 nm was performed using a plate reader (BioKinetic Reader RJ 340; Bio-Tek Instruments Inc., Winooski, VT, USA).

The protein contents of the tissue extracts were measured colorimetrically at 490 nm using bicinchoninic acid protein reagent. The amounts of TS and DPD protein in the tissue extracts were calculated as nanograms of TS and DPD enzyme protein per milligram of tissue protein, using the standard curves for TS and DPD, respectively. Initially, after tissue homogenization, the sample was centrifuged and the supernatant was collected, appropriately diluted, and dispensed into a solid-phased plate with TS antibodies (or DPD antibodies) added and left to react. Subsequently, peroxidase-conjugated antibodies were added to the TS or DPD antibodies to form a sandwich-type complex consisting of TS antibody-TS peroxidase-conjugated TS antibody and DPD antibody-DPD peroxidase-conjugated DPD antibody. Thereafter, orthophenylenediamine (chromogenic substrate) and hydrogen peroxide (peroxidase substrate) were added. The intensity of the color produced by the reaction with the peroxidase in the complex was measured. Afterwards, the TS protein expression level (or DPD protein expression level) in the tissue was measured from the analytical curve created using standard preparations.

Statistical Analysis

Statistical analysis was performed using SPSS II Ver. 22.0. Means were used from the *t* test. DFS was defined as the time from assignment to relapse or death, whichever occurred first. OS was calculated from the date of assignment to the date of death from any cause. Time to event endpoints were calculated using the Kaplan-Meier method and were described. Multivariable Cox models were stratified on the treatment parameter which allowed consideration of the treatment effect on outcomes. The statistical significance of the differences between groups was analyzed using the Kaplan-Meier test and Cox regression. A *p*-value of < 0.05 was considered to indicate a statistically significant difference.

Results

In postoperative chemotherapy, the 5-year DFS rate in stage III cancer was 63.9 %, and there was no significant difference in the DFS rate between the 4 groups. Our specific findings included 76.5% in the LL group, followed by 72.7%, 56.8%, and 52.8% in the HL, LH, and HH groups, respectively ($p = 0.123$) (Figure 1).

Among the 4 groups, disease-free survival was good in 2 groups in which the DPD level was low. However, a statistically significant difference was not observed.

Figure 1: Disease-free survival rates in stage III patients.

A significant difference was observed between the LL and HL groups as well as the LH and HH groups ($p = 0.020$) (Figure 2).

A statistically significant difference was found ($p = 0.020$).

Figure 2: Comparison of disease-free survival rates between the 2 groups in which the DPD levels were low and high.

A significant OS of 79.6% ($p = 0.010$) was observed for the 4 groups, namely, 91.2% in the LL group, followed by 81.8%, 81.8%, and 63.9% in the LH, HL, and HH groups, respectively (Figure 3).

A statistically significant difference in the overall survival was found between the 4 groups

Figure 3: Overall survival of stage III patients.

In the multivariate analysis, there was a significant difference in the DFS rate for stage ($p = 0.002$), TS ($p = 0.003$), and with TS and DPD ($p = 0.012$) (Table 2).

Group	B	SE	Wald	df	P-value	Exp (B)
			7.232	3	.065	
Group (1)	-.613	.581	1.114	1	.291	.542
Group (2)	.945	.521	3.291	1	.070	2.574
Group (3)	.563	.616	.833	1	.361	1.755
Age	.015	.014	1.193	1	.275	1.015
Sex	-.315	.305	1.065	1	.302	.730
Stage	.970	.310	9.802	1	.002	2.637
TS	.048	.016	9.008	1	.003	1.049
DPD	.007	.007	1.022	1	.312	1.007
TS and DPD	-.014	.005	6.374	1	.012	.986

There were statistically significance differences for stage ($p = 0.02$), TS ($p = 0.003$) level, and the combination of TS and DPD levels ($p = 0.012$).

Table 2: Disease-free survival factors affecting adjuvant chemotherapy.

As well as in OS for group ($p = 0.023$), stage ($p = 0.022$), age ($p = 0.03$), TS ($p = 0.001$), and TS and DPD ($p = 0.001$) (Table 3).

Group	B	SE	Wald	df	P-value	Exp (B)
			9.542	3	.023	
Group (1)	-.740	.875	.715	1	.398	.477
Group (2)	1.554	.819	3.597	1	.058	4.730
Group (3)	1.391	.897	2.404	1	.121	4.020
Age	.076	.025	9.048	1	.003	1.079
Sex	-.498	.460	1.173	1	.279	.607
Stage	1.083	.473	5.245	1	.022	2.953
TS	.079	.023	11.859	1	.001	1.082
DPD	.017	.009	3.230	1	.072	1.017
TS and DPD	-.030	.008	14.362	1	.001	.971

There were statistically significance differences for group ($p = 0.023$), age ($p = 0.03$), stage ($p = 0.22$), TS level ($p = 0.001$), and the combination of TS and DPD levels ($p = 0.01$).

Table 3: Overall survival factors affecting adjuvant chemotherapy.

Taken together, measurement of the TS and DPD levels of the primary colorectal cancer lesion as target molecules may be helpful for achieving an effective postoperative adjuvant chemotherapy. In particularly, a low TS level is a good marker. Cases with a high TS level can expect an adjuvant chemotherapy effect if the DPD level is low. However, for cases with high TS and DPD levels, the treatment outcome is poor and more intensive chemotherapy is necessary. With regard to OS, it is necessary to consider not only TS and DPD but also the staging and age as important factors. Prognosis is poor in cases with high levels of TS and DPD, which may be more appropriate as prognostic predictors than staging and age.

Discussion

In advanced stage III colorectal cancer with lymph node metastasis, postoperative adjuvant chemotherapy, including FL

[2], capecitabine [3], or FOLFOX [4] therapy, has been found to result in significant DFS benefit. Fluorinated pyrimidine anticancer agents have an inhibitory effect on cell division by utilizing the difference in nucleic acid metabolizing enzymes within the tumor compared with normal tissue. In matched samples of primary Colorectal Cancer (CRC) and normal colonic tissue, the TS level in the primary CRC was significantly higher than that in the normal colonic tissue, whereas the DPD level in the primary CRC was significantly lower than that in the normal tissue [14]. Therefore, this effect is naturally believed to involve the expression of such enzymes. These enzymes primarily include TS, DPD, and Thymidine Phosphorylase (TP), and have been reported to be target molecules of fluorinated pyrimidine anticancer agents, as well as prognostic indicators of CRC [15-17]. In terms of mechanism of action, TS is an enzyme that plays a key role in DNA synthesis

from thymine, and the TS level has been found to correlate with the degree of cancer malignancy. Notably, the TS inhibition rate is improved in FL therapy by fluorodeoxyuridine monophosphate (FdUMP). On the other hand, DPD is an enzyme that inactivates uracil; therefore, in cancers with an elevated DPD level, the effect of fluorinated pyrimidine anticancer agents is considered to be lower. Furthermore, TP is somewhat similar to the angiogenic growth factor PD-ECGF. TP shows the strongest correlation with the degree of cancer malignancy and is involved in 5'DFUR and capecitabine metabolism.

In 2004 when we started the present clinical trial, the use of chemotherapy combined with capecitabine or oxaliplatin as an adjuvant chemotherapy was not yet approved in Japan, and the standard treatment was primarily the UFT/UZEL combination or FL therapy. In the UFT/UZEL combination, inferiority was proved in clinical trials with FL therapy [3]. Therefore, we selected TS and DPD as the enzymes for measurement. In reference to previous reports, there has not been a definite trend in the outcome prediction with a single factor [18], as examination using 1 factor was inferred to be difficult. Therefore, we examined DFS and OS by comparing 4 groups using 2 enzymes. As previously reported [14], the possibility that these enzymes can be used as prognostic factors for other staged cancers is being considered. In particular, these enzymes can be used as factors for predicting chemotherapy outcomes in advanced stage IVa and IVb cancers, and enzyme measurement is performed in patients who provide consent. The 5-year DFS rate in the present clinical trial was 69.3%, which is not inferior to the DFS rate in the ACTS-CC trial that commenced in 2008 [19]. We believe that although the results are from clinical trials in 2 different facilities, they provide high-quality data. The present results suggest the strong effect of TS on DFS as an independent factor. This is because the FL therapy involves a mechanism to increase TS inhibition, and naturally the effect depends on the TS level. Both patients in the low TS and DPD level group and the high TS and low DPD level group showed good DFS. If there is little effect from the inactivation of DPD, it was suggested that TS inhibition is possibly sufficient. However, it can be inferred that FL therapy is effective in the HL group with low TS levels. This results in patients with a high DPD level, and the low TS group suggests that the effect of the inactivation of DPD was strong, and that TS inhibition was insufficient.

These results indicate that TS and DPD as a combined factor are useful target molecules in adjuvant chemotherapy. Overall, we suggest that this clinical trial was not able to show a sufficient adjuvant chemotherapy effect for groups with a high DPD level. As DPD inactivates uracil, this was considered to decrease the effect of fluoride pyrimidine drugs. In CRC, the DPD level is low, thus the effect of fluoride pyrimidine drugs is expected. It is considered that resistance to fluoride pyrimidine drugs is developed because the DPD level is generally higher in pancreatic and gastric cancers than

in CRC. However, these carcinomas show a response when S-1 is used as adjuvant chemotherapy [20,21]. For CRC, it appears to have a low DPD level and that there is little evidence of S-1 efficacy. S-1 has strong DPD antagonism via tegafur. S-1 includes gimeracil with effects of raising the concentrations in which tegafur is converted into fluorouracil and oteracil leading to a reduction of gastrointestinal toxicity. S-1 is currently used in Europe and Asia.

This clinical trial therefore points to the following question: what agent should be administered to the LH and HH groups. The non-inferiority of S-1 as adjuvant chemotherapy has been proven in clinical trials [19], and although it is expected to be effective in patients with high DPD levels, this remains a topic requiring further examination. It has been demonstrated that the outcomes of adjuvant chemotherapy using FOLFOX therapy following surgery for CRC are better than those of adjuvant chemotherapy using FL therapy. Therefore, adjuvant chemotherapy of CRC has been switched to FOLFOX therapy. However, FOLFOX therapy has 1 problem. Depending on the patient, oxaliplatin neurotoxicity can persist for long periods [22]. Moreover, the prognostic factors and supportive therapy for oxaliplatin neurotoxicity remain unclear. The finding that individualized treatment can be administered according to TS and DPD measurements, such as administration of FL therapy to patients in whom FL therapy is sufficient or oxaliplatin combination chemotherapy to patients in whom FL therapy is inadequate, is beneficial for the patient and underscores the significance of the present clinical trial. Following adjuvant chemotherapy for advanced stage III CRC patients who have undergone FL therapy, DFS can be satisfactorily estimated by combining TS and DPD measurements for the primary CRC lesion. In the LL and LH groups, FL therapy alone offered adequate DFS. However, the results suggested that for the other 2 groups, a period of adjuvant chemotherapy and combination oxaliplatin therapy was needed to improve therapeutic outcomes. Therefore, with respect to FL therapy, we believe that TS and DPD can be used as target molecules in postoperative adjuvant chemotherapy and both can be considered important prognostic factors for achieving precision medicine.

In the examination of biomarkers in the Alliance study, the results of TS are reported. The article reports an association between MMR, BRAF, and TS [23]. TS and other BRAF are not factors that affect the direction of adjuvant chemotherapy, but effective as prognostic biomarkers. The results of the IDEA study [24] are the newest about adjuvant chemotherapy and its results have already been adopted in the NCCN guidelines. This study compares CAPOX and FOLFOX for Stage III colon cancer. In the low risk Stage III colon cancer group, administration is recommended for three months CAPOX. This result shows the advantage of CAPOX over FOLFOX regarding neurotoxicity. Because our study fixed the dosing period, we could not examine the appropriate treatment period. However, in our study, the LL group was the low risk group

for recurrence. In addition, the chemotherapy using an oxaliplatin combination was based on FL therapy. This suggests that the measurement of TS and DPD may be useful in the future.

The OS results were approximately equivalent except in the HH group. The FOLFOX or FOLFIRI therapy in combination with 5FULV2 therapy and irinotecan increases the treatment effect more than a single agent [25]. For oxaliplatin, the treatment effect increases markedly with 5FULV2 combination although it is ineffective as a single agent [12,13]. Naturally, the effect of 5FULV2 therapy is strongly influenced by the effect of TS and DPD. We suggest that the effects of FOLFOX and FOLFIRI therapies increase if the effect of 5FULV2 is strong. Therefore, we can expect TS and DPD to have an effect on chemotherapy as predictors of the recurrence of CRC.

Adjuvant chemotherapy of CRC is administered using pyrimidine fluoride anticancer drugs or oxaliplatin combination. Regarding the choice of the regimen, in stage III cases with extensive lymph node metastases, combination with oxaliplatin is more desirable and the dosing period is not limited to half a year, thus, a sufficient number of cases may be treated in 3 months. In the future, individualization should be carried out for adjuvant chemotherapy. To achieve progress in preventing CRC recurrence, precision medicine should be carried out by searching the responsible gene. The possibility of TS and DPD playing this part in the adjuvant chemotherapy of CRC was suggested.

References

- Haller DG, Catalano PJ, Macdonald JS, O'Rourke MA, Frontiera MS, et al. (2005) Phase III study of fluorouracil, leucovorin, and levamisole in high-risk stage II and III colon: Final report of intergroup 0089. *J Clin Oncol* 23: 8671-8678.
- Labianca R, Marsoni S, Pancera G, Torri V, Zaniboni A, et al. (1995) Efficacy of adjuvant fluorouracil and folinic acid in colon cancer. International Multicentre Pooled Analysis of Colon Cancer Trials (IMPACT) investigators. *Lancet* 345: 939-944.
- Shimada Y, Hamaguchi T, Mizusawa J, Saito N, Kanemitsu Y, et al. (2014) Randomized phase III trial of adjuvant chemotherapy with oral uracil and tegafur plus leucovorin versus intravenous fluorouracil and levofolinate in patients with stage III colorectal cancer who have undergone Japanese D2/D3 lymph node dissection: Final results of JCOG0205. *Eur J Cancer* 50: 2231-2240.
- Twelves C, Wong A, Nowacki MP, Abt M, Burris III H, et al. (2005) Capecitabine as adjuvant treatment for stage III colon cancer. *N Engl J Med* 352: 2696-2704.
- Andre T, Boni C, Mounedji-Boudiaf L, Navarro M, Tabernero J, et al. (2004) Oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment for colon cancer. *N Engl J Med* 350: 2343-2351.
- Allegra CJ, Yothers G, O'Connell MJ, Sharif S, Petrelli NJ, et al. (2011) Phase III trial assessing bevacizumab in stages II and III carcinoma of the colon: Result of NSABP protocol C-08. *J Clin Oncol* 29: 11-16.
- Alberts SR, Sargent DJ, Nair S, Mahoney MR, Mooney M, et al. (2012) Effect of oxaliplatin, fluorouracil, and leucovorin with or without cetuximab on survival among patients with resected stage III colon cancer: a randomized trial. *JAMA* 307: 1383-1393.
- Saltz LB, Niedziecki D, Hollis D, Goldberg RM, Hantel A, et al. (2007) Irinotecan fluorouracil plus leucovorin is not superior to fluorouracil plus leucovorin alone as adjuvant treatment for stage III colon cancer; results of CALGB 89803. *J Clin Oncol* 25: 3456-3461.
- Tournigand C, Andre T, Achille E, Lledo G, Flesh M, et al. (2004) FOLFIRI Followed by FOLFOX6 or the reverse sequence in advanced colorectal cancer: a randomized GERCOR study. *J Clin Oncol* 22: 229-237.
- Saltz LB, Clarke S, Di'az-Rubio E, Scheithauer W, Figer A, et al. (2008) Bevacizumab in combination with oxaliplatin-based chemotherapy as first-line therapy in metastatic colorectal cancer: A randomized phase III study. *J Clin Oncol* 26: 2013-2019.
- Van Cutsem E, Köhne CH, Hitre E, Zaluski J, Chang Chien CR, et al. (2009) Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. *N Engl J Med* 360: 1408-1417.
- Boku N, Ohtsu A, Hyodo I, Shirao K, Miyata Y, et al. (2007) Phase II study of oxaliplatin in Japanese patients with metastatic colorectal cancer refractory to fluoropyrimidines. *Jpn J Oncol* 37: 440-445.
- Rothenberg ML, Oza AM, Bigelow RH, Berlin JD, Marshall JL, et al. (2003) Superiority of oxaliplatin and fluorouracil-leucovorin compared with either therapy alone in patients with progressive colorectal cancer after irinotecan and fluorouracil-leucovorin: Interim results of a phase III trial. *J Clin Oncol* 21: 2059-2069.
- Sumi T, Katsumata K, Tsuchida A, Ishizaki T, Shimazu M, et al. (2010) Correlation of clinicopathological factors with protein expression levels of thymidylate synthase, dihydropyrimidine dehydrogenase and orotate phosphoribosyl transferase in colorectal cancer. *Chemotherapy* 56: 120-126.
- Nishimura G, Terada I, Kobayashi T, Ninomiya I, Kitagawa H, et al. (2002) Thymidine phosphorylase and dihydropyrimidine dehydrogenase levels in primary colorectal cancer show a relationship to clinical effects of 5'-deoxy-5-fluorouridine as adjuvant chemotherapy. *Oncol Rep* 9: 479-482.
- Schüller J, Cassidy J, Dumont E, Roos B, Durston S, et al. (2000) Preferential activation of capecitabine in tumor following oral administration to colorectal cancer patients. *Cancer Chemother Pharmacol* 45: 291-297.
- Ichikawa W, Uetake H, Shiota Y, Yamada H, Nishi N, et al. (2003) Combination of dihydropyrimidine dehydrogenase and thymidylate synthase gene expressions in primary tumors as predictive parameters for the efficacy of fluoropyrimidine-based chemotherapy for metastatic colorectal cancer. *Clin Cancer Res* 9: 786-791.
- Katsumata K, Sumi T, Yamashita S, Takahashi S, Mori M, et al. (2001) The significance of thymidine phosphorylase expression in colorectal cancer. *Oncol Rep* 8: 127-130.
- Yoshida M, Ishiguro M, Ikejiri K, Mochizuki I, Nakamoto Y, et al. (2014) S-1 as adjuvant chemotherapy for stage III colon cancer: A randomized phase III study. *Ann Oncol* 25: 1743-1749.
- Sasako M, Terashima M, Ichikawa W, Ochiai A, Kitada K, et al. (2015) Impact of the expression of thymidylate synthase and dihydropyrimidine dehydrogenase genes on survival in Stage II/III gastric cancer. *Gastric Cancer* 18: 538-548.

21. Uesaka K, Boku N, Fukutomi A, Okamura Y, Konishi M, et al. (2016) Adjuvant chemotherapy of S-1 versus gemcitabine for resected pancreatic cancer: a phase 3, open-label, randomized, non-inferiority trial (JASPAC 01). *Lancet* 388: 248-257.
22. Pachman DR, Qin R, Seisler DK, Smith EM, Beutler AS, et al. (2015) Clinical course of oxaliplatin-induced neuropathy: Results from the randomized phase III Trial N08CB (Alliance). *J Clin Oncol* 23: 3416-3422.
23. Niedzwiecki D, Hasson RM, Lenz HJ, Ye C, Redston M, et al. (2017) A study of Thymidylate Synthase expression as a biomarker for resectable colon cancer: Alliance (Cancer and Leukemia Group B)9581 and 89803. *The oncologist* 22: 107-114.
24. Grothey A, Sobrero AF, Shields AF, Yoshino T, Paul J, et al. (2018) Duration of adjuvant Chemotherapy for Stage III colon cancer. *The New England Journal of Medicine* 378: 1177-1188.
25. Douillard JY, Cunningham D, Roth AD, Navarro M, James RD, et al. (2000) Irinotecan combined with fluorouracil compared with fluorouracil alone as first-line treatment for metastatic colorectal cancer: a multicenter randomized trial. *Lancet* 355: 1041-1047.