Immune-related Genes to Dominate Neutrophil-lymphocyte Ratio (NLR) Associated With Survival of Cetuximab Treatment in Metastatic Colorectal Cancer

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Abstract

Our study, using data of prospective trials, demonstrated that the neutrophil-lymphocyte ratio (NLR) was associated with survival time in patients with *KRAS* wild-type metastatic colorectal cancer treated with first-line chemotherapy with cetuximab. The expression levels of the *LYZ*, *TYMP*, and *CD68* genes differed significantly between the NLR-low and NLR-high groups. Genes encoding for activities on macrophages may affect the NLR.

Background: Few clinical studies have investigated the association between neutrophil-lymphocyte ratio (NLR) and treatment with cetuximab-based chemotherapy in metastatic colorectal cancer (mCRC). The NLR may reflect immune cells modulating specific cytokine signals in the tumor microenvironment; however, which immune-related genes affect the NLR remain unclear. **Patients and Methods:** In 77 patients with *KRAS* exon2 wild-type mCRC from prospective trials of first-line chemotherapy with cetuximab, expression levels of 354 immune-related genes were measured in tissue samples obtained from all patients by the HTG EdgeSeq Oncology Biomarker Panel. The association between the NLR and clinical outcomes was evaluated using the Spearman rank correlation coefficient. In addition, 2-sample *t* tests were performed to investigate which genes among the top 100 genes associated with survival had significantly different expression levels between the NLR-low and NLR-high groups among all measured genes. **Results:** NLR data were available for 71 patients. The NLR was associated with progression-free survival and overall survival ($r = -0.24$; $P = .040$ and $r = -0.29$; $P = .010$, respectively). When stratified by the median value of the NLR, the Kaplan-Meier curve of NLR-low versus NLR-high differed significantly for both progression-free survival (median, 11.8 vs. 9.1 months; $P = .036$) and overall survival (median, 42.8 vs. 26.7 months; $P = .029$). The 2-sample

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t test revealed that the expression levels of the *LYZ*, *TYMP*, and *CD68* genes differed significantly between the NLR-low and NLR-high groups (*t* test *P*-value < .005; false discovery rate *P*-value < .15). **Conclusion:** NLR is significantly associated with survival in patients with mCRC treated with first-line chemotherapy with cetuximab. Genes encoding for activities on macrophages may affect the NLR.

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Introduction

The neutrophil-to-lymphocyte ratio (NLR) is a marker of inflammation, and an elevated NLR reflects enhanced systemic inflammation and is associated with reduced tumor-specific immunity such as decreased tumor-infiltrating lymphocytes (TILs) in the tumor.[1] Neutrophils, monocytes, and platelets have been reported to promote tumor development via different mechanisms, whereas lymphocytes are essential for the elimination of cancer cells.[2] Distinct tumor-suppressive mechanisms are mainly controlled by CD4 and CD8 T lymphocytes, but regulatory T cell contributes to tumor-induced immunotolerance through suppression of the CD4 T lymphocyte. Various cytokines mediate the attraction of immune cells in the tumor microenvironment. A high NLR has been shown to correlate with a distinct cytokine profile including pro-inflammatory cytokines and angiogenic cytokines, which are related to key biological processes involved in carcinogenesis.[3] This may partly explain why an elevated NLR is associated with poor outcomes in cancer.

A systematic review and meta-analysis of 100 studies including 40,559 patients with various solid tumors indicated that NLR was associated with poor survival in all disease subgroups, sites, and stages. Meta-analyses and systemic reviews based on relevant studies showed that NLR has prognostic value in patients with colorectal cancer (CRC). An elevated pretreatment NLR predicted poorer survival in CRC.[4-6] The role of pretreatment inflammatory indices in predicting the outcomes of patients with CRC has been clearly evidenced in patients with radically resected tumors[7] and was more recently suggested in patients with metastatic disease.[8-11] The NLR was a better independent predictor of outcomes than was the lymphocyte-to-monocyte ratio, platelet-to-lymphocyte ratio, and prognostic nutritional index in patients with CRC.[12]

A significant association of the NLR with clinical outcomes has been reported in several retrospective studies of patients with metastatic CRC (mCRC) treated with chemotherapy plus bevacizumab.[13,14] A retrospective study of a large prospective trial, the TRIBE, validated the prognostic value of the NLR in patients with mCRC who received intensive chemotherapy with bevacizumab.[14] However, few clinical studies have investigated the association between the NLR and treatment with cetuximab-based chemotherapy. The NLR may reflect immune cells modulating specific cytokine signals in the tumor microenvironment; however, which immune-related molecular signals affect the NLR remain unclear. We therefore performed a biomarker study to evaluate association between the NLR and the clinical outcomes of cetuximab treatment for mCRC and to investigate which immune-related genes significantly affect the NLR.

Patients and Methods

Study Design and Patient Population

We conducted a retrospective study including patients with *KRAS* exon2 wild-type mCRC whose tissue samples were available for measurement of gene expression levels, from 2 prospective clinical trials evaluating the combination of cetuximab and oxaliplatin-based chemotherapy as first-line treatment, modified-FOLFOX6 (leucovorin, fluorouracil [FU], and oxaliplatin + cetuximab) (JACCRO CC-05 trial (Japan Clinical Cancer Research Organization): N = 28/57, UMIN000004197), and SOX (S-1 and oxaliplatin + cetuximab) (JACCRO CC-06 trial: N = 49/67, UMIN000007022). The identical eligibility criteria of the 2 trials were as follows: adenocarcinoma of the colon or rectum with immunohistologic expression of EGFR; *KRAS* exon 2 wild-type tumor with unresectable metastases; at least 1 measurable lesion of 10 mm or a residual nonmeasurable lesion according to the Response Evaluation Criteria in Solid Tumors, version 1.1; adequate bone marrow function, hepatic function, and renal function; an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0 or 1; and an age of 20 to 79 years. Patients with uncontrolled infection, massive ascites or pleural effusion, symptomatic brain metastases, or other malignancies within 5 years before enrollment (with the exception of early carcinoma that had been treated with curative intent), a history of systemic chemotherapy for mCRC, or who had previously received oxaliplatin or cetuximab were excluded. This biomarker study was conducted in accordance with the Declaration of Helsinki and was approved by the ethical committee of each participating institution. Written informed consent was obtained from all patients before enrollment.

Chemotherapy

In the JACCRO CC-05 trial, patients received modified-FOLFOX6 (oxaliplatin in a dose of 85 mg/m^2 of body surface area [BSA]; leucovorin in a dose of 200 mg/m^2 of BSA; an intravenous bolus of FU in a dose of 400 mg/m^2 of BSA; a continuous 46-hour infusion of FU in a dose of 2400 mg/m^2 of BSA) on day 1 of each 14-day treatment cycle plus cetuximab (a loading dose of 400 mg/m^2 of BSA, followed by 250 mg/m^2 of BSA given weekly thereafter). In the JACCRO CC-06 trial, patients received SOX (a 120-minute infusion of oxaliplatin in a dose of 130 mg/m^2 of BSA on day 1 plus oral S-1 in a dose of 80 mg/m^2 of BSA on days 1-15) on day 1 of each 21-day treatment cycle plus cetuximab. In both trials, treatment was continued until disease progression, unacceptable toxic effects developed, a complete response was achieved, surgical resection became possible, or the patient requested or the physician decided that therapy should be withdrawn.

Assessment of Efficacy

The primary endpoint of the 2 phase II trials was the objective response rate (ORR) (complete or partial response). Secondary endpoints included progression-free survival (PFS), based on disease progression as detected by external review or death from any cause, overall survival (OS), secondary resection of metastases with curative intent, and safety. Responses were evaluated according to Response Evaluation Criteria in Solid Tumors, version 1.1, by the investigators and were then validated by an external review board.

RNA Isolation and Gene Expression Analysis

Formalin-fixed, paraffin-embedded (FFPE) tumor specimens were cut into sections with a thickness of 3 or 10 μm. In a preparation for macrodissection, one 3-μm slide was stained with hematoxylin and eosin and was then evaluated for tumor content and marked for areas with dominant tumor foci by a pathologist. Macrodissection was performed for 10-μm slides by scraping the marked areas with a blade to ensure that as many tumor cells as possible were dissected. Total RNA was extracted from FFPE tissue of the tumor samples on 10-μm slides, using an miRNeasy FFPE Kit (QIAGEN KK, Tokyo, Japan) according to the manufacturer's protocol.

Immune-related gene expression levels were measured with the use of an HTG EdgeSeq Oncology Biomarker Panel, which is comprised of probes targeting 354 genes implicated in the host immune response to tumors. Next generation sequencing was used to quantitatively analyze targeted genes (https://www.htgmolecular.com/assays/io).

DNA Isolation and BRAF Mutation Analysis

Genomic DNA was extracted from the FFPE tissue derived from the tumor samples with the use of a QIAamp DNA FFPE Tissue Kit (QIAGEN KK) according to the manufacturer's protocol. *BRAF* V600E mutations were detected by dye terminator sequencing. Exon 15 of the *BRAF* gene was amplified by polymerase chain reaction, and the polymerase chain reaction products were then visualized using agarose gel electrophoresis with ethidium bromide staining. The products were directly sequenced with the use of an ABI 3130xl Genetic Analyzer (Thermo Fisher Scientific KK, Yokohama, Japan) according to the manufacturer's instructions.

This study was conducted in accordance with the REporting recommendations for tumor MARKer prognostic studies (REMARK).[15] Tissue analyses were performed at HTG Molecular, Inc (Tucson, AZ) after obtaining approval from the Institutional Review Board of each institution that participated in the JACCRO CC-05/06AR trials (UMIN000010635).

Statistical Evaluation

Univariate Cox regression analysis was conducted in cases that passed the internal Quality Control metrics of the HTG EdgeSeq Oncology Biomarker Panel. The association between NLR and PFS and OS in the entire cohort was evaluated using the Spearman rank correlation coefficient. The median of NLR expression was used as the cutoff to stratify patients to NLR-low and NLR-high groups. The association between NLR-low versus NLR-high groups and clinical outcomes was evaluated using the Fisher Exact test for ORR, and the log-rank test for PFS and OS. The multivariable Cox proportional hazards regression model was fitted to reevaluate the association between NLR groups with PFS and OS adjusting for ECOG PS, the number of organs involved, and tumor location. Tumors located from the cecum to the splenic flexure were classified as right-sided, whereas tumors that involved the splenic flexure, descending colon, sigmoid colon, and rectum were classified as left-sided. The 2-sample *t* test was performed to investigate which of the top 100 genes associated with survival had significantly different expression levels between the NLR-low group and NLR-high group among all measured genes by Cox proportional-hazards models using the likelihood ratio test statistic.

All tests were 2-sided with a significance level of 0.05. False discovery rate (FDR) multiple testing was used to adjust the *P* values. FDR *P*-value $< .15$ was considered statistically significant. SAS 9.4 software (SAS Institute, Cary, NC) was used to perform all analyses.

Results

Seventy-seven eligible patients were enrolled in the present study (see Supplemental Figure 1 in the online version). The baseline characteristics of the patients are summarized in Supplemental Table 1 (in the online version). Forty-four (57%) of the patients were men, the median age of the patients was 63 years, and 82% had left-sided tumors. In the patient cohort, the ORR was 71.8%. The median PFS and OS were 11.2 months (95% confidence interval [CI], 9.2-14.5 months) and 36.2 months (95% CI, 26.7 to not reached), respectively. Consequently, a total of 71 patients who passed the internal Quality Control metrics and had available data on NLR were included in this analysis.

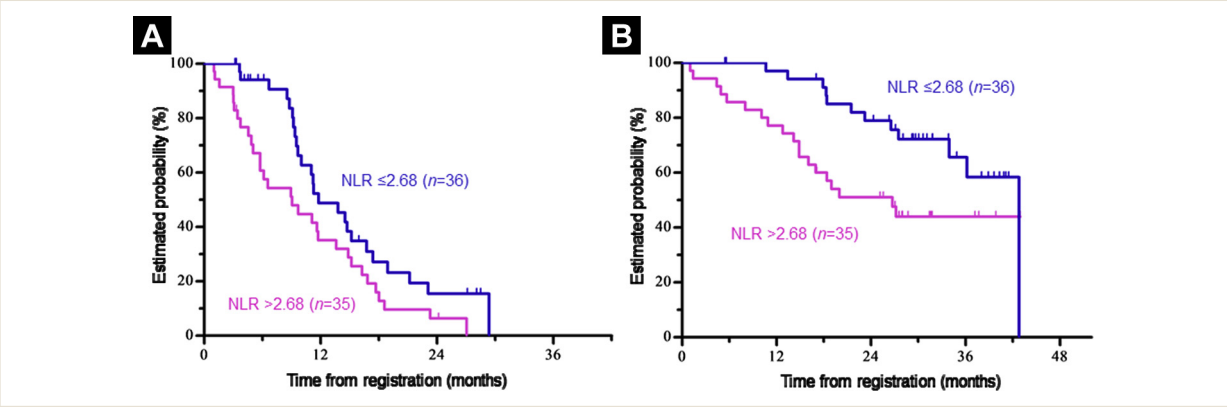
NLR and Patient Characteristics

The median NLR in the 71 patients was 2.68 (range, 0.78-9.95). The patient characteristics were evaluated according to NLR status (ie, low or high according to the cutoff value of 2.68). The distribution of NLR-low or NLR-high did not differ between left-sided tumors and right-sided tumors ($P = .31$). *BRAF* mutations were detected more frequently in patients with NLR-high tumors (18.8% vs. 2.9%; $P = .048$) (see Supplemental Table 2 in the online version).

Association of NLR With Clinical Outcomes

An analysis using the Spearman rank correlation coefficient indicated that the NLR correlated with PFS ($r_s = -0.24$; $P = .040$) as well as with OS ($r_s = -0.29$; $P = .011$) in the entire cohort (see Supplemental Figure 2 in the online version). In the study cohort excluding patients who were censored from the analysis of PFS and OS, the NLR was associated with OS ($r_s = -0.53$; $P = .0024$), but not with PFS ($r_s = -0.20$; $P = .14$). When the patients were stratified according to the median value of the NLR, patients with NLR-low ($n = 36$) had significantly longer PFS than those with NLR-high ($n = 35$) (median, 11.8 months vs. 9.1 months; hazard ratio [HR], 0.57; 95% CI, 0.33-0.97; $P = .036$). The median OS in the patients with NLR-low was significantly longer than that in the patients with NLR-high (median, 42.8 months vs. 26.7 months; HR, 0.45; 95% CI, 0.22-0.94; $P = .029$) (Figure 1). The ORR was slightly but not significantly higher in the NLR-low group than in the NLR-high group (75.0% vs. 68.6%; $P = .60$) (Table 1). The NLR-low group had significantly better outcomes than the NLR-high group in terms of PFS and OS in a multivariable analysis adjusted for ECOG PS, the number of organs involved, and tumor

Figure 1 Kaplan-Meier Curves of Clinical Outcomes according to the Median Value of NLR. A, Progression-free Survival; B, Overall Survival



Abbreviation: NLR = Neutrophil-lymphocyte ratio.

location (HR, 0.55; 95% CI, 0.31-0.95; $P = .033$ and HR, 0.44; 95% CI, 0.20-0.96; $P = .038$, respectively). On the other hand, there was no significant association of outcomes with ECOG PS, the number of organs involved, or tumor location in the multivariable analysis (Table 2).

We performed an exploratory analysis to investigate the association between the NLR and clinical outcomes according to primary tumor sidedness. The frequency of NLR-low was 33% in the right-sided tumor group and 53% in the left-sided tumor group. Patients with NLR-low had slightly but not significantly better clinical outcomes in terms of PFS and OS regardless of tumor sidedness. Both PFS and OS were numerically longer in NLR-low patients than in NLR-high patients, even among patients with right-sided tumors (see Supplemental Table 3 in the online version).

Immune-related Genes Associated With NLR

First, we checked immune-related genes that were associated with survival in 71 patients by Cox proportional hazards models using the likelihood ratio test statistic. Then, 2-sample *t* tests were performed to investigate which of the top 100 genes had significantly different expression levels between the NLR-low and NLR-high groups (see Supplemental Table 4 in the online version). The 2-sample *t* test revealed that the expression levels of the *LYZ*, *TYMP*, and *CD68* genes differently significantly between the 2 groups (*t* test *P*-value < .005; FDR *P*-value < .15) (Table 3). The expression levels of all 3 genes were higher in patients with NLR-high than in patients with NLR-low.

Table 1 Clinical Outcomes According to the NLR Status

Outcomes	NLR > 2.68 (n = 35), n (%)	NLR ≤ 2.68 (n = 36), n (%)	*P* Value
CR	2.9 (1)	5.6 (2)	
PR	65.7 (23)	69.4 (25)	
SD	20.0 (7)	19.4 (7)	
PD	5.7 (2)	0 (0)	
NE	5.7 (2)	5.6 (2)	
ORR	68.6 (24)	75.0 (27)	.60[a]
Median PFS, mos	9.1	11.8	.036[b]
Median OS, mos	26.7	42.8	.029[b]

Abbreviations: CR = Complete response; NE = not evaluable; NLR = neutrophil-lymphocyte ratio; ORR = objective response rate; OS, overall survival; PD = progressive disease; PFS = progression-free survival; PR = partial response; SD = stable disease.
[a]*P*-value was estimated by the Fisher Exact test.
[b]*P*-value was estimated by log-rank test.

Discussion

NLR showed a significant relationship with OS in patients with mCRC treated with first-line cetuximab-based chemotherapy. Our data demonstrated that NLR-low was significantly associated with favorable outcomes as compared with NLR-high when the cutoff value was the median. In addition, this is the first report showing that the expression levels of the *LYZ*, *TYMP*, and *CD68* genes differed significantly between the NLR-low and NLR-high groups. All of the 3 genes had positive correlation with the NLR.

A few studies suggested that the NLR might serve as a predictor of the response to bevacizumab-based chemotherapy in mCRC[16]; however, it is not currently used for treatment decision-making. An Australian multicenter study is ongoing to evaluate the relations between the host inflammatory response as measured by the NLR and treatment outcomes in patients with mCRC who received bevacizumab-based first- and second-line treatment (ClinicalTrials.gov: NCT01588990). On the other hand, a recent retrospective study investigating the role of NLR in patients who received first-line cetuximab treatment for mCRC showed that elevated NLR was an independent predictor of shorter PFS and OS in 95 patients extracted from the clinical database of a single institution.[17] Our results were consistent with those of the previous study, but we could demonstrate that the NLR was significantly associated with the clinical outcomes of cetuximab treatment using data from prospective multicenter trials. The NLR may serve as a prognostic marker in patients with CRC treated with chemotherapy regardless of targeted drugs.

In several studies, the NLR independently predicted survival in patients with mCRC treated with chemotherapy followed by resection or chemotherapy when a cutoff value of 5 was used to

Table 2 Multivariable Analysis for PFS and OS

	PFS			OS		
	HR	95% CI	*P* Value	HR	95% CI	*P* Value
ECOG PS						
0 versus 1	0.79	0.19-3.36	.75	0.29	0.064-1.28	.10
No. of organs involved						
1 versus ≥ 2	0.86	0.49-1.52	.60	0.47	0.20-1.08	.074
Tumor location						
Left versus right	0.60	0.28-1.29	.19	0.41	0.16-1.04	.061
NLR						
Low versus high	0.55	0.31-0.95	.033	0.44	0.20-0.96	.038

Abbreviations: CI = Confidence interval; ECOG PS = Eastern Cooperative Oncology Group performance status; HR = hazard ratio; NLR = neutrophil-lymphocyte ratio; PFS = Progression-free survival; OS = overall survival.

designate NLR-high or NLR-low.[8,18] A few studies showed that a preoperative NLR of greater than 3 correlated with OS and cancer-specific survival in patients with CRC with resectable tumors.[19,20] In a post hoc analysis of the MRC COIN trial, a high derived NLR was defined using a cutoff value of ≥ 2.22.[21] On the other hand, Kubo et al have indicated the impact of the preoperative NLR on long-time survival in patients with CRC using the median value as a cutoff.[22] In our study, we used the median value, 2.68, to divide patients into the NLR-high or NLR-low groups. Accordingly, we performed an exploratory analysis of our cohort using a cutoff value of 5.0 because the value has been used in several previous studies. The patients with high-NLR had a shorter survival time than those with low-NLR (median PFS, 5.8 vs. 11.3 months; median OS, 27.2 vs. 42.8 months); however, the differences were not statistically significant. The cutoff value used to divide patents into the high-NLR or low-NLR groups may differ among patient cohorts and depend on patient characteristics or tumor status.

The antitumor activity of cetuximab may be affected by extracellular immune mechanisms. We have reported that immune-related genes are associated with clinical outcomes in patients with mCRC treated with cetuximab.[23] Therefore, immune-related genes in the tumor microenvironment may contribute to NLR status, which is associated with survival of cetuximab treatment. We could identify 3 genes, *LYZ*, *TYMP*, and *CD68*, which contributed to the level of the NLR. TYMP and CD68 are both expressed in macrophages, particularly tumor-associated macrophages (TAMs).

Table 3 Significant Genes (*P* < .05) Expressed Significantly Differently Between NLR-high (N = 32) and NLR-low (N = 35) Groups

Genes	*t* Test *P* Value	FDR *P* Value
LYZ	.001	.104
TYMP	.004	.145
CD68	.004	.145
IFNGR1	.008	.190
IR4R	.023	.467
STAT1	.031	.490
TNFRSF1B	.037	.490

Abbreviation: NLR = Neutrophil-lymphocyte ratio.

In the tumor microenvironment, TYMP stimulates tumor growth by promoting angiogenesis and evasion of apoptosis.[24] Moreover, it has been reported that TYMP was expressed in tumor-infiltrating macrophages, being associated with tumor angiogenesis and poor prognosis in patients with intestinal-type gastric cancer.[25] Gene expression levels of lysozyme and signal transducer and activator of transcription 1 (STAT1) genes are associated with a macrophage signature.[26] Lysozyme expression levels also positively correlate with the numbers of CD68+pSTAT1+ and CD68+CMAF− macrophages. A recent study demonstrated that the combined use of a macrophage marker including CD68 with pSTAT1 or CMAF can identify TAMs that play a significant role in tumor development.[27] Accumulation of the TAMs in the tumor is associated with a high NLR. We investigated number of immune-related genes including cytokine genes using a multi-gene panel. A high NLR has been shown to correlate with a distinct cytokine profile, including pro-inflammatory cytokines and angiogenic cytokines[3]; however, we found the 3 macrophage-related genes as significant ones for the NLR status, leading that NLR status might depend on macrophage-related genes rather than cytokine-related genes. To the best of our knowledge, this is the first time to report that genes encoding for activities on TAMs may affect the NLR related with the outcomes of cetuximab combination chemotherapy.

In this study, the number of registered samples was relatively small. It is difficult to exactly assess the association between the NLR and clinical outcomes in patients with mCRC treated with chemotherapy. However, the results of our study were consistent with those of previous studies. It would be of great interest to investigate whether the NLR can serve as a predictor of the response to chemotherapy in patients with mCRC, potentially providing clinicians with a useful hint on how to improve treatment in CRC. In our study, it was not possible to evaluate the NLR as a predictive marker for the response to cetuximab treatment because the patient cohort comprised only patients who received cetuximab combination chemotherapy.

Conclusion

The NLR is significantly associated with survival time in patients with mCRC treated with first-line chemotherapy with cetuximab. Genes encoding for activities on macrophages may affect the NLR.

Clinical Practice Points

- Few clinical studies have investigated the association between NLR and treatment with cetuximab-based chemotherapy in mCRC.
- The NLR may reflect immune cells modulating specific cytokine signals in the tumor microenvironment; however, which immune-related genes affect the NLR remain unclear.
- In 77 patients with *KRAS* exon2 wild-type mCRC from prospective trials of first-line chemotherapy with cetuximab, the association between the NLR and clinical outcomes was evaluated using the Spearman rank correlation coefficient. Also, expression levels of 354 immune-related genes were measured in tissue samples by the HTG EdgeSeq Oncology Biomarker Panel.
- The Spearman rank correlation coefficient showed that the NLR was associated with PFS and OS. When stratified by the median value of the NLR, both PFS and OS were significantly different between the NLR-low and NLR-high groups.
- The expression levels of the *LYZ*, *TYMP*, and *CD68* genes differed significantly between the NLR-low and NLR-high groups. Genes encoding for activities on macrophages may affect the NLR.

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Disclosure

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Supplemental Data

Supplemental table accompanying this article can be found in the online version at https://doi.org/10.1016/j.clcc.2018.08.002.

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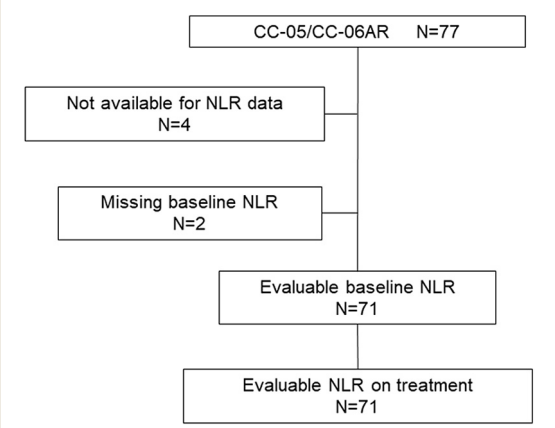
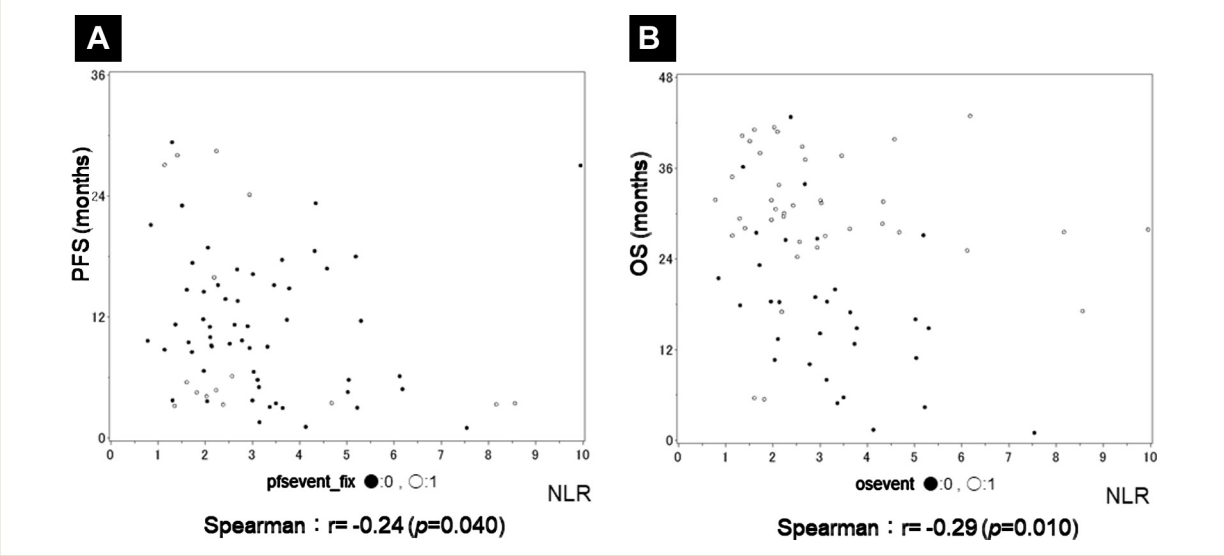
Supplemental Figure 1 Consort Diagram of the Study



Abbreviation: NLR = Neutrophil-lymphocyte ratio.

Supplemental Figure 2 Correlation Between Neutrophil-lymphocyte Ration and Clinical Outcomes by the Spearman Rank Correlation Coefficient. A, PFS; B, OS



Abbreviations: NLR = Neutrophil-lymphocyte Ratio; OS = overall survival; PFS = progression-free survival.

Supplemental Table 1 Patient Characteristics

Characteristic	N = 77, n (%)
Gender	
Male	44 (57)
Female	33 (43)
Age, y	
Median (range)	63 (39-79)
ECOG PS	
0	69 (90)
1	8 (10)
Diagnosis	
Advanced	59 (77)
Relapse	18 (23)
Primary tumor	
Left	63 (82)
Right	13 (17)
Unclassifiable	1 (1)
Metastatic sites	
Liver	49 (64)
Lung	26 (34)
Para-aortic lymph node	22 (29)
Peritoneum	17 (22)
No. organs involved	
1	33 (43)
≥ 2	44 (57)
Previous adjuvant chemotherapy	
Yes	6 (8)
No	71 (92)

Abbreviation: ECOG PS = Eastern Cooperative Oncology Group performance status.

Supplemental Table 2 NLR and Patient Characteristics

	NLR > 2.68 (N = 35), % (n/N)	NLR ≤ 2.68 (N = 36), % (n/N)	*P* Value[a]
Female	42.9% (15/35)	38.9% (14/36)	.81
Right-sided tumor	17.6% (6/34[b])	8.6% (3/35[b])	.31
BRAF mutation	18.8% (6/32[c])	2.9% (1/35[c])	.048
No. organs involved ≥ 2	62.9% (22/35)	52.8% (19/36)	.47

Abbreviation: NLR = Neutrophil-lymphocyte ratio.
[a]*P* value was estimated by the Fisher Exact test.
[b]Excluding unknown cases.
[c]Excluding not available cases.

Supplemental Table 3 Association Between NLR and Clinical Outcomes According to Primary Tumor Sidedness

	NLR > 2.68	NLR ≤ 2.68	*P* Value
Right-sided	N = 6	N = 3	
ORR, %	33.3	100	.17[a]
Median PFS, mos	3.3	9.1	.17[b]
Median OS, mos	12.0	NR	.20[b]
Left-sided	N = 28	N = 32	
ORR, %	75.0	75.0	1.00[a]
Median PFS, mos	11.1	13.8	.10[b]
Median OS, mos	27.2	42.8	.057[b]

Abbreviations: NLR = Neutrophil-lymphocyte ratio; NR = not reached; ORR = objective response rate; OS = overall survival; PFS = progression-free survival.
[a]Fisher Exact test.
[b]Log-rank test.

Supplementary Table 4 Top 100 Genes Associated With Overall Survival in the Entire Population (n = 71)

	Cox Coefficient	Cox *P* Value	FDR *P* Value
IRF3	2.197417	.000597	.125988
BMP4	0.549022	.000857	.135867
JAK2	1.089111	.002221	.175681
RIPK1	0.801924	.00323	.212947
CEACAM5	−0.39164	.003864	.237543
LYZ	0.347785	.004415	.237543
STAT3	−1.33918	.005326	.237543
CXCL2	−0.44702	.011925	.288448
CXADR	−0.34977	.014527	.312784
CCL21	−0.26439	.023218	.38685
TIRAP	0.563616	.025301	.38685
TNNC2	−0.18214	.039303	.451151
STAT1	0.533921	.050418	.526496
TNFRSF21	0.602231	.053818	.537687
NFKBIA	−0.61953	.056441	.537687
IRF6	−0.60738	.064225	.560577
CHI3L1	−0.29482	.065781	.560577
IL1RAP	0.356383	.06636	.560577
TLR4	0.312032	.069231	.560577
STAT6	−0.85658	.07738	.562216
LTBR	0.660447	.094166	.579278
AREG	−0.24499	.099929	.580118
CXCL14	−0.17102	.105026	.580118
CCL24	−0.15232	.141003	.662468
CXCL3	−0.24603	.145895	.666315
ACKR3	−0.41272	.146837	.667727
CMTM3	0.33083	.157089	.684766
IL10RB	0.536558	.182484	.716366
CD276	0.311782	.198794	.721757
IRF9	0.359011	.199766	.721757
CD68	0.379412	.208307	.725026
CEACAM7	−0.11742	.211182	.727183
PIAS3	0.461605	.21506	.731994
IL22RA1	−0.22387	.215642	.731994
CMTM4	0.402386	.219625	.73557
TRADD	0.357101	.243744	.767107
MAF	−0.31135	.251835	.775733
CKLF	0.351129	.26453	.779689
TNFRSF10A	−0.17728	.267842	.779689
CSF1R	0.260926	.28145	.791925
TYMP	0.291872	.303124	.806455
FOS	−0.16708	.31014	.809896
CKMT1A	0.188942	.315581	.822069
IRAK1	0.334769	.323936	.823543
FAF1	−0.30431	.35091	.839921
PPARG	−0.13113	.355127	.84193
FADD	0.349391	.397436	.871715
VEGFA	−0.18581	.414925	.879718
ILK	−0.3381	.436729	.88738
ID3	0.210547	.468076	.891239

Supplementary Table 4 Continued

	Cox Coefficient	Cox *P* Value	FDR *P* Value
CD14	−0.19706	.472899	.891239
TNFSF10	−0.29774	.473607	.891239
MX1	0.095026	.485194	.891239
MAGED1	−0.20066	.486801	.891239
IL2RG	−0.1515	.48883	.891239
HLA_E	0.255818	.494354	.892007
CXCR4	0.111316	.494676	.892007
CD47	−0.20311	.511222	.897991
IL4R	0.255394	.511593	.897991
SPP1	0.066652	.512408	.897991
TYK2	−0.26478	.532175	.905301
HIF1A	0.170423	.53942	.906207
CASP1	−0.13509	.545796	.906207
IRF8	−0.11285	.566713	.92098
IL1RN	−0.08542	.568822	.92098
HLA_A	−0.15644	.575951	.925478
FGL2	0.119752	.580435	.925478
IL10RA	0.099282	.599187	.928256
MIF	−0.16878	.606226	.928256
CXCL16	0.126814	.613832	.92988
IL6ST	0.165385	.617996	.92988
IL1B	0.060208	.624416	.92988
CCT2	−0.21429	.628983	.92988
MMP9	−0.0788	.63006	.92988
CEBPD	0.133206	.639947	.92995
IL18	0.105252	.644345	.933769
C3	−0.07029	.655302	.940603
GPI	−0.17591	.668025	.946824
ID1	−0.06008	.688229	.94841
STAT2	0.084011	.691557	.94841
PTGS2	−0.04776	.69423	.94841
LIF	−0.06599	.697481	.94841
CEBPB	0.147603	.700577	.94841
CCL4	−0.06021	.705189	.94841
TNFRSF14	−0.11415	.712522	.94841
TNFRSF1B	0.088783	.724284	.94841
MYD88	−0.14941	.729562	.948949
RIPK2	−0.09743	.757316	.966105
DDX58	−0.06093	.793263	.988142
IFNGR1	0.111883	.809281	.988142
CCL2	−0.04487	.80977	.988142
MAGED2	−0.04118	.875374	.993754
IL33	−0.02126	.886231	.993754
STAT5B	0.035229	.913199	.993754
IL7R	−0.01807	.928058	.993754
GBP1	0.013208	.952559	.993754
ID2	−0.01303	.967245	.993754
TNFRSF10B	−0.00994	.971908	.993754
IL20RA	0.002061	.988019	.998178
HLA_B	−0.00159	.995277	.998528

Abbreviation: FDR = False discovery rate.