Evaluation of PD-L1 Immunoexpression in Resected Samples of Colorectal Adenocarcinoma: A Study on 64 Cases

*Jahan I,[1] Dey BP,[2] Akhtaruzzaman M,[3] Hossain KH,[4] Alam M,[5]Rahman MZ[6]

Abstract

Background: Colorectal cancer (CRC) is a leading cause of cancer-related deaths worldwide and its incidence is rising steadily in developing nations. The patient prognosis for CRC remains poor, despite advances in surgery and treatment. Additionally, there are currently no reliable prognostic approaches for CRC, despite the use of conventional prognostic factors. Immune checkpoints blockades (ICB) have emerged as a promising treatment strategy and have dramatically improved long-term survival in several malignances. The "PD-1 (programmed cell death-1)"/PD-L1 (programmed cell death-ligand 1) axis plays an important role to control immune suppression by down-regulating T effector cell activities enable tumor cells to escape from the host's anti-tumor immune surveillance. Aim of this study was to evaluate the expression of PD-L1 (28-8 clone) in resected samples of colorectal cancer.

Methods: This was a cross-sectional observational study. A total 64 cases were selected from the patients who were diagnosed as adenocarcinoma from resected samples received in the department of pathology at BSMMU from July 2021 to June 2023. Immuno-histochemical staining for PD-L1 was performed along with appropriate positive control.

Results: In this study PD-L1 immuno-expression was found in 14 (21.9%) out of 64 cases. However, no expression was found in rest of the 50 (78.1%) cases.

Conclusion: Evaluation of expression of PD-L1 may emerge as a new marker and target for the immunotherapy of colorectal cancer.

[Journal of Histopathology and Cytopathology, 2024 Jan; 8 (1):56-64]

Keywords: CRC, ICB, PD-1, PD-L1, IRS.

1. **Dr. Israt Jahan, Lecturer, Department of Pathology, Chandpur Medical College, Chandpur, Bangladesh; isratliza424@gmail.com*
2. *Dr. Bishnu Pada Dey, Associate Professor, Department of Pathology, BSMMU, drbishnu@bsmmu.edu.bd;*
3. *Dr. Md. Akhtaruzzaman, Lecturer, Department of Pathology, Pabna Medical College, Pabna, Bangladesh; akter22nd@gmail.com;*
4. *Dr. Kassim Hagi Hussain, Senior lecturer, Department of pathology, Jazeera University Medical College, Somalia; doctorqaasim@gmail.com*
5. *Dr. Mahabub Alam, Assisstant Professor, Department of Pathology, Bashundhara Ad-din Medical College & Hospital, Dhaka, Bangladesh; mahabub.robin@gmail.com*
6. *Dr. Md Zillur Rahman, Professor, Department of Pathology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Shahbagh, Dhaka-1000, Bangladesh;drzillur@bsmmu.edu.bd;*

**For correspondence*

Introduction

According to Wyss et al. (2019), colorectal cancer (CRC) is a leading cause of cancer-related deaths worldwide, and its incidence is rising steadily in developing nations. The primary method of treating colon cancer needs thorough treatment including surgery and postoperative chemotherapy, however, metastasis and recurrence are the main reasons for treatment failure.[1] The patient prognosis for CRC remains poor, despite advances in surgery and treatment. Additionally, there are currently no reliable prognostic approaches for CRC, despite the use of conventional prognostic factors such as tumor site, tumor size, histological type, grading, and TNM staging. Because of this, identifying more biomarkers would be beneficial for creating accurate prognosis methods for CRC.[2] Many types of human malignancies exhibit immune tolerance, which is a significant obstacle in cancer immunotherapy. The tumor microenvironment has been studied recently to see the significance of the interplay between cancer cells and the immune system in cancer surveillance.[3] Tumor cells use a variety of defense mechanisms within the tumor microenvironment to shield themselves from the body's immune responses.[4] A member of the B7 family of cell-surface glycoproteins, PD-L1 is expressed on the surfaces of a variety of inflammatory cells and is induced by inflammatory mediators and cytokines, including interferon-gamma (IFN).[5] Malignant epithelial tumors, such as colorectal cancer, express the ligand Programmed Death Ligand-1 (PD-L1) on their cell membranes, and activated lymphocytes that express the PD-1 receptor can attach to it specifically. Antigen-stimulated lymphocyte proliferation and cytokine production are down-regulated as a result of the interaction between PD-L1 on tumor cells and its receptor PD-1 on activated T-cells, which inhibits the host immune response.[6] According to Lang-Schwarz et al. (2021), PD-L1 may emerge as a new marker and target for the therapy of colon cancer.[7]

According to studies, PD-L1 overexpression in the tumor micro-environment is linked to an increase in effector T-cell infiltration, and malignancies that are PD-L1 "positive" are more likely to benefit clinically from check-point inhibitor therapy.[8]

For the treatment of colorectal cancer, pembrolizumab, nivolumab, and the combination of nivolumab and ipilimumab are approved by FDA.[9] Studies have shown that nivolumab improved overall survival in patients who had not previously received treatment for colorectal cancer.[10]

Better stratification of patients for anti–PD-1 immunotherapy relies on the measurement of tumor or immune cell PD-L1 expression. Therefore, given the therapeutic application of PD-L1, this study was designed to assess PD-L1 expression in colorectal cancer.

Methods

This study was a cross sectional observational study conducted in the Department of Pathology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, after taking institutional review board clearance. Paraffin blocks of 64 cases of colorectal adenocarcinoma from resected specimens were taken. Biopsy specimen, carcinoma in situ, tumors with extensive areas of necrosis and patients who received neoadjuvant therapies were excluded. Demographical and relevant clinical information such as age, sex, histopathological diagnosis with grade and stage were collected from the departmental records.

Paraffin blocks of all selected cases were retrieved from Department and checked and reviewed. After confirming the diagnosis

immunohistochemical staining for PD-L1 was performed along with appropriate positive control. 3-4 mm thick sections were cut and gently lowered on surface of water bath at 45˚ C and were spread wrinkle free on to the slides coated with 0.1% poly L-lysine for 15 minutes at 37°C and air dried. Then the slides were kept on hot plate at 60˚C for baking for 30 minutes. Dewaxing was done by treating the slides in xylene followed by rehydration in absolute alcohol, 90% alcohol and 70% alcohol. For antigen retrieval slides were put in preheated pressure cooker having citrate buffer, then boiled and allowed to cool naturally. To block the endogenous enzyme activity hydrogen peroxide was added in a moist chamber at room temperature.

Monoclonal Rabbit PD-L1 Clone 28-8 (Abcam) pre-diluted, ready to use was used as primary antibody. Then DAKO REALTM EnVision TM (HRP RABBIT/MOUSE) (ENV) secondary antibody was added. The presence of PD-L1 staining in syncytiotrophoblast of term placenta served as a positive control. (Abcam, 2022). Immune cells (lymphocytes and macrophages in the lymphoid tissue of healthy colorectal tissue) showing PD-L1 expression worked as internal control in this study.[11]

Enhancement of primary antibody was done by adding antibody enhancer (super enhancer) and incubated in moist chamber for 20 min. The peroxidase anti-peroxidase method was followed for secondary staining. DAB was used for coloring the antigen-antibody complex. This was followed by counterstaining with hematoxylin.

Scoring of 'PD-L1 expression' in colorectal cancer

IHC assay is the most common method for detection of 'PD-L1 expression' status. Detection of PD-L1 expression in tumor cells of colorectal cancer helps in patient stratification to guide anti- PD-1/PD-L1 therapy. Evaluation of PD-L1 expression in colorectal cancer is done by immunostaining of the cell membrane of the epithelial tumor cells and the stromal cells. A neoplastic cell is counted as PD-L1-positive if there is a membranous staining, irrespective of staining intensity and whether the membrane depicts complete or partial PD-L1 positivity.

Diffuse positivity (cytoplasmic and/ or membranous staining) is common in immune cells. For this reason, if there is cytoplasmic but no membranous staining; a tumor cell is considered PD-L1 negative.[12]

The percentage of positive cells in total tumor cells (TPS) is scored semi-quantitatively as 0 (<1% positive), 1 (1%-25% positive), 2 (25%-50% positive) and 3 (50%-75% positive) and 4 (>75% positive).[13]

Criteria for assessment of percentage of PD-L1 positive cells in total tumor cells (TPS)[13]

Percentage of PD-L1 positive cells in total tumor cells (TPS)	**Score**
<1%	0
1%-25%	1
25%-50%	2
50%-75%	3
> 75%	4

Intensity score of PD-L1 expression is graded as 0 (negative), 1 (weak), 2 (moderate), or 3 (strong). [14]

Criteria for assessment of intensity score of PD-L1 expression in tumor cells[14]

Staining intensity of PD-L1 positive tumor cells	**Score**
No staining	0
Weak staining	1
Moderate staining	2
Strong staining	3

Immuno-reactivity score (IRS) of PD-L1 expression

The immune-reactivity score (IRS) is used to assess both the percentage of positive cells in total tumor cells (TPS) as well as the intensity score (IS). Immuno-reactivity score (IRS) is calculated by summing these values, culminating in final values ranging from 0 to 7. Positive PD-L1 expression is defined as an IRS value of ≥3 (14). Samples having a final score of ≤4 are considered to be low and those with score of > 4 are considered to be high.[6]

The results of the study were statistically analyzed using the Statistical Package for the Social Sciences (SPSS) version 25.0 (IBM Corp. SPSS statistics, Chicago, Illinois, USA) for windows. Data were expressed as mean ± SD for the quantitative variables, numbers, and percentage. Comparison between multiple groups were made using Chi square test and Fisher's exact test for qualitative data. A value of $P < 0.05$ was taken as significant.

Results

The present study was a cross-sectional observational study. It was conducted in the Department of Pathology, BSMMU. The study population were the patients diagnosed as adenocarcinoma from resected specimens received in the department of pathology at BSMMU during the study period. Patients of all ages and sex were included in the study. Paraffin blocks of total 64 cases were selected. Demographic and histopathological variables (age, sex, grading, staging of tumor etc.) were assessed and immune-histochemical expression of PD-L1 was observed.

A total of 64 cases showed age variations ranging in between 18 years to 75 years. Most of the cases (31.3%) were found in the 6th decade. Mean age of patients was 49.81 (± 13.96) years.

In this study out of total 64 cases, 40 (62.5%) cases were male and 24 (37.5%) cases were female with male to female ratio 1.7:1.

When the laterality of occurrence was considered, it was observed that in 28 (43.8%) cases the tumor existed in right colon while in 36 (56.3%) cases tumor occurred in left colon.

According to location of tumor, most 16 (25%) cases were in rectum. Another 14 (21.9%) cases were in sigmoid colon and 3 (4.7%) cases in descending colon. In right colon, 11 (17.2) cases were in caecum, 12 (18.8%) in ascending colon and 8 (12.55) in hepatic flexure of transverse colon.

In this study only adenocarcinomas were included. Out of 64 cases, the vast majority of the cases were conventional adenocarcinoma (47 cases, 73.4%), followed by 15 (23.4%) cases of mucinous adenocarcinoma. The rest 2 (3.1%) cases were signet ring cell carcinoma.

Among the total 64 cases, PD-L1 expression was positive in 21.9% (14/64) of the cases in total. PD-L1 positive 14 cases were categorized into low expression (IRS≤4) and high expression group (IRS>4). Out of 14 positive cases, 11 (17.2%) cases showed low expression and only 3 (4.7%) cases showed high expression of PD-L1 (Fig 1). No expression was seen in 78.1% (50/64) of cases.

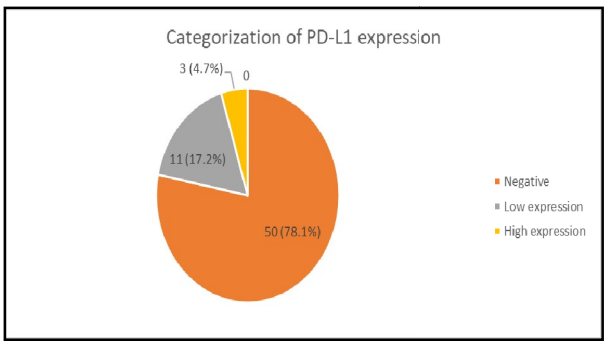


Figure 1. Categorization of PD-L1 positive cases into low and high expression (n=64)

In this study, percentage of PD-L1 positive cells in total tumor cells was assessed. Out of 64 cases, 47 (73.4%) cases showed PD-L1 staining in <1% of tumor cells. It was observed that PD-L1 staining was positive in 1-25% of tumor cells in 9 (12.5%) cases. Six cases (10.9%) showed positive staining in 25-50% of tumor cells and only two (3.1%) cases showed positive staining in 50-75% of tumor cells.

Among 64 cases, no staining for PD-L1 was observed in 47 (73.4%) cases. Moderate staining of PD-L1 was observed in 10 (15.6%) cases (Fig 3) followed by weak intensity in 5 (7.8%) cases(Fig 2). Only 2 (3.1%) cases showed strong intensity of PD-L1 (Fig 4).

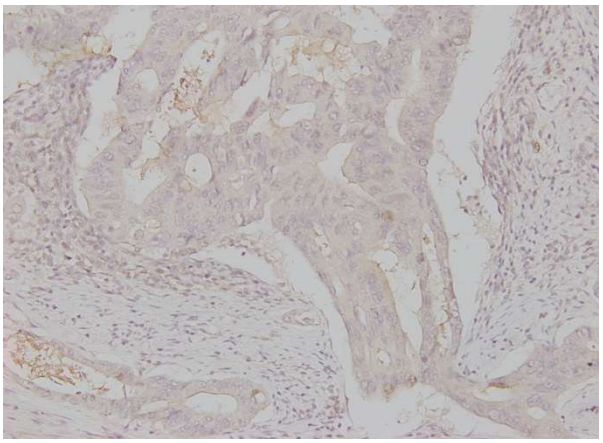


Figure 2. Photomicrograph showing positive PD-L1 stain with weak intensity in tumor cells (case no. 57, IHC, 200x).

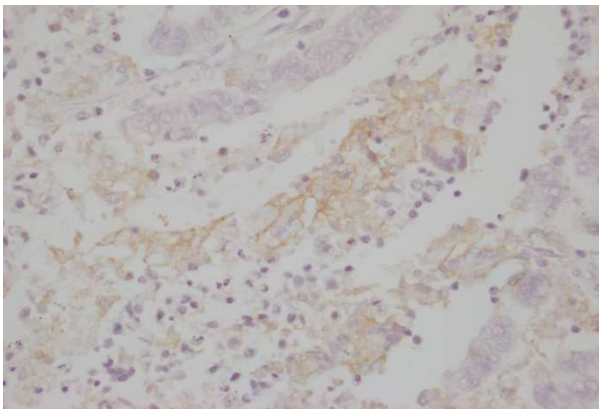
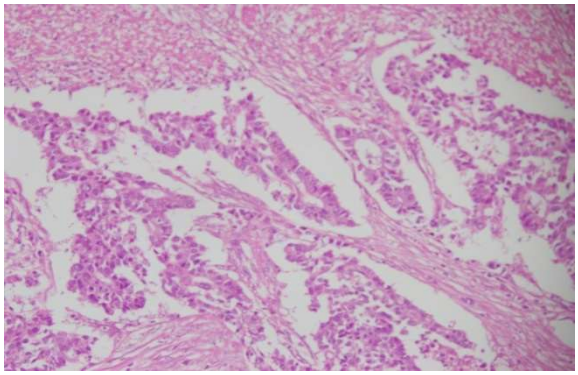
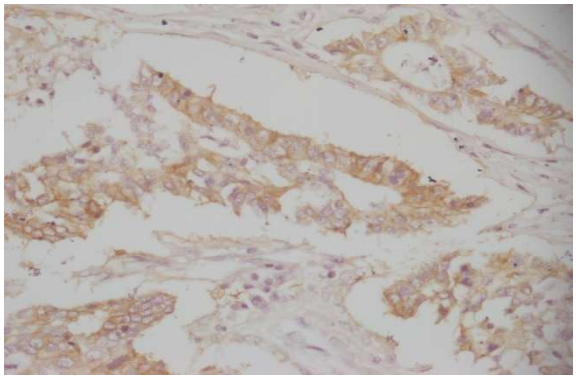


Figure 3. Photomicrograph showing PD-L1 expression in tumor cells with moderate intensity. (Case no. 16, IHC, 400X)



A



B

Figure 4. Photomicrographs show adenocarcinoma of colon under light microscope (A) case no:25, H&E, 100x) and strong positive PD-L1 staining in tumor cells (B) case no: 25, IHC,400x).

In present study, immuno-reactivity score (IRS) of PD-L1 expression was calculated by summation of percentage of PD-L1 positive cells in total tumor cells (TPS) and staining intensity. Out of 64 cases, score:0 was observed in 47 (73.4%) cases. Only 3 (4,7%) cases showed score:2. Among 14 PD-L1 positive cases, score:3 was observed in 7 (10.9%) of cases followed by score:4 in 4 (6.3%) cases and score:5 in 3 (4.7%) cases.

Discussion

Colorectal carcinoma (CRC) is one of the most frequently occurring cancers and one of the leading causes of cancer related death worldwide. The treatment plan and prognosis has so far been mainly based on histologic grading and staging of tumor. But sometimes prognosis, treatment response and recurrence rate varies from patient to patient even within the same stage. Lately studies have been carried out focusing on biomarkers reflecting immune-regulation, which plays an important role in initiation, progression and metastasis. In the metastatic setting, colorectal cancer, has a relatively shorter median survival period. Adding the programmed death-1 (PD-1)-targeting agents Nivolumab or pembrolizumab to chemotherapy was recently shown to improve outcomes in both early-stage and metastatic colorectal cancer.

In this study 64 cases of CRC were included. The mean age of patients was 49.81 ± 13.96 years ranging from 18 to 75 years. The majority (38.5%) of the cases belonged to age group 51-60 years. In another study done in Bangladesh by Raza et al. (2016), the average age of the patient was found 47±14.8 years and the peak age group was in between 50-59 years which is very much similar to current study.[15] One study from USA showed that the median age at the time of diagnosis was 55±15 years.[16] However, the study conducted in India found that CRC patients ranged in age from 19 to 82, with the majority of cases (62.5%) occurring in the sixth and seventh decades of life.[17] In the study of Enkhbat et al., conducted in Japan in 2018, the mean age of CRC patients was 69.7 years (range:41-93 years).[18] One study from China, conducted by Lie et al., (2021) showed that mean age of patients with CRC was 66 years.[6] Another study of in Korea, showed that mean age of CRC patients was 63.1 ± 12.5 years. Two studies from Europe showed that mean age of CRC patients at the time of diagnosis was 62±15 years and 72.2 years.[19,20]

This study showed that, there were male predominance in colorectal cancer. Out of total 64 cases, 40 (62.5%) cases were male and 24 (37.5%) cases were female with male to female ratio 1.7:1. Similar finding was obtained by Raza et al. (2016) having male to female ratio 1.4:1.[15] A study conducted by Gupta et al., in India also showed that, male to female ratio in CRC patients was 1.4:1.[17]

In this study, it was observed that in 34 (53.1%) cases, tumor existed in the left colon while in 30 (46.9%) cases, tumor occurred in the right colon. Most of the malignancies in this investigation were found in the rectum (16 cases, 25%) and sigmoid colon (14 cases, 21.9%) respectively. In their study Raza et al. (2010) observed the similar findings: 74% tumors were located in left colon, while 26% were in right colon. Similar results were discovered in Korea by Lee et al., who found that the left colon was where the majority of cancers (71.4%) were found.[21] One study from Jordan, conducted by Al-Jussani et al., showed that most of the tumors in CRC were located in left colon.[22] Thus, the finding of this study that majority of CRCs are located in left colon is similar with previous studies.

The current study found that the vast majority of the cases were conventional adenocarcinoma, not otherwise specified (n = 47, 73.4%), followed by mucinous

adenocarcinoma (n =15, 23.2%) and signet ring cell carcinoma (n = 2, 3.1%). Lee et al.(2018) also showed similar findings, as their study showed majority of the patients (90.5%) had adenocarcinoma, not otherwise specified.[21] 90% of cases were classified as adenocarcinoma, not otherwise specified; in the study of Eriksen et al.,.[3] One study from Li et al., showed that 88.8% of cases were classified as adenocarcinoma, not otherwise specified.[6] Thus, the finding of this study, that majority of CRCs are of adenocarcinoma (NOS) type are similar with previous studies.

In this study, PD-L1 staining was done with 28-8 clone. Positive expression was seen in 21.9% (14/64) of the cases. On the basis of imuuno-reactivity score of PD-L1 positivity, 17.2% (11/64) cases showed low expression and only 4.7% (3/64) showed high expression. No expression was seen in 78.1% (50/64) of cases. Similar result was found in the study of Noh et al., which showed more cases having low expression of PD-L1 than high expression.[14] Among the 58 CRCs studied by Jung et al., PD- L1 (28-8 clone) expression was detected in 18 (31%) cases.[23] The study of Kim et al., conducted on patients with CRC, showed that 20.9% of cases of CRC were PD-L1 (sp142 clone) positive similar to current study.[24] Another study by Valentini et al. (2018) showed PD-L1 positivity in 25% of tumor cells with E1L3N clone in CRC.[25] In the study of Gupta al. (2020), PD- L1 (Biocare kit) positivity of tumor cells was seen in 35% CRC cases.[17] In the study of Lang-Schwarge et al., PD-L1 (22c3 clone) positivity was seen in 30.5% CRC cases.[7] The variation in PD-L1 expression is due to use of different clones of PD- L1 and cut off values in different studies as shown by the studies.[21] The study of Lee et al. (2022) compared three PD-L1 antibodies (MIH1, E1L3 and 22C3) for staining of tumor cells. The numbers of samples showing positive PD-L1 expression in tumor cells were 14.6%, 5.7% ,4.5% for MIH1, E1L3 and 22C3 clones respectively. Biocare kit in the study of Gupta et al., showed higher PD-L1 positivity in tumor cells than 28-8 clone. But it is not approved by FDA yet. Currently, 22C3 clone and 28-8 clone are the two FDA-approved companion diagnostic tests (8). FDA has approved the anti- PD1 antibodies pembrolizumab (22c3 clone) and nivolumab (28-8) for the treatment of CRC.[26] Different studies showed more PD-L1 positivity using 22c3 clone rather than 28-8 clone. However, Nivolumab is one of the most extensively studied immune checkpoint inhibitor in metastatic CRC.[10] For this reason, 28-8 clone was used in the present study due to the availability of it from the suppliers.

Conclusion

In the present study 21.9% of the tumors showed PD-L1 positivity. On the basis of immuno-reactivity score, majority (17.2%) of the PD-L1 positive tumors showed low expression of PD-L1. High expression of PD-L1 was observed in 4.7% of the PD-L1 positive tumors. According to the results of this study, PD-L1 expression can be recommended as a biomarker for anti-PD-L1 antibody therapy in the treatment of colorectal cancer.

References

1. Shan T, Chen S, Wu T, Yang Y, Li S, Chen X. PD-L1 expression in colon cancer and its relationship with clinical prognosis. Int J Clin Exp Pathol [Internet]. 2019;12(5):1764–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/31933995%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC6947132
2. O'Malley G, Treacy O, Lynch K, Naicker SD, Leonard NA, Lohan P, et al. Stromal cell PD-L1 inhibits CD8+

T-cell antitumor immune responses and promotes colon cancer. Cancer Immunol Res. 2018;6(11):1426–41.
3. Eriksen AC, Sørensen FB, Lindebjerg J, Hager H, Depont Christensen R, Kjær-Frifeldt S, et al. Programmed Death Ligand-1 expression in stage II colon cancer - Experiences from a nationwide populationbased cohort. BMC Cancer. 2019;19(1):1–9.
4. Huang CY, Chiang SF, Ke TW, Chen TW, You YS, Chen WTL, et al. Clinical significance of programmed death 1 ligand-1 (CD274/PD-L1) and intra-tumoral CD8+ T-cell infiltration in stage II–III colorectal cancer. Sci Rep. 2018;8(1):1–10.
5. CS Wong M, Ding H, Wang J, SF Chan P, Huang J. Prevalence and risk factors of colorectal cancer in Asia. Intest Res. 2019;17(3):317–29.
6. Li Y, Liang L, Dai W, Cai G, Xu Y, Li X, et al. Prognostic impact of programed cell death-1 (PD-1) and PD-ligand 1 (PD-L1) expression in cancer cells and tumor infiltrating lymphocytes in colorectal cancer. Mol Cancer. 2016;15(1).
7. Lang-Schwarz C, Melcher B, Hartmann A, Bertz S, Dregelies T, Lang-Schwarz K, et al. Programmed death ligand 1 (PD-L1) in colon cancer and its interaction with budding and tumor-infiltrating lymphocytes (TILs) as tumor-host antagonists. Int J Colorectal Dis [Internet]. 2021;36(11):2497–510. Available from: https://doi.org/10.1007/s00384-021-03985-9
8. Franke AJ, Skelton WP, Starr JS, Parekh H, Lee JJ, Overman MJ, et al. Immunotherapy for Colorectal Cancer: A Review of Current and Novel Therapeutic Approaches. J Natl Cancer Inst. 2019;111(11):1131–41.
9. Kishore C, Bhadra P. Current advancements and future perspectives of immunotherapy in colorectal cancer research. Eur J Pharmacol [Internet]. 2021;893(August 2020):173819. Available from: https://doi.org/10.1016/j.ejphar.2020.173819
10. Jácome AA, Eng C. Role of immune checkpoint inhibitors in the treatment of colorectal cancer: focus on nivolumab. Expert Opin Biol Ther [Internet]. 2019;19(12):1247–63. Available from: https://doi.org/10.1080/14712598.2019.1680636
11. Masugi Y, Nishihara R, Yang J, Mima K, Da Silva A, Shi Y, et al. Tumour CD274 (PD-L1) expression and T cells in colorectal cancer. Gut. 2017;66(8):1463–73.
12. Aziz ZW, Mahmood AM, Yahiya ZO, Taib Al –Nuaimy WM. Correlation Between Programmed Cell Death Ligand1 (PD-L1) Expression and Clinical Parameters in Colorectal Carcinoma. J Contemp Med Sci. 2020;6(4):161–7.
13. Xu J, Yang X, Mao Y, Mei J, Wang H, Ding J, et al. Removal of N-Linked Glycosylation Enhances PD-L1 Detection in Colon Cancer: Validation Research Based on Immunohistochemistry Analysis. Technol Cancer Res Treat. 2021;20(1055):1–8.
14. Noh BJ, Kwak JY, Eom DW. Immune classification for the PD-L1 expression and tumour-infiltrating lymphocytes in colorectal adenocarcinoma. BMC Cancer. 2020;20(1):1–12.
15. Raza AM, Kamal M, Begum F, Yusuf MA, Mohammad D, Begum M, et al. Clinico-demographic Characteristics of Colorectal Carcinoma in Bangladeshi Patients. J Curr Adv Med Res. 2016;3(1):22–5.

16. Lee LH, Cavalcanti MS, Segal NH, Hechtman JF, Weiser MR, Smith JJ, et al. Patterns and prognostic relevance of PD-1 and PD-L1 expression in colorectal carcinoma. Mod Pathol. 2016;29(11):1433–42.
17. Gupta M, Manjari M, Kaur H. PD-L1 Expression in Colorectal Carcinoma: Immunohistochemical Study. Ann Pathol Lab Med. 2020;7(6):A275-281.
18. Enkhbat T, Nishi M, Takasu C, Yoshikawa K, Jun H, Tokunaga T, et al. Programmed cell death ligand 1 expression is an independent prognostic factor in colorectal cancer. Anticancer Res. 2018;38(6):3367–73.
19. Dosset M, Vargas TR, Lagrange A, Boidot R, Végran F, Roussey A, et al. PD-1/PD-L1 pathway: an adaptive immune resistance mechanism to immunogenic chemotherapy in colorectal cancer. Oncoimmunology [Internet]. 2018;7(6):1–14. Available from: https://doi.org/10.1080/2162402X.2018.1433981
20. Azcue P, Encío I, Setas DG, Alecha JS, Galbete A, Mercado M, et al. Pd-l1 as a prognostic factor in early-stage colon carcinoma within the immunohistochemical molecular subtype classification. Cancers (Basel). 2021;13(8):1–18.
21. Lee KS, Kim BH, Oh HK, Kim DW, Kang SB, Kim H, et al. Programmed cell death ligand-1 protein expression and CD274/PD-L1 gene amplification in colorectal cancer: Implications for prognosis. Cancer Sci. 2018;109(9):2957–69.
22. Al-Jussani GN, Alsughayer A, Yousuf MS, Mullahuwash Y, Dabbagh T, Sughayer MA. The clinicopathological features of programmed death ligand-1 expression in colorectal carcinoma. Int J Biol Markers. 2022;37(3):322–7.
23. Jung DH, Park HJ, Jang HH, Kim SH, Jung YJ, Lee WS. Clinical Impact of PD-L1 Expression for Survival in Curatively Resected Colon Cancer. Cancer Invest. 2020;38(7):406–14.
24. Kim ST, Klempner SJ, Park SH, Park JO, Park YS, Lim HY, et al. Correlating programmed death ligand 1 (PD-L 1) expression, mismatch repair deficiency, and outcomes across tumor types: Implications for immunotherapy. Oncotarget. 2017;8(44):77415–23.
25. Valentini AM, Pinto F Di, Cariola F, Guerra V, Giannelli G, Caruso ML, et al. Oncotarget 8584 www.impactjournals.com/oncotarget PD-L1 expression in colorectal cancer defines three subsets of tumor immune microenvironments. Oncotarget [Internet]. 2018;9(9):8584–96. Available from: www.impactjournals.com/oncotarget/
26. Ganesh K, Stadler ZK, Cercek A, Mendelsohn RB, Shia J, Segal NH, et al. Immunotherapy in colorectal cancer: rationale, challenges and potential. Nat Rev Gastroenterol Hepatol [Internet]. 2019;16(6):361–75. Available from: http://dx.doi.org/10.1038/s41575-019-0126-x