1   Immunoscore is a superior prognostic tool in stage II/III colorectal cancer and

2   significantly correlated to PD-L1 expression on tumor-infiltrating mononuclear cells

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9   **Synopsis**

10   Immunoscore was an independent prognostic indicator in CRC. Strong expression of

11   PD-L1 on interstitial tumor-infiltrating mononuclear cells (TIMCs) showed a good

12   prognosis and correlated significantly with Immunoscore. We suggested that PD-L1

13   positive TIMCs may have M2 type macrophages.

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1   【**Abstract**】

2   **Background**: In colorectal cancer (CRC), the indication of immune checkpoint

3   inhibitors is determined by the tumors' microsatellite instability status. However, an

4   optimal biomarker for their indication has not yet been fully identified. We aimed to

5   establish the clinicopathological importance of Immunoscore (IS) in CRC, and clarify

6   the relationships between IS, PD-L1 expression, and tumor-associated macrophages.

7   **Methods**: A total of 132 cases were diagnosed with CRC and surgically treated in our

8   department from 2009 to 2010. Immunohistochemical staining using primary antibodies

9   PD-L1, CD3, CD8, CD68, and CD163 was performed. The IS was determined

10   according to the proposal of an international task force. Statistical analyses were

11   performed to investigate the correlation between IS, clinicopathological variables, and

12   the expression of immune checkpoint molecules.

13   **Results**: The overall survival (OS) and relapse-free survival (RFS) of the high IS group

14   (I3-4) were significantly better than those of the low IS group (I0-2) (OS: P=0.0420,

15   RFS: P=0.0226). The positivity rate of PD-L1 on tumor cells was only 0.8% (tPD-L1),

16   while that of PD-L1 on interstitial tumor-infiltrating mononuclear cells (iPD-L1) was

1 18.2%. The iPD-L1 positive group showed significantly better survival both in terms of

2 OS and RFS than the iPD-L1 negative group (OS: P=0.0278, RFS: P=0.0253). IS and

3 iPD-L1 expression were significantly correlated (P<0.0001).

4 **Conclusions**: We found that high IS was a good indicator of better prognosis and

5 significantly correlated to the iPD-L1 expression in CRC.

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8 【**Introduction**】

9 Colorectal cancer (CRC) is one of the most common malignant diseases worldwide, and

10 is the second leading cause of cancer-related death in Japan.[1] Although a variety of

11 anticancer drugs have been developed, the number of CRC-related deaths has not been

12 significantly reduced.[2–4] Host immune response, including tumor-infiltrating

13 lymphocytes (TILs), plays an important role in CRC prognosis[5,6] and other malignant

14 diseases,[7–9] and interest in the use of immune checkpoint inhibitors as part of a new

15 therapeutic strategy has increased.

16 The mechanism behind immune checkpoint inhibition is the blockade of the

1 programmed death-1 (PD-1) / PD-ligand 1 (PD-L1) pathway.[10] PD-1 is strongly

2 expressed on activated lymphocytes, particularly on TILs, while PD-L1 is expressed not

3 only on antigen-presenting cells but also on tumor cell surfaces. The binding of both

4 molecules causes the suppression of T cells' immune response and results in immune

5 tolerance.[11] The immune checkpoint inhibitors Nivolumab and Pembrolizumab have

6 both been reported effective in malignant melanoma, non small-cell lung cancer, renal

7 cell carcinoma, and malignant lymphoma.[12–16] Galon et al. first proposed the use of the

8 Immunoscore (IS) system, which is defined by the evaluation of TILs, reporting that IS

9 was a significantly better prognostic indicator in CRC.[17,18] However, the use of IS alone

10 for determining the use of immune checkpoint inhibitors needs to be verified.

11 PD-L1 expression is observed not only in tumor cells (TCs) but also in

12 tumor-infiltrating mononuclear cells (TIMCs). The expression of PD-L1 on TCs

13 correlates with poor prognosis in various malignancies[19,20]; however, few studies have

14 investigated the association between PD-L1 expression in TIMCs and the prognosis of

15 CRC patients. The presence of macrophages in the interstitium of PD-L1-expressing

16 tumors has previously been reported;[21,22] studies have also reported that

1 tumor-associated macrophages (TAMs), particularly M2-type macrophages, form an

2 environment, favorable for tumor growth.[23,24]

3 Therefore, the primary purpose of this study was to establish the clinicopathological

4 importance of IS in CRC, and the secondary purpose was to clarify the relationships

5 between IS, PD-L1 expression, and TAMs.

6

7 【**Materials and methods**】

8 *Patients and samples*

9 A total of 192 patients were diagnosed with CRC and surgically treated in our

10 department from 2009 to 2010. After obtaining written informed consent,

11 formalin-fixed paraffin-embedded tissue specimens were obtained from each patient.

12 Cases with neoadjuvant chemotherapy or neoadjuvant chemoradiotherapy and multiple

13 cancer cases were excluded; 132 patients were enrolled in this study.

14 Clinicopathological features of the patients are shown in Supplementary Table S1.

15 Post-operative pathological staging was determined according to the seventh edition of

16 the UICC-TNM classification of malignant tumors. Clinical outcome records and

1 pathological reports were reviewed retrospectively.

2 This study was conducted in accordance with the provisions of the Declaration of

3 Helsinki and approved by the Institutional Review Board of Kurume University

4 Hospital (No. 300).

5 ***Immunohistochemistry***

6 Paraffin-embedded tissue samples were cut at a thickness of 4 μm and spread on coated

7 slide glasses. The slide glasses were labeled with the following antibodies using

8 BenchMark ULTRA (Ventana Automated Systems, Inc., Tucson, AZ, USA) and

9 Bond-Max autostainer (Leica Microsystems, Newcastle, UK). The primary antibodies

10 (with dilutions) used were: CD3 (×300, clone LN10, Leica Microsystems, Newcastle,

11 UK), CD8 (×200, clone 4B11, Leica Microsystems, Newcastle, UK), CD68 (×1200,

12 clone KP1, DakoCytomation, Glostrup, Denmark), CD163 (×100, clone 10D6, Leica

13 Microsystems, Newcastle, UK), and PD-L1 (×100, clone E1L3N, Cell Signaling

14 Technology, Inc., Danvers, MA, USA).

15 Immunostaining with CD3, CD8, CD68, and PD-L1 was performed by the fully

16 automated Bond-III system (Leica Microsystems, Newcastle, UK). Antigen retrieval

1   was performed using onboard heat-induced retrieval with epitope retrieval solution 2

2   (ER2, EDTA-based buffer, pH9.0, Leica Microsystems, Newcastle, UK) for 10 min at

3   99 °C. Slide glasses were incubated with each antibody for 30 min at room temperature.

4   A refine polymer detection system (Leica Microsystems, Newcastle, UK) was used, and

5   slide glasses were incubated with secondary antibody for 30 min at room temperature.

6   All slides were visualized using diaminobenzidine (DAB).

7   BenchMark ULTRA was used to stain CD163. Each slide was heat-treated using

8   Ventana's ULTRA cell conditioning 1 (CC1, Ventana Automated Systems, Inc., Tucson,

9   AZ, USA) retrieval solution for 30 min at 95 °C, and incubated with the CD163

10   antibody for 30 min at 37 °C. This automated system used the streptavidin biotin

11   complex method with 3,3' DAB as the chromogen (Ventana UltraVIEW DAB detection

12   kit).

13   *Image analysis and evaluation of PD-L1 expression*

14   All stained slides were scanned and digitized using NanoZoomer2.0-HT: C9600-13

15   (Hamamatsu Photonics KK, Shizuoka, Japan). The scanned images were analyzed using

16   NDP.view2: U12388-01 software (Hamamatsu Photonics KK, Shizuoka, Japan), and

1   five points of the center of tumor (CT) and invasive margin (IM) each were captured

2   and stored as JPEG images with a x200 field of view. The captured images were

3   processed and quantified using image-processing software, Image J 1.50i.[25] For

4   evaluation, the primary deconvolution of the image was performed, followed by the

5   selection of the red image, and creation of the binary image. The color density threshold

6   was set to be constant, and the auto counting of positive cells was performed using

7   particle count.

8   The median value was calculated from the measured values of the five points measured

9   at the CT and IM. The cutoff value of PD-L1 expression was determined using receiver

10   operating characteristic curves. PD-L1 expression was evaluated by distinguishing

11   between TC expression (tPD-L1) and TIMC expression (iPD-L1).

12   *Evaluation of IS and TAMs*

13   The IS was quantified according to the protocol proposed by the international task force,

14   and classified into five stages according to the density of CD3 and CD8 positive

15   lymphocytes in the CT and IM; specifically, we classified the IS from I0 to I4.[26,27]

16   Similar to the evaluation procedure for PD-L1 expression, the expression of CD3 and

1   CD8 was measured by the CT and IM at five points respectively, and the median of

2   each was taken as the cutoff value. The evaluation of CD68 and CD163 was performed

3   according to the procedure used for PD-L1, CD3, and CD8 evaluations.

4   *Statistical analysis*

5   The correlations between PD-L1 expression, IS, and the clinicopathological

6   characteristics of patients were analyzed using a Chi square test. Survival curves were

7   estimated by the Kaplan-Meier method and statistical significance was evaluated using

8   a log-rank test. Overall survival (OS) and relapse-free survival (RFS) were defined as

9   the time from surgery to death or disease recurrence, respectively. Univariate and

10   multivariate analyses were performed using the Cox hazards model. All statistical

11   analyses were conducted using JMP software version 12.0 (SAS Institute Inc., Cary, NC,

12   USA), and a p-value less than 0.05 was considered statistically significant.

13

14   【**Results**】

15   *Staining results of each marker*

16   Representative PD-L1 stained images are shown in Figs. 1a-1c. PD-L1 was expressed

1   on TCs and TIMCs. Fig. 1a shows a PD-L1 negative case, Fig. 1b shows a tPD-L1

2   positive case, and Fig. 1c shows an iPD-L1 positive case. Only 1 case (0.8%) was

3   positive for tPD-L1, and 24 cases (18.2%) were positive for iPD-L1. There were no

4   PD-L1 positive cases in both tPD-L1 and iPD-L1. Representative examples of CD3 and

5   CD8 positivity in the CT and IM are shown in Figs. 1d-1g.

6   *IS and clinicopathological variables*

7   We first performed IS scoring in the lesion. The IS was classified into five stages, I0 to

8   I4, according to the evaluation of CD3 and CD8 in the CT and IM [I0: 35 cases (26.5%),

9   I1: 21 cases (15.9%), I2: 21 cases (15.9%), I3: 20 cases (15.2%), and I4: 35 cases

10   (26.5%)]. The IS was divided into two groups: a high score group (I3-4) and a low score

11   group (I0-2), and each of these were analyzed. There was no significant association

12   between the IS and each clinicopathological feature (data not shown).

13   *IS and survival analysis*

14   In the I3-4 group, the OS and RFS ratio was significantly higher than that of the I0-2

15   group (OS: P=0.0420, RFS: P=0.0226) (Fig. 2a, 2b). Further examination by TNM

16   stage revealed that there was no significant difference between the OS and RFS in the

11

1   case of stage I and stage IV disease. In stage III cases, those in the I3-4 group had

2   significantly improved prognoses compared to those in the I0-2 group (OS: P=0.0390,

3   RFS: P=0.0125). Even in Stage II cases, the I3-4 group tended to show a good

4   prognosis (OS: P=0.2138, RFS: P=0.0792) (Fig. 2c-2f, Supplementary Fig. S1).

5   To analyze the effects of clinicopathological variables and IS on OS and RFS in stage

6   II/III cases, univariate and multivariate analyses were performed (Table 1). In the

7   univariate analysis of OS, there was a significant difference only in the IS (P=0.0116).

8   In the multivariate analysis, only IS was an independent prognostic factor (hazard ratio

9   [HR]: 2.71, 95% confidence interval [CI]: 2.72-2.87, P=0.0026). In the univariate

10   analysis of RFS, there was a significant difference between IS and N stage (P=0.0019

11   and P=0.0208, respectively), and in the multivariate analysis, IS and N stage were

12   extracted as independent prognostic factors (HR: 11.7, 95% CI: 2.38-210, P=0.0006,

13   and HR: 3.32, 95% CI: 1.17-11.8, P=0.0229, respectively).

14   ***Correlation between iPD-L1 expression, IS, and clinicopathological characteristics***

15   The relationships between iPD-L1 expression, IS, and clinicopathological features are

16   shown in Table 2. In the iPD-L1 positive group, the proportion of right-sided, early

1 TNM stage, T1-2, N0 cases was significantly higher (P=0.0178, P=0.0026, P=0.0035,

2 P=0.0145, respectively). The iPD-L1 positive and I3-4 groups were significantly

3 correlated (P <0.0001).

4 *Localization of PD-L1 expression and survival analysis*

5 We used CD68 as a marker to assess M1 macrophage distribution and CD163 as a

6 marker of M2 macrophage. The results of the immunohistochemical staining of CD68

7 and CD163 performed to clarify the localization of PD-L1 expression, are shown in Fig.

8 3a-3f. PD-L1 positive TIMCs were positive for both CD68 and CD163, suggesting the

9 possibility of the presence of macrophages, especially M2-type macrophages. The OS

10 and RFS were significantly better in the iPD-L1 positive cases than in the iPD-L1

11 negative cases (P=0.0278 and P=0.0253, respectively) (Fig. 3g, 3h).

12

13 【Discussion】

14 Our study showed that IS is an independent prognostic factor for OS/RFS in stage II/III

15 CRCs. We observed an almost complete lack of tPD-L1 expression, and that the iPD-L1

16 positive cases and IS were significantly correlated. Furthermore, iPD-L1 expression was

1   associated with good prognosis, suggesting that PD-L1 may be expressed in M2-type

2   macrophages.

3   IS is determined by the density of CD3 and CD8 in the CT and IM, and is an excellent

4   prognostic factor for CRC.[28–30] Typically, post-operative adjuvant chemotherapy is

5   recommended for stage II high-risk cases and stage III cases[4]. As per regular

6   clinicopathological analysis, stage II low-risk cases are excluded from adjuvant therapy.

7   However, our results suggest that IS can identify truly high-risk cases that could not

8   have been identified by traditional risk analysis. We also compared the significance of

9   IS by stage I and stage IV cases, but could not identify the influence of IS on prognosis

10   in each stage. This could be attributed to the fact that in stage I cases, radical resection

11   is possible and the involvement of immune response should be relatively low. In stage

12   IV cases, even though the effect of pre-surgical factor would be excluded, most cases

13   receive a variety of post-operative combination chemotherapy, possibly causing the true

14   benefit of immune status for OS/RFS to be canceled out.

15   PD-L1 is expressed not only on TCs but also on TIMCs, and tumor PD-L1 expression is

16   correlated to poor prognosis in various carcinomas.[19,20] However, analysis by MSI

1   status is advanced in CRCs, and the clinicopathological evaluation of the expression site

2   of PD-L1 has not been determined. Lee et al. showed that PD-L1 expression in TCs

3   correlated with poor prognosis in mismatch-repair deficient CRCs.[31] In contrast, Li et al.

4   reported that PD-L1 expression in TCs is associated with better prognosis in CRCs.[32]

5   However, few studies have examined the relationship between PD-L1 expression in

6   TIMCs and prognosis in CRC cases. The relationships between PD-L1 in TIMCs

7   expression and clinicopathological features have been reported in other carcinomas.

8   Some reports state that PD-L1 in TIMCs expression correlates with poor prognosis in

9   esophageal   squamous   cell   carcinoma,   gastric   cancer,   and   uterine   cervix

10   adenocarcinoma;[33–35] and favorable prognosis in urothelial carcinoma and head and

11   neck cancer.[36,37] Koganemaru et al. reported that PD-L1 tumor expression is associated

12   with poor prognosis, while high PD-L1 in TIMCs expression is related to better

13   prognosis in stage III CRCs.[38] In addition, Lee et al. reported that PD-L1 in

14   tumor-infiltrating immune cells expression correlated with good prognosis in CRCs.[39]

15   In our study, high iPD-L1 expression correlated significantly to OS improvement,

16   supporting the results of the above-mentioned studies. As for the analysis of PD-L1, in

1   addition to the implication of its expression on tumors or their marginal interstitium, the

2   results may vary depending on the diagnostic reagents used. Therefore, to interpret all

3   analysis results in a similar manner, it is necessary to unify these reagents and make a

4   common diagnosis.

5   TAMs are important to the formation of a tumor microenvironment. When macrophages

6   are activated in the tumor microenvironment, they are polarized into M1 and M2 types;

7   M2 macrophages produce angiogenic factors and cell growth factors and form an

8   environment favorable for cancer growth.[23,24] In this study, the immunohistochemical

9   staining of CD68, which is characteristic of common macrophages, and CD163, which

10 is characteristic of M2 macrophages, suggested that PD-L1 positive TIMCs may have

11 M2-type macrophages. The presence of M2 macrophages is correlated to poor

12 prognosis in various carcinomas.[40–42] However, Edin et al. showed that the high

13 infiltration of M1 and M2 macrophages correlates with good prognosis in CRCs,[43] and

14 that the local role of TAM is controversial. While it has been established that the

15 blockade of the PD-1 / PD-L1 pathway activates T cells, little is known of the role of

16 this pathway in TAMs, and further investigation is warranted.

1 Based on these results, we made the following hypothesis. M2 macrophages may be

2 exhausted and may not be able to fulfill the tumor growth function, just like the

3 lymphocytes may be exhausted and unable to attack the tumor cells. As a result of the

4 exhaustion, there is the possibility that PD-L1 is expressed on macrophages. However,

5 to prove this hypothesis, it will be necessary to investigate the functional mechanism by

6 examining IFNγ, a regulator of PD-L1 expression, and M2 macrophage secretion

7 factors.

8 Our study has several limitations. First, as it was performed in a single center,

9 generalizability of the results may be low. Second, there is a possibility of patient

10 selection bias due to the nature of the retrospective study. Third, the PD-L1 expression

11 on TCs was weak; however, it has been suggested that the antibody we used (E1L3N) in

12 this study, is comparable with other antibodies used in other studies.[44,45] In CRCs,

13 PD-L1 expression on TCs is observed in approximately 12-30% MSI-high cases; the

14 corresponding value is very low in microsatellite stable (MSS) cases.[21,39,46] Although

15 the prevalence of MSI-high is an estimated 10-15% worldwide, it is as low as 3-7% in

16 Japan[47,48]; thus, it is possible that that there was almost no PD-L1 expression on TCs.

1 Fourth, as a result of the low proportion of MSI-high cases in Japan, we did not

2 investigate the MSI status as a routine exam. We plan to study the role of MSI status in

3 IS in future studies.

4

5 【Conclusions】

6 Our findings suggest that IS is a good indicator of better prognosis and is significantly

7 correlated with iPD-L1 expression in CRC. Therefore, the evaluation of PD-L1

8 expression should distinguish between TIMCs and TCs. Further research is necessary to

9 clarify the significance of PD-L1 expression on TIMCs, especially M2 type

10 macrophages.

11

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10

11 **Figure legends**

12 Fig 1

13 Fig 1a-c: Immunohistochemical staining of representative PD-L1 expression ($\times$50 and

14 $\times$200). PD-L1 expression negativity (a). PD-L1 expression positivity on tumor cells (b).

15 PD-L1 expression positivity on tumor-infiltrating mononuclear cells (c).

16 Fig 1d-g: Immunohistochemical staining of representative CD3 and CD8 ($\times$50 and

1 ×200). CD3 positive at the center of the tumor (d). CD3 positive at the invasive margin

2 (e). CD8 positive at the center of the tumor (f). CD8 positive at the invasive margin (g).

3

4 Fig 2

5 Fig 2a, 2b: Kaplan-Meier curves of overall survival (a) and relapse-free survival (b)

6 according to the Immunoscore (IS) in patients with colorectal cancer.

7 Fig 2c-2f: Kaplan-Meier curves of RFS according to the IS by each TNM stage. The

8 solid line represents the group with a high score (I3-4) and the dashed line represents

9 the group with a low score (I0-2).

10

11 Fig 3

12 Fig 3a-3f: Immunohistochemical staining of CD68 and CD163 in tumor-infiltrating

13 mononuclear cells with positive PD-L1 expression (iPD-L1). iPD-L1 positive ×25 (a)

14 and ×200 (b). CD68 expression at the same position ×25 (c) and ×200 (d). CD163

15 expression at the same position ×25 (e) and ×200 (f).

16 Fig 3g, 3h: Kaplan-Meier curves of overall survival (g) and relapse-free survival (h)

1   according to the expression of PD-L1 on the tumor-infiltrating mononuclear cells

2   (iPD-L1) in patients with colorectal cancer.

Table 1. Univariate and multivariate analysis of overall survival and relapse-free survival in stage II/III colorectal cancer

Clinicopathological characteristics		N	Overall survival			Relapse-free survival		
			Univariate analysis	Multivariate analysis		Univariate analysis	Multivariate analysis	
			P-value	HR[a] (95% CI[b])	P-value	P-value	HR (95% CI)	P-value
T stage	T1-2	8	0.3016			0.1439		
	T3-4	74						
N stage	N0	39	0.0563	1	0.0765	0.0208	1	0.0229
	N1-3	43		3.54 (0.88-23.6)			3.32 (1.17-11.8)	
Differentiation	Well, Moderate	74	0.6254			0.9155		
	Others	8						
Lymphatic invasion	Negative	31	0.3365			0.2626		
	Positive	51						

Venous invasion	Negative	17	0.4240				0.1054	
	Positive	65						
Location	Left	55	0.8626				0.8183	
	Right	27						
iPD-L1	Positive	12	0.1664				0.0632	
	Negative	70						
Immunoscore	I3-I4	33	0.0116	1	0.0026	0.0019	1	0.0006
	I0-I2	49		2.71 (2.72-2.87)			11.7 (2.38-210)	

aHR, hazard ratio; bCI, confidence interval

Table 2. Relationship between PD-L1 expression on tumor-infiltrating mononuclear

cells (iPD-L1), clinicopathological features, and Immunoscore in colorectal cancer

Clinicopathological feature	N	iPD-L1 expression		
		Negative (%)	Positive (%)	P-value
Sex				0.2033
Male	67	52 (77.6)	15 (22.4)	
Female	65	56 (86.2)	9 (13.8)	
Location				0.0178
Right	35	24 (68.6)	11 (31.4)	
Left	97	84 (86.6)	13 (13.4)	
TNM stage				0.0026
I	31	19 (61.3)	12 (38.7)	
II	39	32 (82.1)	7 (17.9)	
III	43	38 (88.4)	5 (11.6)	
IV	19	19 (100.0)	0 (0.0)	
T stage				0.0035
T1-2	39	26 (66.7)	13 (33.3)	
T3-4	93	82 (88.2)	11 (11.8)	
N stage				0.0145
N0	75	56 (74.7)	19 (25.3)	
N1-3	57	52 (91.2)	5 (8.7)	

Differentiation				0.3536
Well, moderate	120	97 (80.8)	23 (19.2)	
Others	12	11 (91.7)	1 (8.3)	
Lymphatic invasion				0.1162
Negative	58	44 (75.9)	14 (24.1)	
Positive	74	64 (86.5)	10 (13.5)	
Venous invasion				0.1459
Negative	34	25 (73.5)	9 (26.5)	
Positive	98	83 (84.7)	15 (15.3)	
Recurrence				0.2762
Negative	112	89 (79.5)	23 (20.5)	
Positive	17	16 (94.1)	1 (5.9)	
Immunoscore				<0.0001
I 0-2	77	73 (94.8)	4 (5.2)	
I 3-4	55	35 (63.6)	20 (36.4)	

Fig 1

PD-L1 -	tPD-L1 +	iPD-L1 +
	CD3 CT+	CD3 IM+
x50	CD8 CT+	CD8 IM+
x200		

1

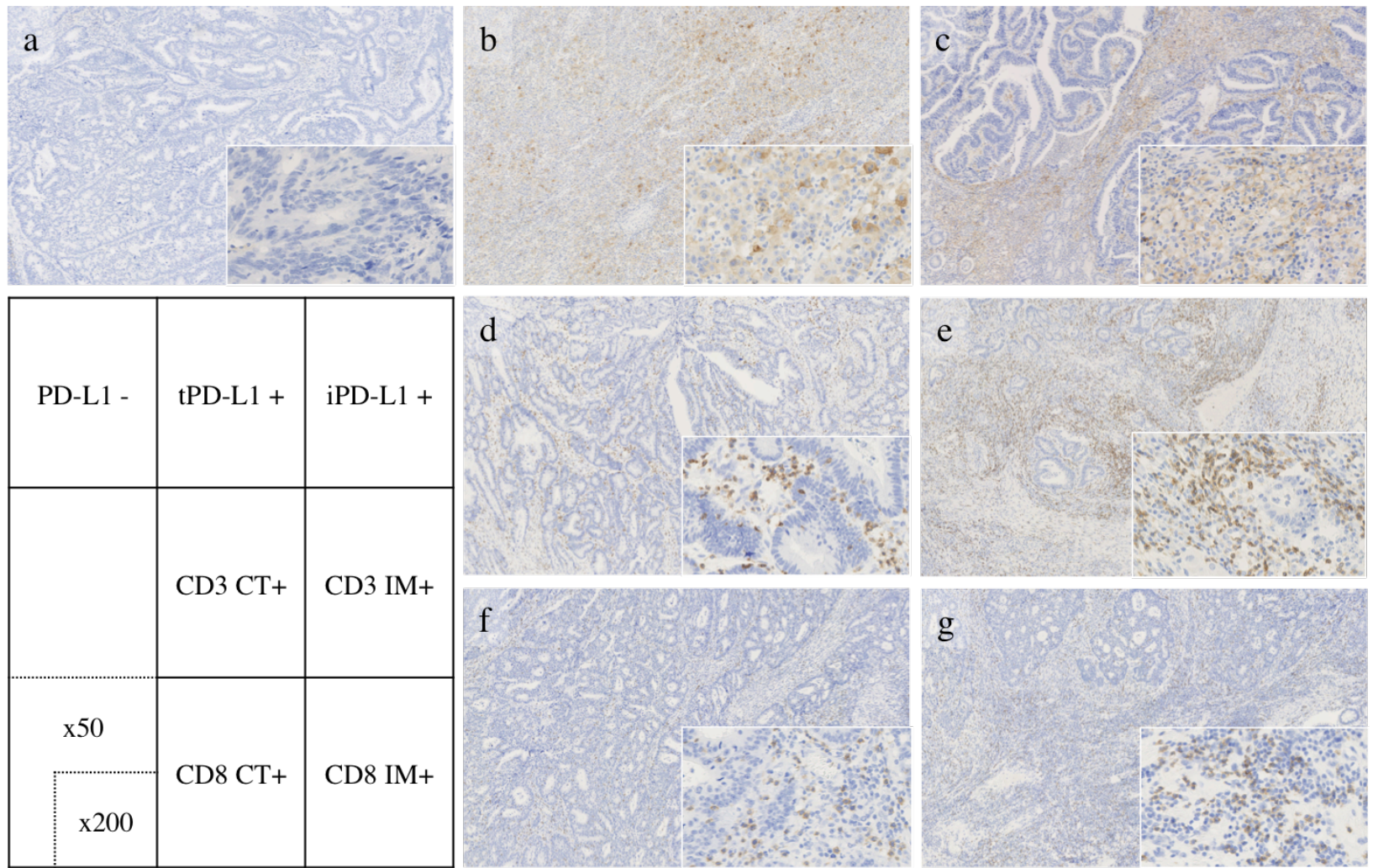Fig 1a-c: Immunohistochemical staining of representative PD-L1 expression (×50 and ×200). PD-L1 expression negativity (a). PD-L1

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CD3 positive at the invasive margin (e). CD8 positive at the center of the tumor (f). CD8 positive at the invasive margin (g).
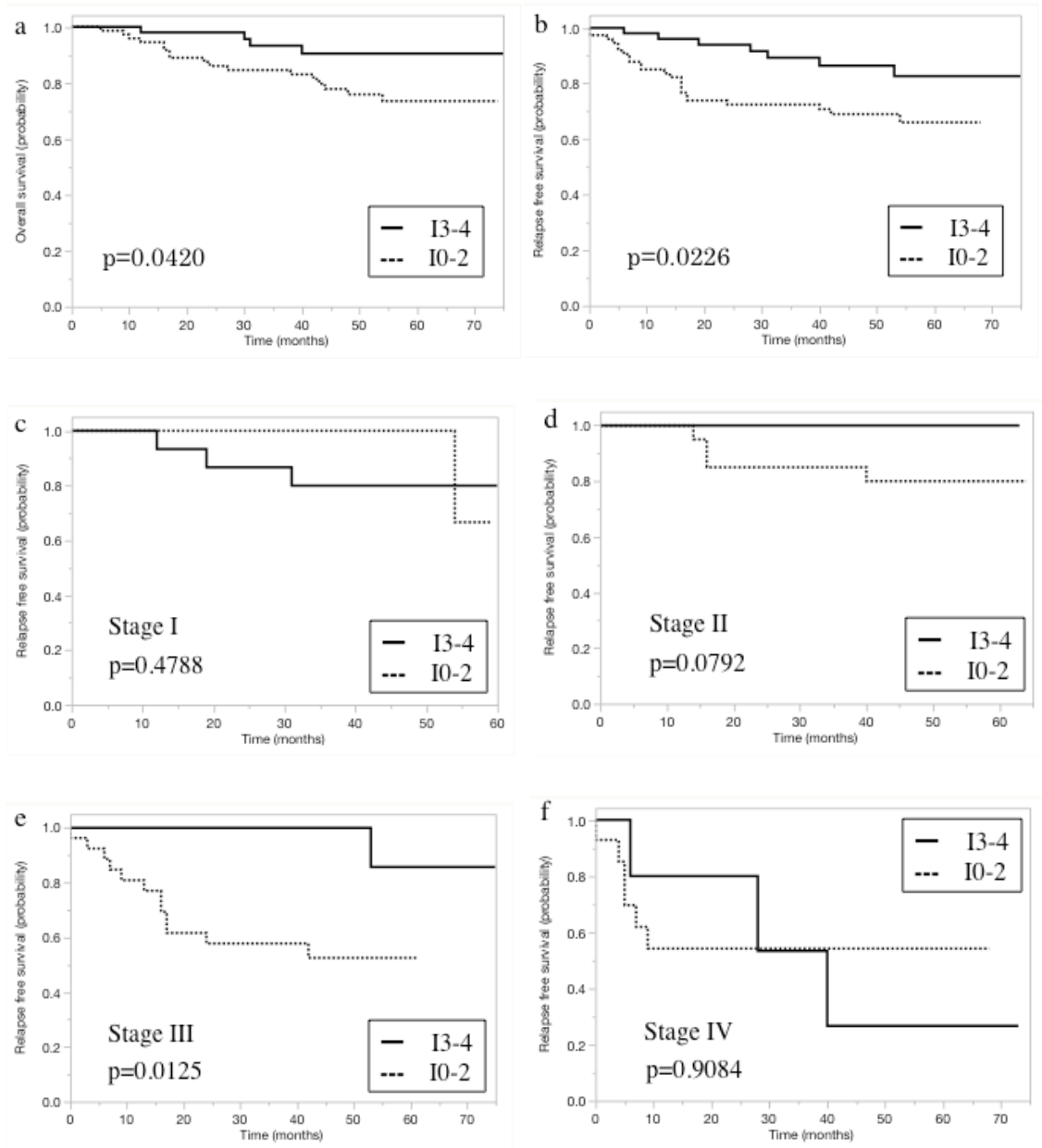
Fig 2

Fig 2a, 2b: Kaplan-Meier curves of overall survival (a) and relapse-free survival (b)

according to the Immunoscore (IS) in patients with colorectal cancer.

Fig 2c-2f: Kaplan-Meier curves of RFS according to the IS by each TNM stage. The

solid line represents the group with a high score (I3-4) and the dashed line represents

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Fig 3

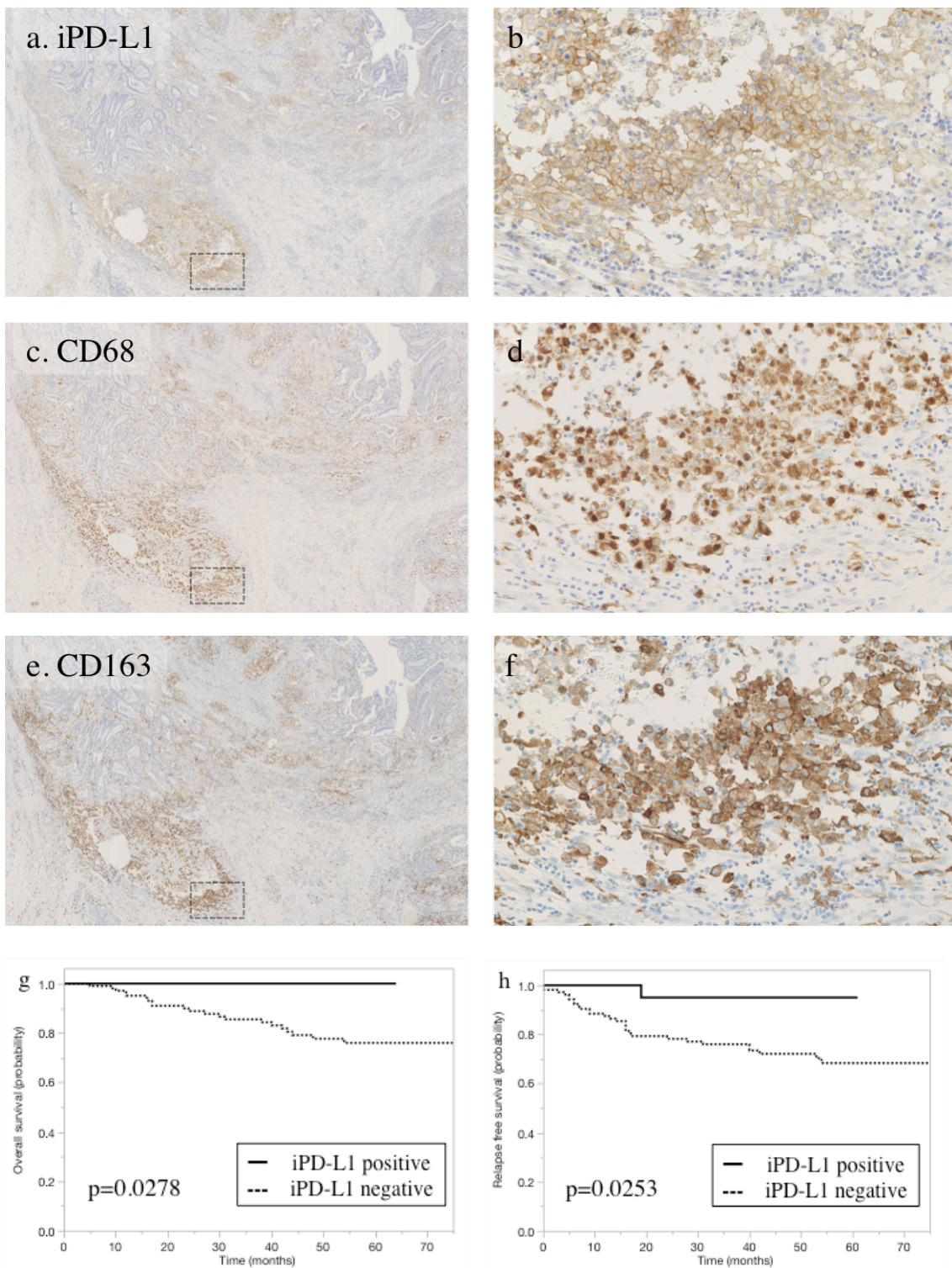

a. iPD-L1

b

c. CD68

d

e. CD163

f

g

h

Fig 3a-3f: Immunohistochemical staining of CD68 and CD163 in tumor-infiltrating mononuclear cells with positive PD-L1 expression (iPD-L1). iPD-L1 positive ×25 (a) and ×200 (b). CD68 expression at the same position ×25 (c) and ×200 (d). CD163 expression at the same position ×25 (e) and ×200 (f).

Fig 3g, 3h: Kaplan-Meier curves of overall survival (g) and relapse-free survival (h) according to the expression of PD-L1 on the tumor-infiltrating mononuclear cells (iPD-L1) in patients with colorectal cancer.