Original Article

Immunohistochemistry Expression of Programmed Death-Ligand 1 in Colorectal Carcinoma among Nigerians

KB Badmos, LA Odukoya[1], GF Khramtsova[2], LA Adebayo[1], OI Olopade[2], FB Abdulkareem

Department of Anatomic and Molecular Pathology, College of Medicine, University of Lagos and Lagos University Teaching Hospital, Idi-Araba, [1]Department of Anatomic and Molecular Pathology, Lagos University Teaching Hospital, Idi-Araba, Lagos, [2]Department of Medicine, Center for Clinical Cancer Genetics and Global Health, University of Chicago, Illinois, Unites States

Received: 05-Jan-2024;
Revision: 08-Mar-2024;
Accepted: 25-Sep-2024;
Published: 04-Dec-2024

Abstract

Background: Colorectal carcinoma (CRC) is the most common gastrointestinal malignancy in Nigeria with a dismal 5-year survival rate. Interactions between the CD8+ T-lymphocytes and the immune checkpoints such as cytotoxic T-lymphocyte antigen-4 (CTLA-4) and programmed death-ligand 1 (PD-L1) expressions are important. Novel antibodies have been developed against these immune checkpoints and have been found to improve clinical outcome in many solid organ malignancies. **Aim:** We aimed to determine immunohistochemical expression of PD-L1 in resected CRC cases assembled on tissue microarray blocks. **Methods:** Representative blocks and clinical information of resected CRC cases between 2010 and 2019 were retrieved from the archives of our department. Tissue microarray (6 × 4) blocks were constructed with 2 mm core needles. Immunohistochemistry using anti-PD-L1 rabbit monoclonal antibody (clone EPR19759 #213524, 1:200 Abcam, MA, USA) was carried out according to manufacturer's instruction. **Results:** The study included 170 cases, of which 144 cases had sufficient tissue for analysis. The peak incidence was observed in the 50–59 age group. Approximately 80.1% of the cases were in T3 and T4 stages. Only 8 (5.6%) out of 144 cases were positive for PD-L1. All the PD-L1 positive cases were either right-sided CRC (6/68) or rectal cancer (2/3). Of the seven positive cases with available histological grading, four were poorly differentiated/mucinous variants and three cases were moderately differentiated. **Conclusion:** PD-L1 expression in CRC was low (5.6%) and showed strong associations with higher tumor grades ($P < 0.013$), right-sided tumors ($P < 0.002$), and rectal cancer. There was no association with age, tumor stage, and lymph node status.

Keywords: *Colorectal cancer, immunohistochemistry, microarray, programmed death-ligand*

Introduction

The mortality resulting from colorectal carcinoma (CRC) in 2020 ranks second to lung cancer, and it accounts for 1 in 10 cancer death across all cancers worldwide.[1] In the same year, about 935,000 deaths were reported to have occurred throughout the world among colorectal cancer patients based on GLOBOCAN estimates. Curbing morbidity and mortality arising from CRC is a top priority in oncology, particularly in countries with a high human development index (HDI), where the prevalence is 4-fold higher. In West Africa, even though the prevalence is low, presentation by patients is usually at an advanced stage, most patients are 1–2 decades younger compared to patients from Western countries, and mortality is disproportionately higher.[2,3] In Nigeria, the incidence of CRC is rising, and it has moved up the scale of cancer prevalence to the fourth position from tenth in the past

Access this article online

Quick Response Code:

Website: www.njcponline.com

DOI: 10.4103/njcp.njcp_18_24

Address for correspondence: Dr. KB Badmos, Department of Anatomic and Molecular Pathology, College of Medicine, University of Lagos and Lagos University Teaching Hospital, Idi-Araba, P.M.B 12003, Lagos, Nigeria.
E-mail: kbadmos@unilag.edu.ng

How to cite this article: Badmos KB, Odukoya LA, Khramtsova GF, Adebayo LA, Olopade OI, Abdulkareem FB. Immunohistochemistry expression of programmed death-ligand 1 in colorectal carcinoma among Nigerians. Niger J Clin Pract 2024;27:1239-44.

few decades based on cancer registry reports.[2] In addition to surgery and chemoradiation treatment, immunotherapy is assuming an important role in the treatment and control of cancer generally. Of specific importance in the past decade among the immunotherapy options are the immune checkpoint inhibitors.

Immune checkpoints interact with specific receptors on T-cells to generate coinhibitory signals crucial to maintenance of peripheral immune tolerance and preventing autoimmunity.[4,5] Among these checkpoints, the two well-studied include cytotoxic T-lymphocyte antigen-4 (CTLA-4) and programmed cell death 1/programmed death-ligand 1 (PD-1/PD-L1). The PD-1 and its ligand, PD-L1, are present on immune and neoplastic cells. PD-L1 is found on T- and B-cells, macrophages, and dendritic cells, and expression is increased by inflammatory mediators and cytokines. The engagement of PD-1 by its ligand PD-L1 on tumor cells invokes a negative feedback mechanism that represses Th1 cytotoxic immune cells and impairs production of cytokines such as IFN-y and IL-12. This ultimately leads to lymphocyte depletion and immune tolerance of the tumor cells. PD-L1 expression on tumor cells implies a weakened host immune responses and consequent poor prognosis. Overexpression of PD-L1 has been reported in different tumor types such as melanoma (40–100%), non-small-cell lung carcinoma (35–95%), glioblastoma (100%), ovarian cancer (33–80%), and colorectal carcinoma (53%).[6] The impact of expression of PD-L1 in CRC is less clear as there are many conflicting reports from different studies.

Expression of PD-L1 by tumor cells is one of the mechanisms by which tumor evades host immune responses. Development of monoclonal antibodies that bind these ligands may restore the host immunity and consequently control tumor growth or even induce regression. Recent clinical studies in melanoma, renal cell carcinoma, and non-small-cell lung cancer have reported significant positive responses to PD-1 checkpoint targeting.[7] In contrast, results in CRC have been disappointing and evidence is pointing to MSI status as a predictor of response to PD-1 blockade.[8] This has necessitated several clinical trials looking at MSI status and response to PD-1/PD-L1 checkpoint inhibitors, particularly among those CRC patients with metastatic disease that is refractory to standard chemotherapy combinations.[8] We studied the immunohistochemistry expression profile of PD-L1 on array blocks of resection specimens of CRC cases seen at our Teaching Hospital between 2000 and 2019. The clinicopathological parameters of these cases were correlated with the immunohistochemistry expression.

Materials and Method

The materials for the study included colorectal resection specimens diagnosed between 2010 and 2019 obtained from the Pathology archives of our University Teaching Hospital. The biodata of the cases consisting of unique identifiers, age, and sex among others as well as clinical information of resected colorectal carcinoma cases were obtained from the pathology records. Pathological information extracted includes the tumor stage, grade, lymph node, and metastatic status. Also considered was tumor location; the right-sided CRC consists of cecal, ascending, and transverse colonic tumors up to splenic flexure, whereas left-sided CRC consists of descending, sigmoid, and rectal tumors. Tissue blocks from these resection specimens with complete clinical information (n = 170 cases) were extracted from the Departmental archives; hematoxylin and eosin sections were cut to assess for adequacy of adenocarcinoma in the tissue blocks for TMA by K.B and LA. Out of the 170 cases, only 144 had enough tumor samples for TMA and representative areas of the block selected were marked.

Colorectal tumor microarray

The TMA instrument is used to acquire two 2 mm cylindrical cores of tissue manually from each donor block of CRC. This is then placed in the empty recipient paraffin block. The core is placed at a specifically assigned coordinate (X-Y guide), which is accurately recorded on a spreadsheet. The TMA blocks (6 × 4) consist of two cores of each CRC cases and two cores of control tissue (tonsils and placenta). The cores were constructed by GK and KB. A total of 144 CRC cases were arrayed for PD-L1 immunohistochemistry.

PD-L1 immunohistochemistry

The primary antibody used is Abcam clone EPR19759, rabbit anti PD-L1 monoclonal antibody (mAb), 1:200 (Abcam). Representative sections of the array blocks were cut at 5 μm. The slides were stained on a Leica Bond RX automatic stainer using the protocol 'No Post Primary 1 h Bond DAB refine'. Epitope retrieval solution II (Leica Biosystems, AR9640) was used for 40-minute heat-induced antigen retrieval treatment. Anti-PDL-1 antibody (1:200) was applied on tissue sections for 60 minutes incubation, and the antigen–antibody binding was detected with Bond polymer refine detection (Leica Biosystems, DS9800). The tissue sections were mounted with cover glasses after staining.

PD-L1 scoring

The scoring for PD-L1 was assessed mainly on tumor cells by assessing any form of membrane staining. PD-L1 is considered positive whether the membrane stain is partial or complete, weak, or strong. Positive

cells are counted as a percentage of total tumor cells in up to 10 high-power fields. The percentage of positive tumor cells was semiquantitatively scored as 0, absence of positive tumor cells; 1, <1% positive tumor cells; and 2, ≥1% positive tumor cells. Tumor was considered positive for PD-L1 expression with a score of 2, while others were considered negative.

Statistical analysis

To analyze the relationship between PD-L1 expression and clinicopathological parameters, Chi-square and two-tailed Fischer's exact tests were used. Statistical analysis was performed using Stata IC 15.1 statistical software (TX USA). All categorical variables were summarized using frequency and percentages. A *P* value of <0.05 was considered statistically significant for all analyses performed.

Ethics approval

This study was approved by the Health Research Ethics committee of the College of Medicine of our University with approval number – CMUL/HREC/02/19/491.

Results

The 144 resection colorectal samples whose materials were arrayed for PD-L1 comprise 53/144 (36.8%) cases who were under 50 years of age, 87/144 (60.4%) cases who were above 50 years, and 4/144 cases whose age was not recorded [Table 1]. These cases were almost evenly distributed among males and females (71 vs 72). Most of the cases, 68/144 (47.2%), were well-differentiated adenocarcinoma, 52/144 (36.1%) were moderately differentiated, and 32/144 (22.2%) were poorly differentiated carcinoma. The remaining three cases were not graded. Most of these cases were stage T3 (n = 77) and T4 (n = 39) constituting 73.6% of the total. Right-sided tumor was 68/144 (47.2%), left-sided tumor was 45/144 (31.3%), and in 31/144 (21.5%) cases, the tumor location was not stated.

Programmed-death ligand 1 was positive in 8 cases (5.6%), while the remaining 136 cases (94.4%) were negative [Figure 1 and Table 2]. Correlation between PD-L1

Table 1: Pathological characteristics of resected CRC

Variables	PDL-1 Status					
	n	Positive *n* (%)	Negative *n* (%)	Pearson χ^2	*P*	Fisher's exact (2-sided)
Age (years)				2.319	0.123	0.259
<50	53	1 (1.9%)	52 (98.1%)			
≥50	87	7 (8.1%)	80 (92.0%)			
Not stated	4	0	4			
Gender				0.0597	0.971	1
Male	71	4 (5.6%)	67 (94.4%)			
Female	72	4 (5.6%)	68 (94.4%)			
Not stated	1	0	1			
Tumor site				16.866	0.002	0.007
RCC	68	6 (8.8%)	62 (91.2%)			
LCC	40	0 (0%)	40 (100%)			
RecC	5	2 (40.0%)	3 (60.0%)			
Not stated	31	0	31			
Tumor grade/Differentiation				10.7104	0.013	0.009
Well Differentiated AdenoCa	57	0 (0%)	57 (100%)			
Moderate Differentiated AdenoCa	52	3 (5.8%)	49 (94.2%)			
Poorly Differentiated Carcinoma	32	4 (12.5%)	28 (87.5%)			
Not stated	3	1	2			
pT stage				1.7897	0.774	0.648
pT1	1	0 (0%)	1 (100%)			
pT2	18	0 (0%)	18 (100%)			
pT3	77	5 (6.5%)	72 (93.5%)			
pT4	39	2 (5.1%)	37 (94.9%)			
Not stated	9	1	8			
Lymph node involvement				1.0382	0.792	0.851
N0	48	2 (4.2%)	46 (95.8%)			
N1	42	2 (4.8%)	40 (95.2%)			
N2	33	3 (9.1%)	30 (90.9%)			
Nx/not stated	21	1	20			

RCC=Right-sided Colon cancer, LCC=Left-sided Colon Cancer, RecC=Rectal Cancer, AdenoCa=Adenocarcinoma

Table 2: Programmed-death ligand 1 (PD-L1) immunohistochemical expression in CRC

PD-L1	Frequency (%)
Positive	8 (5.56%)
Negative	136 (94.44%)
Total	144 (100%)

Photomicrograph of tonsil showing positive control for PD-L1 (x20). Photomicrograph of CRC with weak and focal PD-L1 membrane staining in the tumor cells (x20). Photomicrograph of CRC showing strong PD-L1 membrane staining in the tumor cells (x20)

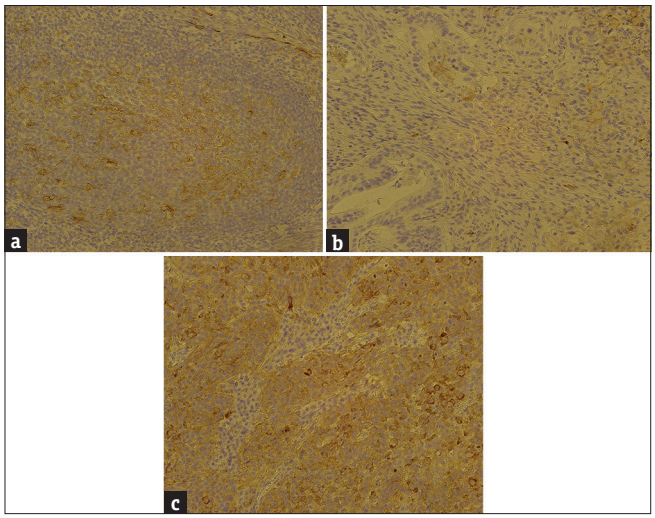

Figure 1: a-c: Photomicrographs of PD-L1 membrane staining

expression and high tumor grade showed significant association (X^2 = 10.7104, P = 0.013, Fisher's exact P = 0.009). Seven out of the eight positive cases were moderate to poorly differentiated adenocarcinoma, while none of the well differentiated cases was positive. PD-L1 was positive in 6/68 (8.8%) right-sided CRC and in 2/45 (4.4%) left-sided CRC. The rest of the cases (31/144) with unstated tumor location were all PD-L1-negative. There was a strong association between right-sided CRC and PD-L1 expression (X^2 = 16.866, P = 0.002, Fisher's exact P = 0.007). There was no association between PD-L1 expression and age of the patient (X^2 = 2.319, P = 0.123, Fisher's exact P = 0.259), tumor stage (X^2 = 1.7897, P = 0.774, Fisher's exact P = 0.648), or lymph node status (X^2 = 1.0382, P = 0.792, Fisher's exact P = 0.851).

Discussion

CRC treatment in Nigeria is fraught with challenges of late-stage presentation in patients who are at least 1 decade younger compared to patients in developed countries. A significant percentage of the resected CRC patients from this study (n = 53, 36.8%) were less than 50 years of age, while the rest (n = 67, 60.4%) were 50 years and above. The distribution mirrors the general pattern of presentation in earlier publications on CRC across Nigeria where the mean age is between 39 and 50.7 years.[9,10] The younger age of occurrence has a profound negative economic impact since this is the prime of working population and people affected are at the peak of their career. In United States, rising incidence of early onset CRC was first noted in 2003 and the carcinogenic pathway is similar to that of patients who are 50 years and older.[11] Apart from known environmental risk factors such as smoking, obesity, heavy alcohol intake, and red meat consumption, alteration in the composition of gut microbiota is being interrogated in many scientific studies.[11] Many cases of early onset CRC are associated with positive family history in first-degree relations and/or presence of familial cancer syndromes such as familial adenomatous polyposis (FAP) and hereditary non-polyposis cancer syndrome (HNPCC).[11] In Nigeria, studies have reported high frequency of occurrence of CRC in the young, while a few case reports provided genetic linkage.[9,12] Generally, in most West African countries, the effects of diets that are mainly starch-based, vegetable-based, spicy, and peppery food are suggested as being protective in the observed low incidence of CRC in all age groups, but a surge in dietary shift toward Western diet may need to be evaluated as a possible risk associated with the increased frequency noted in the young Nigerians.[2]

In this tissue microarray study of 144 CRC, PD-L1 expression detected by immunohistochemistry is low (n = 8, 5.6%) and this is comparable to reports of low-level PD-L1 expression in CRC from earlier studies.[13-15] Lee *et al*.,[13] in an immunohistochemistry study of PD-1 and PD-L1 of CRC using tissue microarray, reported that out of the 394 CRCs evaluated, 19% had high PD-1-positive tumor-infiltrating lymphocytes and 5% had high-level PD-L1 expression in the tumor cells. In another PD-L1 study of 181 CRC among Americans, 9% of the cases were positive and were associated with signature of serrated pathways, BRAF mutation, MSI, poor differentiation, and frequent tumor-infiltrating lymphocytes.[14] Another study on 454 well-characterized CRCs reported 12% positive rate, although this study combined both membrane and cytoplasmic staining of between 5 and 100% as positive.[15] Variations reported for CRC are influenced by cutoff point for positive cells as well as strict membranous or combined membrane and cytoplasmic staining. Our study considered only membrane staining of any intensity but in up to and more than 1% of the tumor cell population as positive, which might have accounted for the lower frequency observed.

PD-L1 expression in this study shows a strong association with high tumor grade and right-sided tumor.

The high-grade tumor comprises poorly differentiated carcinoma forming sheets of medullary-like pattern morphologically, mucinous and signet ring-cell carcinoma. It is quite astonishing that none of the well-differentiated adenocarcinoma (n = 68, 47.2%) was PD-L1 positive. High-grade CRC is frequently mismatch repairs deficient (dMMR), and it is this particular subset of CRC classified as consensus molecular subtype 1 (CMS1) that has a higher frequency of expression of PD-L1 positivity.[16,17] Recent publications including an earlier study from our center on CRC among Nigerians have detected high levels of MMR deficiency ranging from 23 to 43%.[18,19] Some therapeutic clinical trials have documented the benefits of anti-PD-L1 therapy, such as pembrolizumab, in carefully selected CRC patients who had high PD 1/PD-L1 expression. Le *et al*. in an open phase II multicenter clinical trial of pembrolizumab treatment involving 124 patients with advanced CRC that are MSI-High/dMMR found durable clinical benefits with an objective response rate of 31.1% in cohort A and 38.1% in cohort B.[20] Also, a phase III clinical trial by André *et al*.[8] comparing pembrolizumab as a first-line treatment versus chemotherapy in 307 CRC patients with MSI-H-dMMR observed an overall response rate of 43.8% against 33.1% recorded for the chemotherapy group. Pembrolizumab led to significantly longer progression-free survival than chemotherapy when received as a first-line therapy.

Tumor location or sidedness has been subjected to scientific interrogation looking at the roles of tumor immunogenesis and disease outcomes and to what extent this should be considered in therapeutic decisions. This study has shown that there is higher PD-L1 expression in right-sided tumor compared to left-sided tumor location. Indeed, the left-sided PD-L1 positive tumors in this study were rectal (2/5), while the rest were PD-L1-negative (0/40). The observation of higher PD-L1 expression in right-sided tumor location has been documented in some studies together with mismatch repair deficiency when compared with left-sided tumors.[21,22]

The significance of this PD-L1 expression on survival outcome and disease-free survival in CRC has been conflicting. Takasu *et al*.[22] in a study of 112 stage II and III CRC cases reported that PD-L1 was highly expressed by right-sided tumors, but PD-L1 among other immune-related molecules was an independent prognostic factor for OS and DFS in left-sided CRC. Some publications have suggested that high PD-L1 expression in deficient MMR and right-sided colon cancer is reportedly associated with less aggressive tumor biology and better outcome.[21] Others have pointed out that PD-L1 expression in tumor cells are associated with aggressive tumor biology, although there is no impact on the outcome.[23]

The present study showed no association between the age of patients, stage of the tumor, lymph node metastasis, and PD-L1 expression. The meta-analysis by Yan Li *et al*.[24] evaluating the clinicopathological and prognostic significance of PD-L1 expression in CRC patients reported that no significant association was found between PD-L1 expression and age, and TNM stage. Although the TNM stage is a major consideration in the determination of prognosis, treatment, and survival outcome, the meta-analysis and some other studies showed that it has no association with PD-L1 expression.

Conclusion

The frequency of PD-L1 expression in Nigerian cohorts of CRC patients is low, but there are definite associations such as right-sided tumor location, high-grade tumor, and rectal tumor location with some overlap of cases that were previously identified as mismatch repairs deficient. A larger cohort of prospectively followed-up CRC cases where the immune checkpoint status of tumor cells including the surrounding stromal and associated tumor infiltrating lymphocytes are determined will provide a basis for immune-modulatory therapeutic options in addition to current treatment protocols.

Financial support and sponsorship

This research was supported by the Fogarty International Center of the National Institutes of Health under award number D43TW010134.

Conflicts of interest

There are no conflicts of interest.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, *et al*. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021;71:209-49.
2. Irabor DO. Colorectal carcinoma: Why is there a lower incidence in Nigerians when compared to Caucasians? J Cancer Epidemiol 2011;2011:675154.
3. Abdulkareem FB, Abudu EK, Awolola NA, Elesha SO, Rotimi O, Akinde OR, *et al*. Colorectal carcinoma in Lagos and Sagamu, Southwest Nigeria: A histopathological review. World J Gastroenterol 2008;14:6531-5.
4. Singh PP, Sharma PK, Krishnan G, Lockart AC. Immune checkpoints and immunotherapy for colorectal cancer. Gastroenterol Rep 2015;3:289-97.
5. Fumet JD, Isambert N, Hervieu A, Zanetta S, Guion JF, Hennequin A, *et al*. Phase Ib/II trial evaluating the safety, tolerability and immunological activity of durvalumab

(MEDI4736) (anti-PD-L1) plus tremelimumab (anti-CTLA-4) combined with FOLFOX in patients with metastatic colorectal cancer. ESMO Open 2018;3:e000375.

6. Chen DS, Irving BA, Hodi FS. Molecular pathways: Next-generation immunotherapy—inhibiting programmed death ligand 1 and programmed death-1. Clin Cancer Res 2012;18:6580–7.
7. Dunne PD, McArt DG, O'Reilly PG, Coleman HG, Allen WL, Loughrey M, *et al.* Immune-Derived PD-L1 gene expression defines a subgroup of stage II/III colorectal cancer patients with favorable prognosis who may be harmed by adjuvant chemotherapy. Cancer Immunol Res 2016;4:582-91.
8. André T, Shiu KK, Kim TW, Jensen BV, Jensen LH, Punt C, *et al*. Pembrolizumab in microsatellite-instability-high advanced colorectal cancer. N Engl J Med 2020;383:2207-18.
9. Rotimi O, Abdulkareem FB. Fifty-three years of reporting colorectal carcer in Nigerians – A systematic of the published literature. Niger Postgrad Med J 2014;21:68-73.
10. Ibrahim KO, Anjorin AS, Afolayan AE, Badmos KB. Morphology of colorectal carcinoma among Nigerians: A 30-year review. Niger J Clin Pract 2011;14:432-5.
11. Siegel RL, Jakubowski CD, Fedewa SA, Davis A, Azad NS. Colorectal cancer in the young: Epidemiology, prevention, management. Am Soc Clin Oncol Educ Book 2020;40:1-14.
12. Adebamowo CA, Adeyi O, Pyatt R, Prior TW, Chadwick RB, de la Chapelle A. Case report on hereditary non-polyposis colon cancer (HNPCC) in Nigeria. Afr J Med Med Sci 2000;29:71-3.
13. 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;29:1433-42.
14. Rosenbaum MW, Bledsoe JR, Morales-Oyarvide V, Huynh TG, Mino-Kenudson M. PD-L1 expression in colorectal cancer is associated with microsatellite instability, BRAF mutation, medullary morphology and cytotoxic tumor-infiltrating lymphocytes. Mod Pathol 2016;29:1104-12.
15. Inaguma S, Lasota J, Wang Z, Felisiak-Golabek A, Ikeda H, Miettinen M. Clinicopathologic profile, immunophenotype, and genotype of CD274 (PD-L1)-positive colorectal carcinomas. Mod Pathol 2017;30:278-85.
16. Guinney J, Dienstmann R, Wang X, de Reyniès A, Schlicker A, Soneson C, *et al*. The consensus molecular subtypes of colorectal cancer. Nat Med 2015;21:1350-6.
17. Abdulkareem FB, Khramtsova G, Odukoya LA, Badmos KB, Tunde A, Rotimi O. Consensus molecular subtyping of colorectal cancer by immunohistochemistry, an imperative for a resource limited setting: Report of a Nigerian study. Niger J Gastroenterol Hepatol 2021;13:33-9.
18. Badmos KB, Adebayo LA, Osinowo AO, Lawal AO, Habeebu MY, Rotimi O, *et al*. Mismatch repair protein expressions in cohort of colorectal carcinoma patients in Lagos. Niger J Clin Pract 2021;24:1294-9.
19. Alatise OI, Knapp GC, Sharma A, Chatila WK, Arowolo OA, Olasehinde O, *et al*. Molecular and phenotypic profiling of colorectal cancer patients in West Africa reveals biological insights. Nat Commun 2021;12:6821.
20. Le DT, Kim TW, Van Cutsem E, Geva R, Jäger D, Hara H, *et al*. Phase II open-label study of pembrolizumab in treatment-refractory, microsatellite instability-high/mismatch repair-deficient metastatic colorectal cancer: KEYNOTE-164. J Clin Oncol 2020;38:11-9.
21. Jung DH, Park HJ, Jang HH, Kim SH, Jung Y, Lee WS. Clinical Impact of PD-L1 expression for survival in curatively resected colon cancer. Cancer Invest 2020;38:406-14.
22. Takasu C, Nishi M, Yoshikawa K, Tokunaga T, Kashihara H, Yoshimoto T, *et al*. Impact of sidedness of colorectal cancer on tumor immunity. PLoS One 2020;15:e0240408.
23. Wyss J, Dislich B, Koelzer VH, Galván JA, Dawson H, Hädrich M, *et al*. Stromal PD-1/PD-L1 expression predicts outcome in colon cancer patients. Clin Colorectal Cancer 2019;18:e20-38.
24. Li Y, He M, Zhou Y, Yang C, Wei S, Bian X, *et al*. The prognostic and clinicopathological roles of PD-L1 expression in colorectal cancer: A systematic review and meta-analysis. Front Pharmacol 2019;10:139.