REVIEW ARTICLE

AGSurg Annals of Gastroenterological Surgery — Open Access — WILEY

Programmed death ligand-1 expression in gastrointestinal cancer: Clinical significance and future challenges

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Funding information
Japan Society for the Promotion of Science, Grant/Award Number: 20K16308

Abstract

Cancer immunotherapy has caused a paradigm shift from conventional therapies that directly target cancer cells to innovative therapies that utilize the host immune system. In particular, programmed cell death-1 (PD-1)/programmed death ligand-1 (PD-L1) inhibitors have achieved an impressive breakthrough and been approved for clinical use in several types of cancer including gastrointestinal (GI) cancer. To identify and develop predictive biomarkers for PD-1 inhibitors is of great concern in clinical practice. Although PD-L1 expression is considered a logical biomarker as PD-L1 is a substantial target of the immune checkpoint inhibitors, its clinical significance in GI cancer remains unclear. In this review, we summarize the current evidence for PD-L1 expression as a prognostic and predictive biomarker for PD-1/PD-L1 inhibitors in GI cancer from recent publications, and emerging evidence from recent key clinical trials on the efficacy of PD-1/PD-L1 inhibitors. Challenging clinical issues for PD-L1 assessment are then discussed from the viewpoint of the methodology for PD-L1 evaluation including the differences in PD-L1 detection assays and evaluation criteria for PD-L1 positivity. Moreover, we highlight the biological features of PD-L1 expression in terms of tumor spatial and temporal heterogeneity, which suggests important implications for biomarker analysis. Finally, we describe future perspectives using liquid biopsy for better assessment of PD-L1 status. This new information should improve our understanding of the clinical significance of PD-L1 in GI cancer, leading to optimal patient selection and treatment strategy for the clinical use of PD-1/PD-L1 inhibitors in patients with GI cancer.

KEYWORDS
gastrointestinal cancer, heterogeneity, PD-L1, predictive biomarker

1 | INTRODUCTION

The concept of cancer immunity has given rise to new insights into oncology.[1] Notably, cancer immunotherapy has caused a paradigm shift from conventional therapies that directly target cancer cells to innovative therapies that utilize the host immune system.[2] Immune checkpoint inhibitors (ICIs), which target inhibitory receptors on immune effector cells and reactivate the immune response, have been highlighted over the past several years.[3] The programmed cell death-1 (PD-1)/programmed death ligand-1 (PD-L1) axis has been attracting particular interest as a promising target for ICIs since it was first described in 1992.[4] Subsequently, blockade of the PD-1/PD-L1

axis has demonstrated favorable antitumor effects and achieved an impressive breakthrough in cancer immune therapy for several types of cancer including melanoma, non-small cell lung cancer (NSCLC), and gastrointestinal (GI) cancer.[5–8]

To identify and develop predictive markers for ICIs is of great concern in clinical practice. To date, several predictive markers for PD-1/PD-L1 inhibitors, such as tumor mutation burden (TMB) and mismatch repair deficiency (dMMR)/microsatellite instability (MSI), have been reported.[9–11] Above all, PD-L1 expression is considered a logical biomarker because PD-L1 is a substantial target of the ICIs. An initial phase I study on the use of nivolumab, one of the PD-1 inhibitors, supported a potential role for assessment of PD-L1 expression on tumor cells in patients with several types of solid tumor including melanoma, NSCLC, renal cell carcinoma, and colorectal cancer (CRC).[12] However, in contrast to the clinical use of PD-L1 assessment in patients with melanoma and NSCLC,[13–15] the clinical significance of PD-L1 expression in GI cancer remains unclear from contradictory outcomes in multiple studies on the correlation between PD-L1 expression and the ICI response or prognosis.

In this review, we focus on PD-L1 expression in GI cancer and summarize its clinical significance as a prognostic biomarker and as a predictive biomarker for PD-1 inhibitors. In addition, we discuss challenging issues for PD-L1 assessment from the viewpoint of methodology for PD-L1 evaluation, and biological features of PD-L1 expression that display spatial and temporal heterogeneity, with future perspectives using liquid biopsy for better assessment of PD-L1 status. This new information should improve our understanding of the clinical significance of PD-L1 in GI cancer, leading to optimal patient selection and treatment strategy for the clinical use of PD-1/PD-L1 inhibitors in patients with GI cancer.

2 | CLINICAL SIGNIFICANCE OF PD-L1 EXPRESSION IN GI CANCER

2.1 | Prognostic significance of PD-L1 expression in GI cancer

Multiple studies on the relationship between PD-L1 expression and patient survival in GI cancer have been reported. Recent studies involving large numbers of patients are summarized in Table 1.[16–29] Although the differences in patient background and assessment methods of PD-L1 expression were major limitations, the prognostic significance of PD-L1 expression in GI cancer was highly heterogeneous in each study. Most of the studies demonstrated that PD-L1 positivity is a poor prognostic biomarker as PD-L1 contributes to immune evasion. However, other recent studies did not confirm this; indeed, several studies indicated that PD-L1 positivity is a better prognostic factor. The latter studies discussed that PD-L1 expression does not necessarily represent an immunosuppressive state in the tumor microenvironment, but rather acts as a surrogate marker of immune activation because PD-L1 is upregulated by some

inflammatory cytokines such as interferon-γ, which is secreted from activated immune effector cells.[30] In fact, high PD-L1 expression was associated with a high density of tumor-infiltrating lymphocytes (TILs), which is regarded as the preferred state of the host immune response.[24] In addition, some specific situations such as Epstein–Barr virus infection and dMMR in GI cancer are also reported to be associated with high PD-L1 status.[31,32] Although these molecular subtypes and genetic profiles themselves have been reported to be promising predictive biomarkers for PD-1 inhibitors,[33,34] prognostic prediction for such cases by assessment of PD-L1 alone apparently remains difficult given the contradictory results from several studies.[35-37]

Taken together, the prognostic significance of PD-L1 expression in GI cancer is still unknown. It may be challenging to consider PD-L1 expression as the result of preferable host immune response or as a predisposition to cause immune evasion. Therefore, comprehensive analysis with other immune markers and immune profiles will be required to better assess the role of PD-L1 expression as a prognostic factor.

2.2 | Predictive role of PD-L1 expression for PD-1 inhibitors in GI cancer

To date, PD-1/PD-L1 inhibitors, such as nivolumab and pembrolizumab, for clinical use in GI cancer have been approved based on the results of several important clinical trials. The recent key trials on the efficacy of the PD-1/PD-L1 inhibitors in GI cancer are summarized in Table 2.[38–49] Here, we introduce the current clinical evidence and the significance of PD-L1 expression as a predictive biomarker for the ICIs in each clinical trial. Several clinical trials on the efficacy of combination therapy of ICIs and PD-L1 inhibitors are ongoing and are not discussed in detail here.

2.2.1 | Esophageal cancer

First, the two single-arm trials ATTRACTION-01 and KEYNOTE-028 were reported for esophageal cancer (EC). ATTRACTION-01 was a phase II trial on the efficacy of nivolumab and showed 17% objective response rate (ORR) and 10.8 months for median overall survival (OS) for EC without PD-L1 assessment.[41] In contrast, patients with PD-L1-positive advanced solid tumors including EC were eligible in the phase Ib KEYNOTE-028 trial, in which pembrolizumab showed 30% ORR and 7.0 months for median OS.[38] Subsequently, phase II and III trials for the efficacy of nivolumab and pembrolizumab, respectively, were conducted. KEYNOTE-180 was a phase II trial of the efficacy of pembrolizumab for patients with advanced or recurrent EC with two or more prior treatments. In this trial, PD-L1 expression was evaluated by the combined positive score (CPS), which scores PD-L1 expression on tumor cells and immune cells, with a cut-off value of 10. The ORR was 13.8%

TABLE 1 Recent studies on the relationship between PD-L1 expression and patient survival in GI cancer

Authors	Year	Journal	N	Ab Clone	Cut-off value	PD-L1 Positive rate	Prognostic outcome
Esophageal cancer							
Tanaka K et al[16]	2016	Cancer Sci	180	27A2	NA[a]	29.4%	Worse OS
Kim R et al[17]	2017	World J Gastroenterol	200	E1L3N	TCs > 10%	33.5%	No impact
Zhang W et al[18]	2017	Cancer Sci	344	SP142	TCs, ICs > 5%	TCs 14.5%, ICs 24.7%	Better OS and DFS (ICs)
Kollmann D et al[19]	2018	Oncoimmunology	168	E1L3N	TCs, TILs > 1%	TCs 43.5%, TILs 69%	Better OS and DFS (TCs, TILs)
Yagi T et al[20]	2019	Ann Surg	305	E1L3N	TCs > 25%[a]	17.4%	Worse OS and DFS
Gastric cancer							
Eto S et al[21]	2016	Gastric Cancer	105	EPR1161-2	TCs > 50%	24.8%	Worse OS (NS)
Kim JW et al[22]	2016	Gastric Cancer	243	NA	TCs > 10%[a]	TCs 43.6%	Better OS and DFS
Dai C et al[23]	2016	Mol Oncol	444	MKP1A07310	TCs > 5%[a]	TCs 14.1%	Better OS (NS)
Kawazoe A et al[24]	2017	Gastric Cancer	487	SP142	TCs, ICs > 1%	TCs 12%, ICs 44%	No impact
Wang L et al[25]	2018	Cancer Med	550	28-8	TCs, ICs > 1%	TCs 17.3%, ICs 34.5%	No impact
Yamashita K et al[26]	2020	Gastric Cancer	191	E1L3N	TPS, CPS > 1	TPS 20.4%, CPS 71.7%	Worse OS and DFS (CPS)
Colorectal cancer							
Lee LH et al[27]	2016	Mod Pathol	394	E1L3N	NA[a]	5%	No impact
Koganemaru S et al[28]	2017	Cancer Sci	235	SP142	TCs, ICs > 5%	TCs 8.1%, ICs 15.3%	Worse DFS (TCs), better DFS (ICs)
Huang CY et al[29]	2018	Sci Rep	867	28-8	TCs > 5%	44%	Better DFS

Note: Abbreviations: CPS, Combined positive score; DFS, Disease-free survival; ICs, Immune cells; NA, Not available; NS, Not statistically significant; OS, Overall survival; TCs, Tumor cells; TILs, tumor-infiltrating lymphocytes; TPS, Tumor proportion score.

[a]PD-L1 positivity is defined by a combination of stained area and staining intensity in each study.

among patients with PD-L1+ tumors and 6.3% among patients with PD-L1− tumors.[39] Finally, the phase III KEYNOTE-181 trial demonstrated that pembrolizumab significantly improved OS compared with chemotherapy in patients with advanced EC with PD-L1 CPS ≥ 10 (median, 9.3 months vs 6.7 months; HR, 0.69; 95% CI, 0.52-0.93; *P* = .0074), while median OS was 7.1 months for both treatment groups in the intention to treat group (HR, 0.89; 95% CI, 0.75-1.05; *P* = .0560).[40] These results support the predictive significance of PD-L1 expression for pembrolizumab, and the US Food and Drug Administration (FDA) has approved pembrolizumab for patients with recurrent, locally advanced or metastatic EC with PD-L1 of CPS ≥ 10 as 2 or more therapy line. On the other hand, the ATTRACTION-3 trial, which was a phase III trial of the efficacy of nivolumab, demonstrated that nivolumab significantly improved OS compared with chemotherapy (median, 10.9 months for nivolumab vs 8.4 months for chemotherapy; HR, 0.77; 95% CI 0.62-0.96;

P = .019).[42] However, in this trial, the survival benefit occurred regardless of PD-L1 expression on the tumor with several cut-off values of 1, 5, and 10, although patients with PD-L1 ≥ 1%, tumor cells had a 15% greater reduction in the risk of death than those with PD-L1 < 1%. Thus, nivolumab has just been approved in Japan for clinical use for all-comer populations of advanced unresectable EC who received prior treatment.

2.2.2 | Gastric and GEJ cancer

For gastric cancer (GC) and gastroesophageal junction (GEJ) cancer, the initial phase Ib trial KEYNOTE-012 showed 22% ORR in patient with PD-L1 + advanced GC and triggered the initiation of further trials.[43] In this trial, there was no association between the response to pembrolizumab and higher PD-L1 expression on tumor cells, while a weak association between high PD-L1 + mononuclear

TABLE 2 Recent key trials on the efficacy of PD-1/PD-L1 inhibitors in GI cancer

Trial	Agent	N	Patients (treatment line)	Study design	Remarks
Esophageal cancer (EC)					
KEYNOTE-028[38] (P-Ib)	Pem	23	ESCC, EAC, GEJ AC (≥2)	NA	ORR 30%, median OS 7.0 mo
KEYNOTE-180[39] (P-II)	Pem	121	EC (≥3)	NA	ORR 13.8% (PD-L1+), 6.3% (PD-L1–)
KEYNOTE-181[40] (P-III)	Pem	628	ESCC, EAC (≥2)	Pem vs. CT (PTX or DTX or IRI)	PD-L1+, improved OS; ITT, no significance
ATTRACTION-1[41] (P-II)	Niv	64	ESCC (≥2)	NA	ORR 17%, median OS 10.8 mo
ATTRACTION-3[42] (P-III)	Niv	419	ESCC (≥2)	Niv vs. CT (PTX or DTX)	Niv improved OS regardless of PD-L1 status
Gastric and GEJ cancer (GC and GEJ C)					
KEYNOTE-012[43] (P-Ib)	Pem	39	GAC and GEJ C (NA)	NA	ORR 22%
KEYNOTE-059[44] (P-II)	Pem	259	GC and GEJ C (≥2)	NA	ORR 15.5% (PD-L1+), 6.4% (PD-L1–)
KEYNOTE-061[45] (P-III)	Pem	592	GC and GEJ C (≥2)	Pem vs. PTX	Pem did not significantly improve OS (≥CPS 1)
Pem showed better OS (≥CPS 10)					
ATTRACTION-2[46] (P-III)	Niv	493	GC and GEJ C (≥3)	Niv vs. placebo	Niv improved OS regardless of PD-L1 status
(dMMR/MSI-H) Colorectal cancer (CRC)					
KEYNOTE-016[47] (P-II)	Pem	41	dMMR or pMMR CRC (NA)	NA	ORR 40%; PD-L1, not associated with PFS or OS
KEYNOTE-164[48] (P-II)	Pem	128	dMMR/MSI-H CRC (≥)	NA	ORR 33%
CheckMate 142[49] (P-II)	Niv	74	dMMR/MSI-H CRC (≥2)	NA	ORR 29% (PD-L1+), 28% (PD-L1–)

Abbreviations: CPS, combined positive score; dMMR, mismatch repair deficient; DTX, docetaxel; EAC, esophageal adenocarcinoma; ESCC, esophageal squamous cell carcinoma; GAC, gastric adenocarcinoma; IRI, irinotecan; ITT, intention to treat; MSI-H, microsatellite instability-high; NA, not available; Niv, nivolumab; ORR, objective response rate; OS, overall survival; Pem, pembrolizumab; pMMR, mismatch repair procifient; PTX, paclitaxel.

inflammatory cell densities and the response was observed. In the following phase II trial KEYNOTE-059, the ORR of the pembrolizumab group was 11.6% in all enrolled patients, and durable responses were observed in patients with PD-L1 + and PD-L1– tumors with CPS ≥ 1 in subgroup analysis (PD-L1 + ORR, 15.5% vs PD-L1– ORR, 6.4%).[44] Based on this trial, the FDA granted accelerated approval to pembrolizumab for patients with recurrent locally advanced or metastatic GC or GEJ adenocarcinoma with PD-L1 CPS ≥ 1. Subsequently, the phase III KEYNOTE-061 trial, which was a randomized controlled trial of pembrolizumab versus paclitaxel, was conducted.[45] However, this trial failed to demonstrate a survival benefit for pembrolizumab compared with paclitaxel as second-line therapy for patients with PD-L1 CPS ≥ 1 (median OS, 9.1 months for pembrolizumab vs 8.4 months for paclitaxel; HR, 0.82; 95% CI 0.66-1.03; one-sided P = .0421). Notably, post-hoc subgroup analyses suggested that the treatment effect of pembrolizumab was greater for patients with a PD-L1 CPS ≥ 10 (median OS, 10.4 months for pembrolizumab vs 8.0 months for paclitaxel; HR, 0.64, 95% CI 0.41-1.02). For nivolumab, the phase III ATTRACTION-02 trial was conducted.[46] This was a randomized, placebo-controlled trial in an Asian population with GC or GEJ cancer who received two or more prior therapies. This trial demonstrated better OS in the nivolumab group than in the placebo group (median OS, 5.26 months for nivolumab vs 4.14 months for placebo; HR, 0.63, 95% CI 0.51-0.78, P < .0001). However, the survival benefit was shown regardless of PD-L1 positivity, defined as staining in 1% or more of tumor cells (PD-L1+: median OS, 5.22 months for nivolumab vs 3.83 months for placebo, HR 0.51; PD-L1–: median OS, 6.05 months for nivolumab vs 4.19 months for placebo, HR 0.72). Therefore, nivolumab was first approved in Japan for clinical use of ICIs for all-comer populations of advanced GC and EGJ cancer who received at least two prior treatments. However, it should be noted that only about 40% of the samples were available for evaluation of PD-L1 expression in this trial. Therefore, the predictive significance of PD-L1 expression for nivolumab is unclear and further analysis is needed.

2.2.3 | Colorectal cancer (dMMR/MSI-H colorectal cancer)

Although ICIs had been expected to be less effective for CRC, the KEYNOTE-016 trial first demonstrated the effectiveness of pembrolizumab for patients with metastatic dMMR CRC.[47] In this phase II trial, PD-L1 expression was observed only in patients with dMMR cancer, and PD-L1 expression was not significantly associated with patient survival. The subsequent phase II KEYNOTE-164 trial demonstrated the efficacy of pembrolizumab for patients with MSI-H/dMMR CRC (the ORR 33%).[48] Unfortunately, this trial lacked any biomarker analysis, including PD-L1 expression, due to limited tissue samples. Accordingly, the FDA first granted tissue/site-agnostic approval to pembrolizumab for patients with unresectable or metastatic MSI-H/dMMR solid tumors based on the results of the five trials (KEYNOTE-016, KEYNOTE-164, KEYNOTE-012, KEYNOTE-028, and KEYNOTE-158[50]). However, the predictive value of PD-L1 expression for pembrolizumab in MSI-H/dMMR solid tumors remains unknown, and further analysis is needed. On the other hand, the efficacy of nivolumab in patients with metastatic MSI-H/dMMR CRC was reported in the CheckMate 142 trial.[49] In this trial, nivolumab monotherapy demonstrated durable response and disease control in pretreated patients with dMMR/MSI-H metastatic CRC regardless of PD-L1 expression (ORR, 29% for PD-L1 ≥ 1% vs 28% for PD-L1 < 1). Thus, the predictive significance of PD-L1 expression for nivolumab, as well as for pembrolizumab, in dMMR/MSI-H metastatic CRC is unestablished.

3 | CLINICAL ISSUES FOR THE ASSESSMENT OF PD-L1 EXPRESSION

For the assessment of PD-L1 expression in clinical practice, immunohistochemistry staining (IHC) assays for PD-L1 are usually performed using pretreatment tissue samples obtained by biopsy. However, several studies have pointed out that some clinical issues can affect the accurate assessment of PD-L1 status in individuals. These issues are potentially associated with the significance of PD-L1 expression as a prognostic or a predictive biomarker for PD-1/PD-L1 inhibitors. We discuss the clinical issues concerning PD-L1 evaluation in terms of their technical and biological aspects.

3.1 | Evaluation methods for PD-L1 expression

3.1.1 | PD-L1 IHC detecting assay

Various monoclonal primary antibodies against PD-L1 with laboratory-developed or standardized assays have been used in experimental studies and clinical trials (Table 3). Several studies noted that differences in primary antibodies and detection assay for PD-L1 could affect the degree of tissue staining in NSCLC, melanoma, and GI cancer.[51-53] Accordingly, standardized PD-L1 IHC detecting assays for clinical use have been developed and approved by the FDA as a companion diagnostic for certain PD-1/PD-L1 inhibitors.[54] In GI cancer, the PD-L1 IHC 22C3 pharmDx assay was approved as a companion diagnostic for pembrolizumab in advanced EC (based on

TABLE 3 PD-L1 IHC assay in recent key trials of the efficacy of PD-1/PD-L1 inhibitors in GI cancer

Trial	Agent	N	PD-L1 IHC assay	Cut-off value	PD-L1 positivity
Esophageal cancer					
KEYNOTE-028[38] (P-Ib)	Pem	23	22C3 laboratory-developed testing	≥ 1% scorable cells	All patients
KEYNOTE-180[39] (P-II)	Pem	121	22C3 pharmDx assay	≥ CPS 10	47.9%
KEYNOTE-181[40] (P-III)	Pem	628	22C3 pharmDx assay	≥ CPS 10	35.4%
ATTRACTION-1[41] (P-II)	Niv	64	NA	NA	NA
ATTRACTION-3[42] (P-III)	Niv	419	28-8 pharmDx assay	≥ 1% TCs	48.4%
Gastric and GEJ cancer					
KEYNOTE-012[43] (P-Ib)	Pem	39	22C3 pharmDx assay	≥ 1% scorable cells	All patients
KEYNOTE-059[44] (P-II)	Pem	259	22C3 pharmDx assay	≥ CPS 1	57.1%
KEYNOTE-061[45] (P-III)	Pem	592	22C3 pharmDx assay	≥ CPS 1	66.7%
ATTRACTION-2[46] (P-III)	Niv	493	28-8 pharmDx assay	≥ 1% TCs	13.5%
(dMMR/MSI-H) Colorectal cancer					
KEYNOTE-016[47] (P-II)	Pem	41	NA	NA	NA
KEYNOTE-164[48] (P-II)	Pem	128	NA	NA	NA
CheckMate 142[49] (P-II)	Niv	74	28-8 pharmDx assay	≥ 1% TCs	30.9%

Abbreviations: CPS, combined positive score; dMMR, mismatch repair deficient; NA, not available; Niv, nivolumab; Pem, pembrolizumab; pMMR, mismatch repair proficient; TCs, tumor cells.

the KEYNOTE-180 and KEYNOTE-181 trials) and in advanced GC and GEJ cancer (based on the KEYNOTE-059 trial). On the other hand, the 28-8 pharmDx assay was used for PD-L1 evaluation in clinical trials of nivolumab (ATTRACTION-2, ATTRACTION-3). Although some reports directly compared stainability between the two assays using the same tissue samples of NSCLC,[55] few studies made such a comparison using the same samples of GI cancer. Further comparative studies will be required for accurate diagnosis of PD-L1 expression.

3.1.2 | Assessment methods for PD-L1 expression

Classically, the assessment methods for PD-L1 expression varied in each study. Some studies used their own criteria based on a combination of stained area and staining intensity, while others evaluated the percentage of stained cells with several cut-off values. These distinct criteria should be associated with a wide range of PD-L1 positivity even in the same cancer type. Thus, two scoring systems have been adopted in clinical trials: the percentage of stained tumor cells, which is substantially the same as the tumor proportion score (TPS) used in NSCLC, and CPS, which assesses all PD-L1+ cells including tumor cells, lymphocytes, and macrophages (Table 3). Although it is not established which of these is better for the assessment of PD-L1 expression, several studies have indicated the usefulness of CPS.[56] We recently demonstrated the utility of CPS as a prognostic biomarker in GC.[26] Interestingly, Herbst et al reported that the PD-1/PD-L1 pathway was inhibited, especially when PD-L1 was expressed by tumor-infiltrating immune cells.[57] From these considerations and the results of recent clinical trials, CPS may become the standard method for PD-L1 evaluation as a prognostic and predictive biomarker.

Elucidation of the optimal cut-off value is another concern. A meta-analysis in solid tumors demonstrated a positive dose-response relationship between PD-L1 positivity (with cut-off value of 1, 5, and 10) and survival benefit.[58] As with NSCLC, in which TPS with cut-off value of 50% is adopted for clinical use of PD-1 inhibitors, future studies will determine optimal organ-specific cut-off values of PD-L1 expression in GI cancer.

3.2 | Tumor heterogeneity of PD-L1 expression

Tumor heterogeneity is one of the most crucial hallmarks of cancer.[59] As a result of this heterogeneity, the bulk tumor may attain a diversity of distinct molecular features with differential levels of sensitivity to treatment.[60] Moreover, tumor heterogeneity often poses substantial issues in biomarker analysis, and thus a better understanding of this issue should have important implications for clinical practice.

3.2.1 | Spatial heterogeneity of PD-L1 expression

Spatial heterogeneity is described as the non-uniform distribution of diverse tumor subpopulations within a single disease site or across different disease sites (Figure 1). For PD-L1 expression, several publications have demonstrated spatial heterogeneity within a primary site in GI cancer.[61,62] This spatial heterogeneity raises the important clinical question of whether a standard biopsy from the primary tumor site can reflect the PD-L1 expression of the whole tumor bulk in a patient. Indeed, we recently reported that the low concordance rate of PD-L1 expression between biopsy and resected samples from the same GC cases and single biopsy was associated with such a discordance.[63] Moreover, Van den Eynde et al also demonstrated the heterogeneous immune diversity between primary and metastatic CRC and the inaccuracy of PD-L1 evaluation by single biopsy.[64] Importantly, the spatial heterogeneity of PD-L1 expression between primary and metastatic tumor is also reported in NSCLC and breast cancer,[65,66] in which evaluation of PD-L1 expression is required for clinical use of PD-1 inhibitors. Therefore, such spatial heterogeneity should be considered in the treatments with PD-1 inhibitors for GI cancer as well. Although multiple biopsies may cover
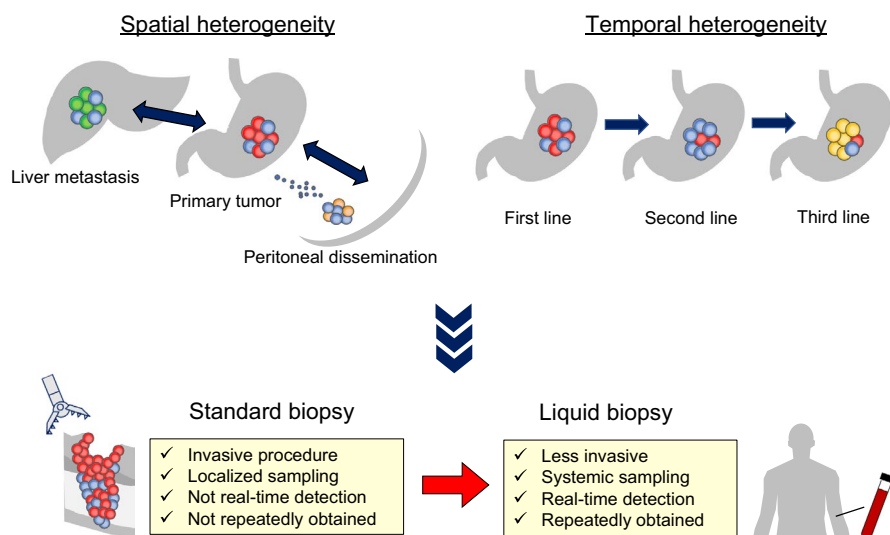

Spatial heterogeneity

Liver metastasis
Primary tumor
Peritoneal dissemination

Temporal heterogeneity

First line
Second line
Third line

Standard biopsy
- ✓ Invasive procedure
- ✓ Localized sampling
- ✓ Not real-time detection
- ✓ Not repeatedly obtained

Liquid biopsy
- ✓ Less invasive
- ✓ Systemic sampling
- ✓ Real-time detection
- ✓ Repeatedly obtained

FIGURE 1 Spatial and temporal heterogeneity of PD-L1 expression. Liquid biopsy is a promising option to solve several clinical issues associated with tumor heterogeneity

the heterogeneity, the optimal number of biopsies and the need for biopsies for metastatic lesions are pivotal issues to be considered.

3.2.2 | Temporal heterogeneity of PD-L1 expression

Temporal heterogeneity is described as the dynamic variation in the genetic diversity during the course of disease. In addition to genetic and phenotypic alterations in the process of tumor progression, the effect of cancer therapies on PD-L1 expression should be considered (Figure 1). Several reports have demonstrated that some cytotoxic agents including fluorouracil, paclitaxel, and radiation therapy can upregulate PD-L1 expression via cell signaling pathways in GI cancer.[67-69] Notably, Yang et al reported that GC patients with a preferable response to chemotherapy displayed PD-L1 downregulation and showed better RFS, whereas pretreatment PD-L1 status was not associated with survival.[70] Ogura et al also demonstrated equivalent results in patients with rectal cancer who received CRT.[71] These impressive results suggest the importance of temporal assessment of PD-L1 expression during the course of treatment. Nevertheless, previous clinical trials on the efficacy of PD-1/PD-L1 inhibitors for patients who received prior therapies used pretreatment samples for PD-L1 assessment. Therefore, a re-biopsy strategy should be established to account for the current PD-L1 status after treatment, leading to a better appreciation of the true significance of PD-L1 expression as a biomarker.

4 | FUTURE PERSPECTIVES

To overcome the clinical issues described above for the assessment of PD-L1 expression, several strategies have been considered, including the development of companion diagnostics, as well as multiple and repeated biopsies from both primary and metastatic tumors. However, this may not be feasible in clinical practice, as tissue biopsies are limited to very few sampling points and accessible metastatic sites. Given this situation, liquid biopsy (LB) is a promising option to solve these problems because LB is less invasive, more systemic, and can be obtained at multiple time points during the treatment course (Figure 1).[72] LB includes a variety of analytes such as circulating tumor DNA, cell-free RNA, and circulating tumor cells (CTCs). Among them, PD-L1 expression on CTCs can be a potential predictive biomarker for PD-1 inhibitors. In fact, several studies have demonstrated that PD-L1 status in CTCs correlated with PD-L1 status in tumor tissue and helps to predict the therapeutic effect of PD-1 inhibitors in NSCLC and melanoma.[73,74] For GI cancer, Yue et al reported that the abundance of PD-L1[high] CTCs was a predictive biomarker of PD-1/PD-L1 inhibitors and the dynamic changes of PD-L1[high] CTCs correlated with disease outcomes.[75] In addition, assessment of systemic PD-L1 status using other analytes as LB may be applicable. Chen et al reported the utility of exosomal PD-L1 on extracellular vesicles as a predictive biomarker for PD-1 inhibitors in melanoma.[76] Moreover, soluble

PD-L1 is also reportedly an available analyte for survival analysis in GI cancer.[77-79] Interestingly, a functional analysis described by Takeuch et al demonstrated glycosylation of soluble PD-L1 plays an important role in its binding to PD-1 receptor.[80] Such functional analysis may be help for biomarker analysis of PD-L1 using LB. Although LB has some limitations such as low detection rate and unestablished standard protocols for clinical use,[81] further studies will reveal clinical utilities of LB in assessment of PD-L1 expression in GI cancer.

5 | CONCLUSIONS

We reviewed the current evidence for PD-L1 expression as a prognostic and predictive biomarker for PD-1/PD-L1 inhibitors. Although PD-L1 is a promising biomarker, some associated clinical issues remain to be addressed. Accurate assessment of PD-L1 expression will reveal its true clinical significance and lead to the establishment of more effective strategies for the clinical use of ICIs.

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