ANTICANCER RESEARCH *39*: 1997-2005 (2019)
doi:10.21873/anticanres.13310

Induction of CD3+ and FoxP3+ T Cells in Left-sided Colorectal Tumors After UFT/LV Chemotherapy

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Abstract. *Background/Aim: Immune checkpoint inhibitors are mainly used for right-sided, microsatellite instability-high colorectal tumors. In this study, the effects of oral uracil–tegafur plus leucovorin (UFT/LV) chemotherapy on the gene expressions of four immunotherapy targets and the amounts of tumor-infiltrating lymphocytes (TILs) were investigated. Patients and Methods: Data of 260 patients with stage II or stage III colorectal cancer were analyzed. Gene expression and amount of TILs were evaluated using real-time reverse transcription polymerase chain reaction (CRT-PCR) assay and immunohistochemical staining, respectively. Results: Expression of CTLA4 and LAG3 in tumor tissues was significantly increased after UFT/LV chemotherapy, but only in left-sided tumors. The percentage of high-TIL, high-CD3 and high-FoxP3 patients in the UFT/LV group was significantly higher than that in the control group, only in left-sided tumors. Conclusion: The increase in TILs count, especially of CD3+ T cells and FoxP3+ regulatory T cells, after UFT/LV chemotherapy were specific to left-sided colorectal cancers.*

The therapeutic regimen of 5-fluorouracil (5-FU) plus leucovorin (LV) combined with oxaliplatin or irinotecan has been established as a standard chemotherapeutic regimen for metastatic colorectal cancer (1, 2). Because oral fluoropyrimidine is highly convenient to use, oral uracil and tegafur (UFT) combined with LV, capecitabine or S-1 is now extensively prescribed (3-5).

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Key Words: Colorectal cancer, uracil-tegafur plus leucovorin (UFT/LV) chemotherapy, tumor-infiltrating lymphocytes (TILs), CD3+ T cells, FoxP3+ regulatory T cells.

Use of immune checkpoint inhibitors, namely, pembrolizumab and nivolumab (anti-PD-1 antibodies), has been approved for the treatment of unresectable or metastatic, microsatellite instability-high (MSI-H) or mismatch repair-deficient (dMMR) colorectal cancer following failure of standard chemotherapy (6, 7). Recently, nivolumab plus low-dose ipilimumab (an anti-CTLA-4 antibody) was approved for the same subset of patients in the USA (8). Thus, the MSI-H/dMMR status has been shown to be correlated with the response to immune checkpoint inhibitors in patients with colorectal cancer.

The right-sided colon originates from the midgut, while the left-sided part is derived from the hindgut. Differences in the clinicopathological characteristics, MSI-H/dMMR status, prognosis, and response to epidermal growth factor receptor-targeted monoclonal antibodies have been reported between right-sided and left-sided colorectal cancers (9-12). Because of the low frequency of the MSI-high/dMMR status in left-sided tumors, which is an indication for immunotherapy (13, 14), immunotherapy for colorectal cancer has been mainly performed for right-sided tumors. Predictors of response to immunotherapy, other than MSI-high/dMMR, are thought to be high levels of expression of target molecules such as PD-L1 and a high number of tumor-infiltrating lymphocytes (TILs) in the tumor tissues, while changes in these factors after standard chemotherapy may influence the response to subsequent immunotherapy. However, there are few reports regarding changes in these factors after chemotherapy.

We had previously investigated the differences in the expression levels of 5-FU and leucovorin-related genes between right-sided and left-sided tumors using biopsy or resected samples in patients with colorectal cancer receiving UFT/LV as a neoadjuvant chemotherapy (15, 16).

In this study, we investigated the changes in the expression levels of four target genes, namely, *CD274* (PD-L1), *CTLA4, IDO1* and *LAG3*, in right-sided and left-sided tumor tissues before and after neoadjuvant chemotherapy with UFT/LV. Moreover, the counts of TILs and the subtypes

of TILs in the tumor tissues after and in the absence of neoadjuvant chemotherapy with UFT/LV were determined, and also the effects of UFT/LV chemotherapy on the TILs separately in right- and left-sided tumors were investigated.

Patients and Methods

Patients. Data of 260 patients with clinical stage II or stage III colorectal adenocarcinoma who underwent surgery at the Tokai University Hospital between 2011 and 2016 were analyzed in this study. Ninety patients (right-sided: n=42; left-sided: n=48) received preoperative UFT/LV chemotherapy for 2 weeks (UFT/LV group), and 170 patients (right-sided: n=79; left-sided: n=91) did not receive preoperative chemotherapy (control group). In the UFT/LV group, the patients received oral treatment with UFT (300 mg/m^2/day, t.i.d.) and LV (75 mg/day, t.i.d.) for two weeks, with surgery undertaken three days after the final administration. Right-sided tumors included tumors arising from the right-sided colon involving the cecum, ascending colon, hepatic flexure or transverse colon, and left-sided tumors included tumors arising from the descending colon, sigmoid colon or rectum. Cancers arising from the splenic flexure, which represents the boundary between the right- and left-sided colon, were excluded from this study.

This study was conducted with the approval of the Ethics Committees of Tokai University School of Medicine (17R-175) and Taiho Pharmaceutical Co., Ltd (S13-004). All the patients provided written informed consent. The patient characteristics are shown in Table I. There were no differences in the clinical parameters, sex, age, primary tumor site or histological tumor grade between the control and UFT/LV groups.

Table I. *Patient characteristics in the control and UFT/LV groups.*

Characteristic	n (%)				*p*-Value
	Control		UFT/LV		
Total patients	170	(100.0)	90	(100.0)	
Gender					
Male	91	(53.5)	55	(61.1)	0.359
Female	79	(46.5)	35	(38.9)	
Age (years)					
Median:71, Range=33-90					
<75	100	(58.8)	56	(62.2)	0.690
≥75	70	(41.2)	34	(37.8)	
Primary tumor site[a]					
Right (Proximal)	79	(46.5)	42	(46.7)	1.000
Left (Distal)	91	(53.5)	48	(53.3)	
Histological grade					
Well	66	(38.8)	25	(27.8)	0.075
Moderate	91	(53.5)	55	(61.1)	
Poor	2	(1.2)	5	(5.6)	
Mucinous	11	(6.5)	5	(5.6)	

p-Values were calculated by Fisher's exact test. [a]Since tumors arising from the splenic flexure, which represents the boundary between the right- and left-sided colon, were excluded in this study, right-sided colorectal cancer included tumors arising from the cecum, ascending colon, hepatic flexure and transvers colon, while left-sided colorectal cancer included tumors arising from the descending colon, sigmoid colon and rectum.

Gene expression analysis of the immunotherapy targets. Biopsy specimens obtained prior to chemotherapy were used as the pre-chemotherapy samples. A total of 6 colonoscopic biopsy specimens were obtained from each patient. Surgery was performed 3 days after the completion of UFT/LV chemotherapy. The resected tumor specimens were used as the post-chemotherapy samples. Whole tumor tissues, including stromal cells, were used in the gene expression analysis. All the samples were immediately immersed in RNAlater solution (Thermo Fisher Scientific, Waltham, MA, USA) and incubated overnight at 4˚C. They were then removed from the RNAlater solution and stored at −80˚C.

Gene expression analysis was performed in 78 out of the 90 patients of the UFT/LV group for whom both pre-chemotherapy and post-chemotherapy samples were available. Gene expression of 4 immunotherapy targets, CD274 molecule (PD-L1; *CD274*), cytotoxic T-lymphocyte-associated protein 4 (*CTLA4*), lymphocyte-activation gene 3 (*LAG3*) and indoleamine 2,3-dioxygenase 1 (*IDO1*), were quantitatively evaluated using a real-time reverse transcription polymerase chain reaction (RT-PCR) assay, as described below. Total RNA was isolated from the tissues using the RNeasy mini kit (Qiagen, Valencia, CA, USA) and reverse-transcribed using a high-capacity cDNA reverse transcription kit (Thermo Fisher Scientific). A real-time RT-PCR was performed with the ABI PRISM 7900HT sequence detection system using TaqMan Array Cards (Thermo Fisher Scientific), which included duplicated wells of a reference gene and the 4 target genes. The gene expression levels were normalized to those of the reference gene, beta-actin (*ACTB*). The relative gene expression levels were calculated using the delta threshold cycle (Ct) method according to the formula shown below. The expression levels of the target genes were expressed as $2^{-(\text{delta Ct})} \times 10000$, to simplify the calculation.

Expression levels of target gene=$2^{-(\text{delta Ct})} \times 10000$.

Delta Ct=(Ct of target gene) − (Ct of ACTB).

The data of 4, 6, 2 and 4 patients in whom the expression levels of *CD274, CTLA4, IDO1* and *LAG3*, respectively, were below the detection limit in either the pre- or post-chemotherapy samples were excluded from the analysis.

Analyses of the counts of tumor-infiltrating lymphocytes. The TIL counts were quantitatively evaluated using hematoxylin and eosin (H-E)-stained tumor tissue specimens. The subtypes of TILs were evaluated by immunohistochemical staining with the surface markers of lymphocytes (CD3, CD4, CD8 and FoxP3), as follows. Formalin-fixed paraffin-embedded sections were used. Antibodies for the pan T cell marker CD3 (Ready to use, Clone 2GV6, Roche, Basel, Switzerland), helper T-cell marker CD4 (diluted 1:100, clone 4B12, DAKO, Glostrup, Denmark), cytolytic T-cell marker CD8 (diluted 1:40, C8/144B, DAKO) and regulatory T-cell marker FoxP3 (diluted 1:100, Clone SP97, Thermo Fisher Scientific) were used as the primary monoclonal antibodies. An avidin-biotin-peroxidase complex method with diaminobenzidine as chromogen was used according to the manufacturer's instructions. For the CD3 and CD8 immunostaining, the Ventana DISCOVERY ULTRA Slide Staining System (Roche Diagnostics) was used. For the CD4 and FoxP3 immunostaining, the Leica BOND-MAX fully automatic immunohistochemistry system (Leica Biosystems, Nussloch, Germany) was used. Ventana Cell Conditioning Solution (CC1) was used for 60 min for the CD3 and CD8 staining. BOND Epitope Retrieval Solution 2 (AR9640) was used for 20 min for the CD4 and FoxP3 staining.

Table II. *Changes in the expression levels of immunotherapy target genes in tumors during preoperative UFT/LV chemotherapy.*

Gene	n	Relative gene expression level to that of *ACTB* (mean)		Post/Pre ratio	*p*-Value
		Pre	Post		
Right-sided tumors					
CD274	29	4.97	4.68	0.94	0.8115
CTLA4	27	4.26	5.61	1.32	0.2919
IDO1	30	18.60	22.97	1.24	0.5451
LAG3	29	3.03	3.80	1.26	0.3699
Left-sided tumors					
CD274	45	3.88	3.98	1.03	0.8895
CTLA4	45	3.29	6.64	2.02	0.0001**
IDO1	46	17.13	22.96	1.34	0.2122
LAG3	45	2.60	4.03	1.55	0.0056**

p-Values were calculated by a paired *t*-test. **$p<0.01$.

Quantifications of the TIL counts and of the CD3+, CD4+ and CD8+ T cells were performed using the semiquantitative grading system for lymphocytic infiltration in breast cancer proposed by Black *et al*. (17) as follows:
Grade 0: No lymphoid infiltrate seen in the primary tumor.
Grade 1: A few scattered lymphocytes seen in the primary tumor.
Grade 2: A definite scattering of lymphocytes seen in association with invading cords of tumor cells, with predominance of the latter.
Grade 3: Dense lymphocytic infiltration, with an overall lymphoid appearance of the lesion.
Grade 4: Lymphocytic infiltration intimately related to individual tumor cells, imparting a lymphoid structure even at first glance.
Groups classified as grades 0-2 and 3-4 were defined as low and high groups, respectively.

The aforementioned evaluation of TILs and of infiltration by CD3+, CD4+ and CD8+ T-cells was performed by two physicians (SS, HK) under blinded (in terms of patient identification and clinical data) conditions. The evaluation was performed in at least 6 fields at high power magnification (×200) in both the central tumor and at the invasive margin. If different results of evaluation were obtained, microscopic examinations were repeated by the same two physicians to determine the final results.

For FoxP3, the positively stained cells were counted microscopically in 10 fields at high-power magnification (×400) and averaged. More than 30 positive cells and ≤30 positive cells/high power field, on average, were defined as high and low groups, respectively. The numbers of stained cells were comprehensively evaluated in both the central tumor and at the invasive margin.

Statistical analysis. The association between the gene expression levels before and after chemotherapy was calculated by the paired *t*-test. The association between the gene expression levels in right- and left-sided colorectal tumors was calculated by the student t-test. The association between gene expression levels in tumor tissues and high-TIL tumors were evaluated using univariate logistic regression analysis. The odds ratios were calculated as the value per one unit change in gene expression level. *p*-Values were calculated using the Wald test. Fisher's exact test was used for the other statistical analyses in this study. The JMP 9.0.2 statistical software (SAS Institute Inc., Cary, NC, USA) was used for the analyses. Differences were considered significant at $p<0.05$.

Results

Changes in the gene expression levels of the immunotherapy targets after UFT/LV chemotherapy. The gene expression levels of four immunotherapy targets, namely, *CD274* (PD-L1), *CTLA4, IDO1* and *LAG3*, were determined in the pre-chemotherapy samples and post-chemotherapy samples obtained after UFT/LV neoadjuvant chemotherapy. In pre-chemotherapy samples, no significant differences in the expression levels of these genes were seen between right- and left-sided tumors (statistical data not shown). In the comparison between pre- and post-chemotherapy samples, there were no differences in the expression levels of any of the four genes in patients with right-sided tumors (Table II). On the other hand, in left-sided tumors, the expression levels of *CTLA4* and *LAG3*, that are mainly expressed in T-lymphocytes, were significantly increased after UFT/LV chemotherapy ($p=0.0001$ and $p=0.0056$, respectively), whereas those of *CD274* and *IDO1* showed no significant changes after UFT/LV chemotherapy (Table II).

Association between high counts of TILs and gene expression levels of the immunotherapy targets. The amount of TILs was evaluated using hematoxylin and eosin (H-E)-stained tumor tissue specimens. The associations between gene expression levels of the immunotherapy targets and high counts of TILs were determined using univariate logistic regression analysis (Figure 1). In the pre-chemotherapy tumors, no significant associations between the patients with high-TIL and the expression levels of four genes, *CD274, CTLA4, IDO1* and *LAG3*, were seen. On the other hand, in the post-chemotherapy tumors, high expressions of the four genes were significantly associated with high-TIL tumors. In particular, the expression levels of *CTLA4* and *LAG3*, which are mainly expressed in T-lymphocytes, were strongly associated factors with high-TIL tumors.

Comparison of the percentages of patients with high counts of TILs and of CD3+, CD4+, CD8+, and POXP3+ lymphocytes between the UFT/LV and control groups. The amount of TILs was evaluated using H-E-stained tumor tissue specimens. The amounts of CD3+, CD4+, CD8+ and FoxP3+ T-lymphocytes were evaluated by immunohistochemical staining. Representative examples of H-E staining and immunohistochemical staining for FoxP3+ TILs in right-sided and left-sided tumors in the UFT/LV group are shown in Figure 2. The percentage of patients in the high groups with respect to the TIL counts and counts of lymphocytes showing expression of each lymphocyte marker were compared between the UFT/LV and control groups. The percentage of patients with high TIL counts in the UFT/LV group was significantly higher than that in the control group (34.4% *vs*. 15.3%, p=0.0008) (Table III).

The percentage of patients with high counts of FoxP3-Tregs was significantly higher in the UFT/LV group than that in the control group (41.1% *vs*. 22.4%, p=0.0024, Table III). No differences were observed in the expression of other lymphocytic markers, *i.e*., CD3, CD4, and CD8 (Table III). We then analyzed the data separately for right-sided and left-sided tumors. In left-sided tumors, the percentage of patients with high TIL counts in the UFT/LV group was significantly higher than that in the control group (45.8% *vs*. 14.3%, p<0.0001, Table III). On the other hand, in right-sided tumors, no significant differences were observed between the patients that received and those that did not receive chemotherapy (Table III). The percentage of patients with high CD3 and FoxP3 expression levels in the TILs were significantly higher in the UFT/LV group as compared to the control group in left-sided tumors (77.1% *vs*. 56.0%, p<0.0165 and 43.8% *vs*. 20.9%, p=0.0060, respectively (Table III). There were no significant differences between patients who received and those that did not receive chemotherapy for right-sided tumors (Table III).

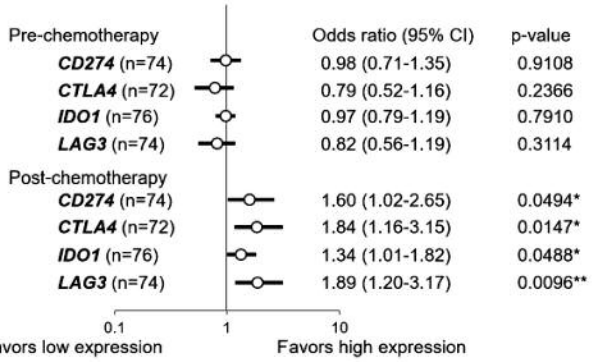

Figure 1. *Univariate logistic regression analysis for the prediction of patients with high-TIL using gene expression levels in tumor tissues before or after UFT/LV chemotherapy. Odds ratio are the value per one unit change in gene expression level. p-Values were calculated by Wald test. 95%CI=95% confidence interval.*

Discussion

The effect of UFT/LV neoadjuvant chemotherapy on the gene expression levels of four immunotherapy targets, *CD274, CTLA4, IDO1* and *LAG3*, in the tumor tissues of patients with colorectal cancer was first investigated. The expression levels of *CTLA4* and *LAG3* were significantly increased after UFT/LV chemotherapy in left-sided tumors, but not in right-sided tumors. CTLA4 is expressed on the surface of activated T cells, and its homologue CD28 is also expressed on the activated T cells (18). T cell activation requires the signal provided by the interaction between CD28 on the T cell and the B7 molecules (CD80 and CD86) on the antigen-presenting cells (APCs). CTLA4 attenuates ongoing immune responses by blocking the interaction of CD80 with CD28 (19). LAG-3 is selectively expressed on activated NK and T lymphocytes (20), and is another vital checkpoint that may have a synergistic interaction with PD-1/PD-L1 (21). Increased CD274, LAG3 and IDO1 expression in tumor-infiltrating immune cells is reported to be associated with a better prognosis in patients with MSI-H colon cancer (22). Therefore, induction of LAG3 by UFT/LV chemotherapy may result in a better prognosis. CTLA4 and LAG3 are mainly expressed in T-lymphocytes, as described above. Therefore, it is suggested that UFT/LV chemotherapy may induce TILs, including T-lymphocytes, but only in left-sided tumors. On the other hand, the expression levels of *CD274* (PD-L1) and *IDO1* did not change after UFT/LV chemotherapy in either right-sided or left-sided colorectal cancer. CD274 is mainly expressed on tumor cells and is known to be a predictor of the response to PD-1/PD-L1 inhibitors, namely, pembrolizumab, nivolumab and atezolizumab, in patients with non-small-cell lung cancer (23-26). IDO-1 is mainly expressed in macrophages and dendritic cells, and is a rate-limiting enzyme involved in the kynurenine pathway (27). IDO1 induces immunosuppression through the breakdown of tryptophan in the tumor microenvironment and tumor-draining lymph nodes (28). It seems that UFT/LV chemotherapy did not affect the expression levels of the immunotherapy target genes that are mainly expressed in cells other than the T-cells, in either right-sided or left-sided tumors.

In this study, the expression levels of *CTLA4* and *LAG3* were significantly increased after UFT/LV chemotherapy in left-sided tumors, and high expression levels of *CTLA4* and *LAG3* were significantly associated with high-TIL tumors. These results suggest that UFT/LV chemotherapy may increase the number of T-cells infiltrating the tumor tissue in left-sided tumors. Therefore, the amount of TILs in the tumor tissues between patients who received surgery alone

Table III. *Comparison of the rate of patients with high-TIL or high-each lymphocytic marker between control and UFT/LV groups.*

Factor	High, n (%)				*p*-Value	Odds ratio (95%CI)	
	Control		UFT/LV				
Total patients	n=170		n=90				
TIL	26	(15.3)	31	(34.4)	0.0008**	2.91	(1.59-5.32)
CD3	84	(49.4)	54	(60.0)	0.1177	1.54	(0.91-2.58)
CD4	72	(42.4)	44	(48.9)	0.3591	1.30	(0.78-2.18)
CD8	21	(12.4)	10	(11.1)	0.8427	0.89	(0.40-1.97)
FoxP3	38	(22.4)	37	(41.1)	0.0024**	2.43	(1.39-4.22)
Right-sided tumors	n=79		n=42				
TIL	13	(16.5)	9	(21.4)	0.6212	1.38	(0.54-3.57)
CD3	33	(41.8)	17	(40.5)	1.0000	0.95	(0.44-2.03)
CD4	22	(27.8)	14	(33.3)	0.5381	1.30	(0.57-2.91)
CD8	7	(8.9)	4	(9.5)	1.0000	1.08	(0.30-3.93)
FoxP3	19	(24.1)	16	(38.1)	0.1402	1.94	(0.87-4.36)
Left-sided tumors	n=91		n=48				
TIL	13	(14.3)	22	(45.8)	<0.0001**	5.08	(2.24-11.49)
CD3	51	(56.0)	37	(77.1)	0.0165*	2.64	(1.20-5.81)
CD4	50	(54.9)	30	(62.5)	0.4712	1.37	(0.67-2.80)
CD8	14	(15.4)	6	(12.5)	0.8009	0.79	(0.28-2.20)
FoxP3	19	(20.9)	21	(43.8)	0.0060**	2.95	(1.38-6.32)

p-Values were calculated by the Fisher's exact test. *$p<0.05$, **$p<0.01$. The amounts of tumor-infiltrating lymphocytes (TILs) were evaluated using hematoxylin and eosin (HE)-stained tumor tissues. The amounts of CD3+, CD4+, CD8+ and FoxP3+ T-lymphocytes were evaluated by immunohistochemical analyses. For the amounts of TILs and lymphocytes showing CD3, CD4 or CD8 expression, grades 3-4 were defined as the high group. For FoxP3 expression, >30 positive cells/high power field was defined as the high group.

(control group) and those who received neoadjuvant chemotherapy (UFT/LV group) was compared. The percentage of patients with high TIL counts was higher in the UFT/LV group than that in the control group in left-sided tumors, but not in right-sided tumors (Table III). These results suggest that UFT/LV chemotherapy for 2 weeks induces TILs only in left-sided tumors, consistent with the findings obtained by the gene expression analyses described above (Table II).

In the analysis of the T-cell subtypes conducted using immunohistochemical staining, the percentage of patients with T-cells showing high FoxP3+ and CD3+ expression was higher in the UFT/LV group than that in the control group in left-sided colorectal cancer patients, but not in right-sided cancer patients, suggesting that UFT/LV chemotherapy for 2 weeks induces infiltration by CD3+ T-cells and FoxP3+ regulatory T-cells (Treg), but only in cases of left-sided colorectal cancer. High counts of CD3+ TILs have been reported to be associated with improved survival in patients with colorectal cancer (29-31). Two meta-analyses have recently revealed that high FoxP3+ Treg infiltration indicates a tendency towards favorable prognosis in patients with colorectal cancer (32, 33). Therefore, the induction of tumor-infiltrating CD3+ and FoxP3+ T-cells by UFT/LV chemotherapy may lead to a favorable prognosis in patients with left-sided colorectal cancer.

Roxburgh *et al*. recently reported the effects of FOLFOX chemotherapy on the number of T cell subsets (CD3+, CD8+, FoxP3+ and so on) in 8 patients with locally advanced rectal cancer (34). However, the study was too small to draw any conclusions. In the present study, we demonstrated, for the first time, the effect of standard chemotherapy including 5-FU on the counts of tumor-infiltrating CD3+ T-cells and FoxP3+ Tregs in a large-scale setting. It is unknown why UFT/LV treatment affects the number of CD3+ T cells and FoxP3+ Tregs, but not CD4+ and CD8+ T cells. Moreover, the association between MSI status and the induction of these cells by UFT/LV treatment remains unknown because the MSI status of the patients in this study was not determined. It has been reported that TIL infiltrates and gene expression of immunotherapy targets were higher in MSI tumors than those in microsatellite stable (MSS) tumors in colorectal cancer (35, 36). Therefore, further investigation regarding the association between MSI status and the induction of TILs by UFT/LV treatment is required.

Among patients with stage III colorectal cancer who had received adjuvant chemotherapy including 5-FU, a survival benefit was observed in patients with right-sided tumors, but not in those with left-sided tumors (37). We have previously reported that the response rate of right-sided tumors to UFT/LV chemotherapy was significantly higher than that of

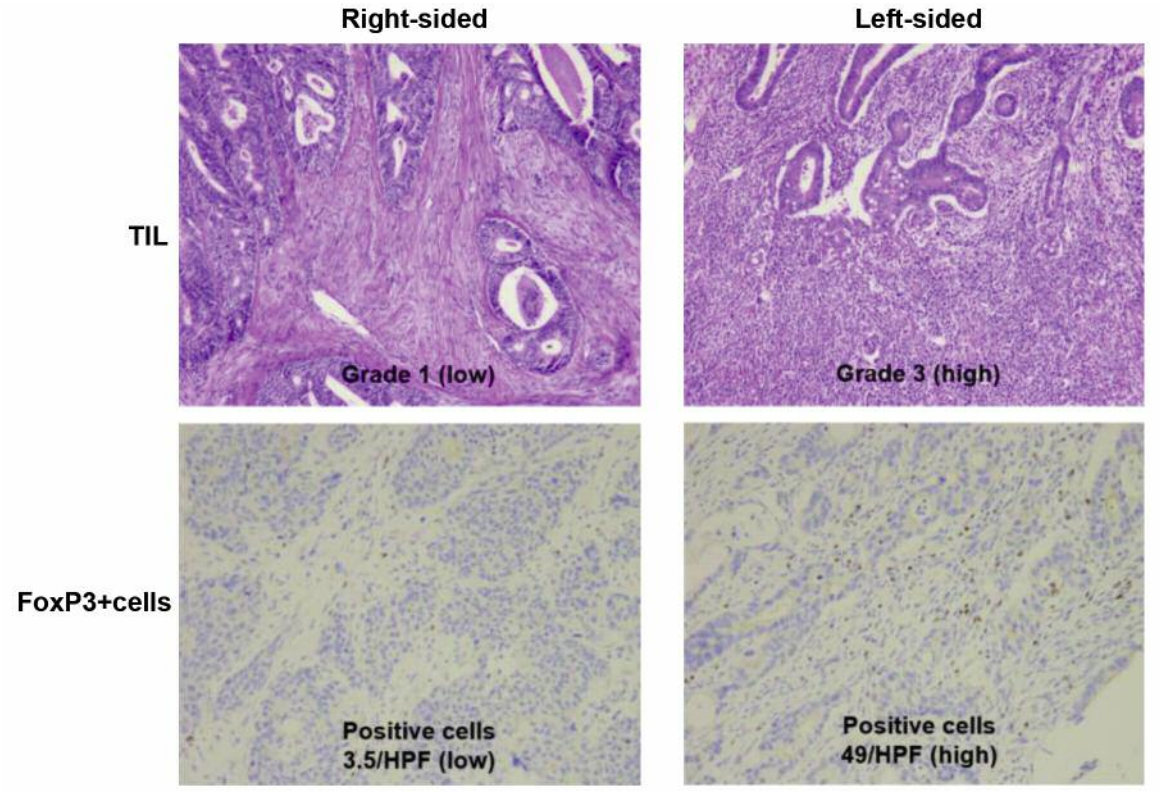

Figure 2. *Photomicrographs of HE-stained tumor tissues and immunohistochemical analysis of FoxP3+ cells in patients with colorectal cancer who had received UFT/LV neoadjuvant chemotherapy. The panels on the left and on the right show the results for left-sided and right-sided colorectal cancer, respectively. In quantifications of the TIL counts, the semiquantitative grading system for lymphocytic infiltration was used. Grade 1 means a few scattered lymphocytes were seen in the primary tumor, and grade 3 means dense lymphocytic infiltration, with an overall lymphoid appearance of the lesion. Groups classified as grades 0-2 and 3-4 were defined as low and high groups, respectively. For FoxP3, the positively stained cells were counted microscopically in 10 fields at high-power magnification (×400) and averaged. More than 30 positive cells and ≤30 positive cells/high power field, on average, were defined as high and low groups, respectively.*

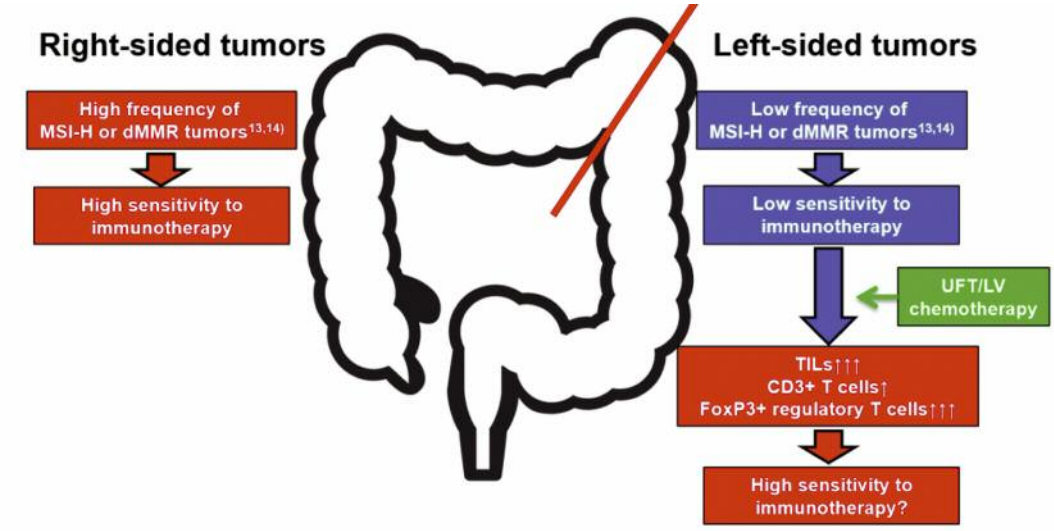

Figure 3. *Immunotherapeutic strategy for patients with right- and left-sided colorectal cancer. The frequency of MSI-H or dMMR in left-sided tumors has been reported to be lower than that in left-sided tumors (13, 14), and the sensitivity to immunotherapy in left-sided tumors seems to be low. However, in left-sided tumors, UFT/LV chemotherapy induces increase in the amount of TILs, especially of CD3+ T cells and FoxP3+ regulatory T cells, associated with an increase in the sensitivity to immunotherapy in left-sided tumors.*

left-sided tumors among patients with stage II or III colorectal cancer (16). Therefore, left-sided tumors seem to be resistant to chemotherapy, including 5-FU. The results of this study suggested that UFT/LV chemotherapy induced FoxP3+ Tregs specifically in left-sided tumors. FoxP3 is a surface marker of Tregs, and the induction of Tregs by UFT/LV in left-sided tumors may lead to suppression of tumor immunity. This may be one of the reasons why left-sided tumors are resistant to chemotherapy, including 5-FU. However, as described above, high FoxP3+ Treg infiltration has been reported to be associated with a favorable prognosis in patients with colorectal cancer (32, 33). Moreover, Saito *et al*. reported that functionally distinct subpopulations of FoxP3+ T-cells contribute opposing effects on prognosis in colorectal cancer (38). The role of FoxP3+ Tregs on the efficacy of chemotherapy needs to be further investigated.

On the other hand, the induction of FoxP3+ Tregs and CD3+ T cells by UFT/LV chemotherapy in left-sided tumors may lead to a higher sensitivity to immunotherapy. Therefore, the combined UFT/LV plus immune checkpoint inhibitor therapy or sequential therapy with UFT/LV followed by immune checkpoint inhibitors can be useful for patients with left-sided tumors, who have a low frequency of MSI-H or dMMR (13, 14) (Figure 3). Further clinical investigation is required to clarify the efficacy of immunotherapy in patients with left-sided colorectal cancer.

Conclusion

Increases in the counts of TILs, especially CD3+ T cells and FoxP3+ regulatory T cells, after UFT/LV chemotherapy may be specific to left-sided colorectal cancer, suggesting that combined UFT/LV plus immune checkpoint inhibitors or sequential therapy with UFT/LV followed by immune checkpoint inhibitors can be useful for patients with left-sided colorectal cancer.

Conflicts of Interest

H. Nagase is an employee of Taiho Pharmaceutical Co., Ltd. The other Authors declare that they have no conflict of interest.

Authors' Contributions

Conception and design: SS, TS. Provision of study materials or patients: KO, GS, HM, LFC, YK, AT. Collection and assembly of data: GS, HM, HK. Data analysis and interpretation: HM, SS. Manuscript writing: SS, HN. All Authors read and approved the final manuscript.

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Received March 3, 2019
Revised March 17, 2019
Accepted March 18, 2019