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Expression of PD-L1 clones (22C3 and 28-8) in hepatocellular carcinoma: a tertiary cancer care hospital experience

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Abstract

Background Hepatocellular carcinoma (HCC) is a highly aggressive and rapidly progressing form of cancer with a poor prognosis. Recent advances in the management of HCC focused on the novel immunotherapeutic modalities for patients with advanced disease. PD-L1 has emerged as a promising immunotherapeutic approach for HCC. The evaluation of PD-L1 expression aids in identifying patients who can derive maximum benefits from these therapies. This study aims to examine and compare the expression of PD-L1 using two clones (22C3 and 28-8) in HCC patients.

Methods Forty-six patients with HCC were selected between 2005 and 2022 from the Shaukat Khanum Memorial Cancer Hospital and Research Centre (SKMCH&RC) in Lahore, Pakistan. The patients' formalin-fixed paraffin-embedded (FFPE) tissue samples were retrieved from the department of pathology to conduct immunohistochemical analysis. Moreover, the clinicopathological data of these patients were gathered from the hospital information system (HIS). To assess the relationship between variables, bivariate analysis was carried out using either the chi-square test or Fisher exact test when necessary.

Results Among the 46 tissue specimens analyzed, the presence of clone 22C3 was detected in 20 HCC patients, with 10 patients showing high expression (21.7%) and another 10 patients showing low expression (21.7%). 22C3 expression was not observed in 26 patients (56.5%). On the other hand, clone 28-8 was expressed in 10 patients, all of whom exhibited low expression (21.7%), while no expression of clone 28-8 was observed in 36 patients (78.3%). An association was found between the expression of 22C3 and 28-8 PD-L1 clones (*p*-value 0.01). Furthermore, upon closer examination, it was revealed that 12 cases exhibited positive results for 22C3 but negative results for 28-8. Interestingly, two cases displayed positive results for 28-8 but negative results for 22C3.

Conclusion We obserevd that the PD-L1 clones, 22C3 and 28-8, are comparable. If PD-L1 expression using 22C3 is negative, considering the use of 28-8 for evaluating expression in HCC patients may be beneficial. However, further validation in a larger cohort is necessary.

Keywords Programmed cell death ligand-1, 22C3, 28-8, Biomarkers, Histopathology, Hepatocellular carcinoma

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Introduction

Hepatocellular carcinoma (HCC) is the commonest form of liver malignancies, constituting over 90% of the primary tumors of the liver [1]. It is a leading cause of cancer-associated morbidity and mortality across the globe [2]. It continues to pose a significant public health challenge, with projections indicating an estimated incidence of over 1 million deaths by 2030 [3]. Despite promising trials, systemic therapies for HCC remain limited, emphasizing an unmet clinical need [4]. However, recent advancements in cancer immunotherapy offer new therapeutic modalities for HCC [4]. One of the latest approaches in immunotherapy involves using monoclonal antibodies (mAbs) that target immune checkpoint molecules, such as programmed cell death ligand-1 (PD-L1) [5–7]. Several monoclonal therapies have been developed based on clone-specific tissue expression of PD-L1, determined through immunohistochemistry (IHC), to identify patients who are more likely to respond to immunotherapies [8].

The assessment of PD-L1 expression through IHC analysis plays a pivotal role in routine clinical practice to determine a patient's suitability for PD-1/PD-L1 immunotherapy [9]. Furthermore, quantitative evaluation of PD-L1 expression can be valuable for monitoring the response to this therapeutic approach [9]. Initial investigations utilizing IHC in formalin-fixed and paraffin-embedded (FFPE) tissue samples have demonstrated that PD-L1 expression in human cancers has the potential to serve as a predictive marker for clinical responses to PD-1/PD-L1 immunotherapy [10–13]. Notably, PD-L1 protein expression by IHC is a promising biomarker for PD-1/PD-L1blockade in HCC [14].

Four commercially available PD-L1 clones (22C3, 28-8, SP142, and SP263) are associated with specific IHC staining platforms and used as diagnostic tests for specific monoclonal antibody therapies [8]. However, all four PD-L1 assays are not offered by most of the pathology laboratories due to cost and availability of resources of each standardized assay. Furthermore, pathological diagnosis is not obligatory for the majority of HCC patients, particularly those in advanced stages [15, 16]. This is further compounded by the fact that these patients often have only a small biopsy as the available specimen, making it impractical and costly to conduct multiple PD-L1 tests [17]. Given the diversity of testing platforms, global initiatives are taken to "harmonize" PD-L1 testing in order to facilitate in making the clinical decision [18]. In a study by Pinato et al., it was observed that the 22C3 and 28-8 clones exhibited a higher prevalence of PD-L1 expression in HCC patients compared to SP142 and SP263 [19]. This finding underscores the significance of clone selection in accurately assessing PD-L1 expression levels in HCC patients. Therefore, in the current study, we examined and compared the expression of PD-L1 using two clones (22C3 and 28-8) on FFPE specimens from HCC patients through immunohistochemical analysis.

Material and methods

Case selection

Forty-six patients with HCC who had no previous history of viral hepatitis were selected between 2005 and 2022 from the Shaukat Khanum Memorial Cancer Hospital and Research Center (SKMCH&RC) in Lahore, Pakistan. It was a rare group of patients who tested negative for both HBV and HCV. The FFPE tissue samples of these patients were retrieved from the archives of the Department of Pathology for further analysis. The histopathologists reviewed and confirmed the tumor tissue block for each sample, with preference given to the most suitable block in cases where multiple tumor blocks were available. Comprehensive clinicopathological features of HCC patients were obtained from the hospital information system (HIS). The current study received approval from the Institutional Review Board (IRB) of SKMCH & RC, with a waiver granted for informed consent in compliance with the ethical principles outlined in the Declaration of Helsinki.

Table 1 PD-L1clones (22C3 and 28-8) expression in HCC patients

	PD-L1clones (22C3)			
		Negative 26 (56.5)	**Low 10 (21.7%)**	**High 10 (21.7%)**	***P*-value**
PD-L1clone (28-8)	Categories				0.01
	Negative 36 (78.3%)	24 (66.7)	7 (19.4)	5 (13.9)	
	Low 10 (21.7%)	2 (20.0)	3 (30.0)	5 (50.0)	

Table 2 Case-wise description of PD-L1clones (22C3 and 28-8) expression and clinicopathological features of HCC patients

Case	22C3	28-8	Alcohol status	Metastasis	Recurrence	Tumor size	BCLC Staging	Survival	Histological grade
01	+	-	-	-	-	3.5	A	UNK	Well differentiated
02	-	-	-	-	UNK	1.7	0	deceased	Well differentiated
03	-	-	-	+	UNK	7.5	B	deceased	Well differentiated
04	-	-	-	-	UNK	11	UNK	deceased	Well differentiated
05	-	-	-	-	UNK	UNK	UNK	UNK	Well differentiated
06	-	-	-	-	UNK	0.9	UNK	deceased	Well differentiated
07	-	-	-	-	UNK	5.9	B	deceased	Well differentiated
08	-	-	-	-	UNK	UNK	C	UNK	Moderately differentiated
09	-	-	+	-	-	2.9	A1	alive	Moderately differentiated
10	-	-	-	-	UNK	9.5	UNK	deceased	Well differentiated
11	+	+	-	-	UNK	6.5	B	UNK	Moderately differentiated
12	+	-	-	-	+	15.7	B	deceased	Poorly differentiated
13	-	-	-	+	UNK	12	C	deceased	Well differentiated
14	-	-	-	-	UNK	9.6	B	UNK	Well differentiated
15	+	-	-	-	+	11	B	deceased	Well differentiated
16	-	-	-	-	UNK	9.6	C	deceased	Well differentiated
17	-	+	-	-	-	5.6	C	UNK	Well differentiated
18	-	-	-	-	+	7.7	C	deceased	Well differentiated
19	-	-	-	-	-	8.8	C	deceased	Well differentiated
20	-	-	-	-	+	11	B	deceased	Well differentiated
21	+	-	-	-	-	3.8	A	UNK	Well differentiated
22	-	-	-	+	UNK	10	C	deceased	Moderately differentiated
23	+	+	-	+	UNK	7.3	C	deceased	Well differentiated
24	+	-	-	+	UNK	2.6	0	deceased	Moderately differentiated
25	-	-	-	+	UNK	9.5	C	deceased	Moderately differentiated
26	-	-	-	+	UNK	15	C	deceased	Well differentiated
27	-	-	+	-	UNK	14	B	UNK	Well differentiated
28	-	-	-	-	UNK	15.1	C	deceased	Moderately differentiated
29	+	+	-	-	+	5	UNK	UNK	Well differentiated
30	-	-	-	-	UNK	3	B	UNK	Poorly differentiated
31	+	-	-	+	UNK	7.9	C	deceased	Well differentiated
32	+	-	-	-	UNK	8	B	deceased	Well differentiated
33	-	-	-	-	+	1.9	B	deceased	Well differentiated
34	-	+	-	-	UNK	8.9	B	deceased	Poorly differentiated
35	+	+	-	-	UNK	11	B	deceased	Well differentiated
36	-	-	-	-	UNK	UNK	C	deceased	Well differentiated
37	-	-	-	-	+	3.3	C	alive	Well-differentiated
38	+	-	-	-	-	12	B	alive	Well-differentiated
39	+	-	-	-	UNK	6	B	deceased	Moderately differentiated
40	+	-	-	+	UNK	6.3	C	alive	Moderately differentiated
41	+	+	-	-	UNK	4	A	UNK	Moderately differentiated
42	+	+	UNK	-	UNK	4.8	UNK	UNK	Well-differentiated
43	+	+	-	-	+	2.5	C	alive	Poorly differentiated
44	+	-	-	-	+	3.9	B	alive	Moderately differentiated
45	+	-	-	-	-	14	A	alive	Moderately differentiated
46	+	+	-	-	-	14.5	B	alive	Moderately differentiated

Abbreviation: UNK Unknown

Immunohistochemistry

PD-L1 expression was assessed in two different clones (22C3 and 28-8) using IHC analysis. Tumor specimens from the same patients were sectioned at a thickness of 4 µm, resulting in two FFPE sections. These sections were subjected to IHC testing using specific PD-L1 antibodies (22C3; Cat# M3653) and (28-8; Cat# ab205921). Staining of the slides was performed using an autostainer Link 48 (Dako Denmark) following the manufacturer's protocol. Deparaffinization and antigen retrieval were carried out simultaneously using the target retrieval solution (#GV805 Dako). Visualization of PD-L1 labeling was achieved using the Envision Flex detection kit DAKO (K8000). Normal human tonsils were utilized as positive controls for both clones. The slides were examined under an optical microscope (Provis AX-70, Olympus, Melville, NY).

Scoring

The pathologists conducted a blind histopathological evaluation. In cases where discrepancies arose between the pathologists, they collaborated to reach a consensus, considering the mean score of both as the decisive score. The calculation of PD-L1 immunohistochemical expression followed the previously described method [20]. PD-L1 staining intensity was assessed as high (3), moderate (2), weak (1), or negative (0). The percentage of tumor cells with positive staining was categorized according to the following formula: PD-L1 expression score (H score) (range, 0–9)=0×% of non-stained tumor cells +1×% of weakly stained tumor cells +2×% of moderately stained tumor cells +3×% of strongly stained tumor cells.

Statistical analysis

Statistical analysis was performed by using SPSS software (version 20.0; SPSS, Chicago, IL, USA). Bivariate analysis was done using chi-square or Fisher exact test (where necessary). Statistical significance was defined as a two-tailed *P*-value of 0.05.

Results

PD-L1 clones (22C3 and 28-8) expression, histological grading and clinical staging of HCC patients

Forty-six patients diagnosed with HCC were selected from SKMCH&RC in Lahore, Pakistan. Immunohistochemical analysis revealed that clone 22C3 was detected in 20 patients. Among these patients, 10 showed high expression (21.7%), while the other 10 showed low expression (21.7%). However, clone 22C3 was not observed in 26 patients (56.5%) as shown in Table 1. On the other hand, clone 28-8 was expressed in 10 patients, all of whom exhibited low expression (21.7%). However, no expression of clone 28-8 was observed in 36 patients (78.3%) as shown in Table 1. These findings suggest that clone 28-8 may be less prevalent than clone 22C3 in HCC patients. Notably, an association was found between the expression of the two PD-L1 clones, 22C3 and 28-8, as indicated by a *p*-value of 0.01. These findings suggest a correlation or potential similarity in the performance or detection of PD-L1 expression by these two clones in HCC patients as demonstrated in Table 1. Upon closer examination, it was observed that 12 cases exhibited positive results for clone 22C3 but negative results for clone 28-8. In contrast, two cases displayed positive results for clone 28-8 but negative results for clone 22C3 as shown in Table 2. The discrepancy between clone 22C3 and clone 28-8 suggests that clone 22C3 may detect PD-L1-positive cases missed by clone 28-8, while clone 28-8 may identify PD-L1 expression in cases that go undetected by clone 22C3. Moreover, Table 2 summarizes detailed clinicopathological information, comprising survival outcomes, tumor size, BCLC staging, alcohol status, as well as details on metastasis and recurrence for the patients.

In this study, immunohistochemical analysis was conducted on FFPE tissues obtained from 46 patients diagnosed with HCC to evaluate the expression of PD-L1 clones (22C3 and 28-8). Our observations revealed that PD-L1 22C3 clone was not expressed in 26 patients, while PD-L1 28-8 clone was not expressed in 36 patients. Notably, an interesting finding emerged where the expression of PD-L1 22C3 clone was detected in the same section where the expression of PD-L1 28-8 clone was absent, as depicted in Fig. 1(A, B). Similarly, Fig. 1(C, D) demonstrated that PD-L1 28-8 clone expression was present in the same section where PD-L1 22C3 clone was absent, highlighting an association between the two clones. In addition to our findings, we observed that 8 patients exhibited positive expression for both PD-L1 clones, as illustrated in Fig. 1(E, F). Moreover, Fig. 1(G, H) depicts the positive controls for both PD-L1 clones.

Discussion

HCC is a complex and multifaceted disease characterized by diverse clinical, molecular, and genetic features [21, 22]. Recent advances in immunotherapy have significantly transformed the treatment landscape for HCC, offering new hope and improved outcomes for patients [22, 23]. Immunotherapy has shown promising results by enhancing survival rates and providing long-term cancer control in HCC patients, while also minimizing the side effects commonly associated with traditional treatments [22, 24]. Furthermore, the identification of molecular markers, such as PD-L1 expression, has provided valuable insights into the immunological aspects of HCC [25]. This understanding has opened up new avenues for targeted therapies and immunotherapies, as PD-L1 expression can serve as a potential target for personalized treatment approaches in HCC patients [26]. These advancements in immunotherapy and the utilization of

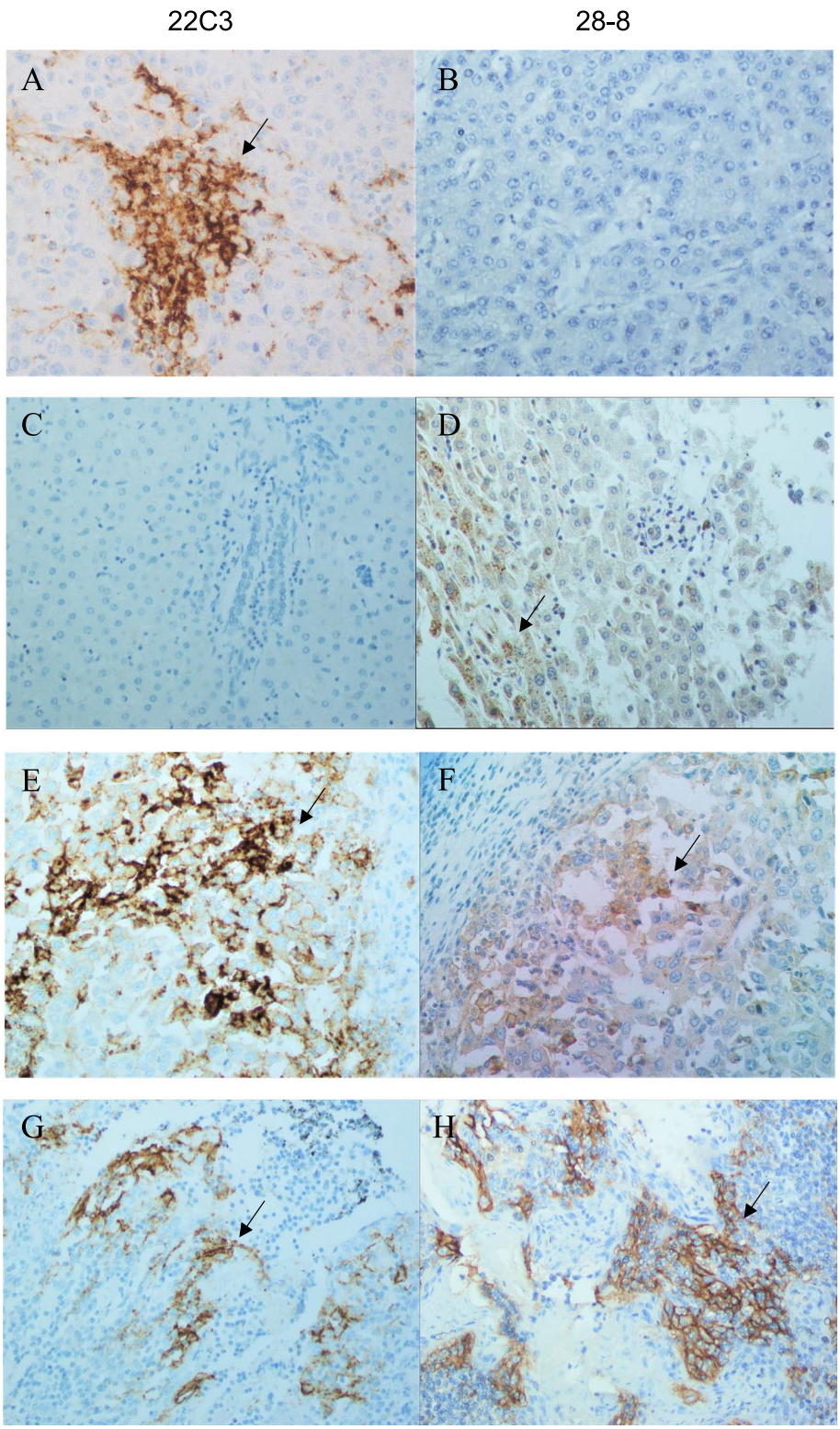

Fig. 1 Expression of PD-L1 clones (22C3 and 28-8) detected by immunohistochemical staining. Representative images of immunohistochemical staining of PD-L1 clones (22C3 and 28-8) in HCC cases. **A**: Positive strong membranous PD-L1clone 22C3 staining in tumor cells with high PD-L1 expression; **B**: Negative expression of PD-L1 clone 28-8; **C**: Negative expression of PD-L1 clone 22C3; **D**: Positive strong membranous PD-L1clone 28-8 staining in tumor cells; **E**, **F**: Positive expression of PD-L1 clones (22C3 and 28-8) in the same tissue specimen. **G**, **H** Positive controls (tonsils) for both clones. All images were captured at 40X magnification

molecular markers have the potential to revolutionize the management and prognosis of HCC, offering more effective and tailored treatment options for patients in the future [27].

The assessment of PD-L1 expression has become a common practice in clinical settings; however, it presents challenges due to the intratumoral heterogeneity observed in PD-L1 expression [28]. Four commercially available PD-L1 clones, namely 22C3, 28-8, SP142, and SP263, play an essential role in IHC staining for evaluating PD-L1 expression and guiding monoclonal antibody therapy selection [8]. Each clone has unique characteristics and has been validated for specific clinical contexts [8]. Clone 22C3 is associated with FDA-approved anti-PD-L1 therapy pembrolizumab, extensively studied and validated in clinical trials to predict response to pembrolizumab treatment [29]. In contrast, clone 28-8 is validated for use with other anti-PD-L1 therapies, aiding treatment decisions and patient stratification in clinical practice [30]. Additionally, clones SP142 and SP263 demonstrate utility in specific clinical contexts, with SP142 associated with atezolizumab therapy and SP263 associated with durvalumab therapy [30]. These selected and validated clones ensure accurate and reliable assessment of PD-L1 expression, thereby assisting in treatment decisions and disease management.

Pinato et al. observed a higher prevalence of PD-L1 expression in HCC patients using the 22C3 and 28-8 clones compared to SP142 and SP263 in their study [19]. In our data set, we examined and compared the expression of PD-L1 using two clones (22C3 and 28-8) through immunohistochemical analysis on FFPE tissues obtained from 46 patients diagnosed with HCC. Our results revealed the presence of clone 22C3 in HCC patients, showing variations in expression levels. While clone 28-8 was detected in these patients, predominantly exhibiting low expression. These findings indicate a potential disparity in the prevalence of clone 22C3 and clone 28-8 in HCC patients. Furthermore, a significant association was observed between the expression of PD-L1 clones 22C3 and 28-8. Interestingly, we identified discrepancies between the two clones, with cases positive for one clone but negative for the other, implying differential abilities to detect PD-L1-positive cases. Additionally, a subset of patients displayed positive expression for both PD-L1 clones. We identified the high prevalence of clone 22C3 in our data set and the expression of both clones showed concordance in PD-L1 scoring. Our results are in compliance with previously published data [14, 19, 31].

Despite the valuable insights gained, our study has limitations. The small sample size may affect the generalizability of our findings. However, this is the first investigation in our region. Another limitation stems from the retrospective nature of our data, which may introduce inherent biases and limitations associated with retrospective analyses. Additionally, our study focused on only two PD-L1 clones, 22C3 and 28-8. Further studies incorporating additional clones can provide a more comprehensive understanding of PD-L1 expression in HCC. Nevertheless, our study contributes valuable insights into the role of PD-L1 in HCC, laying the foundation for future research.

Conclusion

The study significantly contributes to our understanding of PD-L1 expression in HCC, with implications for patient stratification and therapeutic decision-making. The high prevalence of clone 22C3 and concordant expression of both clones provide important insights into PD-L1 landscape. Understanding PD-L1 expression patterns can improve treatment strategies. Further research is needed to explore clinical significance, investigate additional clones, and enhance knowledge in this field.

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Authors' contributions
All authors contributed equally to this work; Asghar K and Bashir S: Generated the hypotheses, designed experiments, data analysis and wrote the manuscript. Bashir S, Hameed M and Loya A: Evaluate the IHC slides and scoring, data analysis. Abu-bakar M, Hassan M and Farooq A: Statistical analysis performed, analyzed the data, and participated in the writing of manuscript. Bilal S, Mehmood S, Hassan M, Farooq A, and Asghar K were involved in acquisition of the data, analyzed the data, and participated in the writing of manuscript: Asghar K, Mehmood S, Hameed M and Loya A critically reviewed and participated in the manuscript.

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Availability of data and materials
The data generated in the present study may be requested from the corresponding author

Declarations

Ethics approval and consent to participate
The institutional review board (IRB) of SKMCH&RC approved the current retrospective study (#EXMPT-09-03-18-01). IRB granted the waiver for informed consent for this study, which is in accordance with the Declaration of Helsinki. The patient data accessed complied with relevant data protection and privacy regulations.

Competing interests
The authors report no conflicts of interest in this work.

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