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Review

The clinical impact of precise assessment of predictive biomarkers in gastroesophageal cancer: focus on the PD-L1 combined positive score (CPS) and tumor area positivity (TAP) systems

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Summary
Accurate assessment of PD-L1 expression is crucial for therapeutic decision-making in esophageal, esophago-gastric junction, and gastric cancers, where immune checkpoint inhibitors have become integral to first-line treatment in selected patients. This review provides an updated, practice-oriented summary on PD-L1 immunohistochemistry evaluation, with emphasis on the emerging Tumor Area Positivity (TAP) scoring system together with established Combined Positive Score (CPS) and Tumor Proportion Score. First, we examine the clinical relevance and use in clinical trials of each scoring method, and the pre-analytical and analytical variables influencing PD-L1 interpretation. Then, we address advantages and disadvantages of each scoring system, including a thorough analysis and pictorial interpretation guide of the recently introduced TAP score. Indeed, thanks to a visual-estimation-based assessment of PD-L1 expression, TAP has improved reproducibility and reduced scoring time, but large-scale validation is ongoing and certain interpretive challenges remain. Finally, we propose a standardized reporting template to enhance consistency in diagnostic practice, together with our perspective on future improvements and challenges of PD-L1 assessment.

Key words: gastro-esophageal cancer, immune-checkpoint inhibitors; clinical trials; digital pathology; artificial intelligence.

Introduction

PD-L1 assessment in gastroesophageal cancer: what's new? Does it matter? Reasons behind the need for a diagnostic practice update

The increasing involvement of pathologists in clinical trials, multidisci-

plinary discussions, and therapeutic management has demonstrated evident benefits on diagnostic accuracy, treatment personalization, and overall patient outcomes across several oncologic domains [1-3]. This evolving role, however, requires pathologists to maintain an up-to-date and in-depth expertise in emerging biomarkers, their associated scoring systems, and relevant therapeutic targets. In this setting, the histopathologic assessment of CD274 (also known as Programmed Death Ligand-1 – PD-L1) has been recently updated for locally advanced and metastatic esophageal, esophago-gastric junction, and gastric carcinoma (GEC) [4-6], thus warranting a revised, updated and comprehensive review on this topic.

The assessment of PD-L1 protein expression via immunohistochemistry (IHC) has emerged as a key predictive biomarker for guiding treatment with immune checkpoint inhibitors (ICIs) in GEC, as currently recommended by European Society of Medical Oncology (ESMO) guidelines [7-9]. While PD-L1 IHC assessment may seem straightforward, some caveats and practical controversies concerning its complexity, especially in this category of tumors, have emerged [4,5]. Specifically, PD-L1 evaluation can be influenced by both (I) significant intra-tumoral heterogeneity and (II) GEC histopathological subtypes [4,10]. Such observations highlight the critical need for robust, standardized, and reproducible scoring systems for PD-L1 IHC interpretation. In 2022, we addressed some of these caveats, specifically focusing on the Combined Positive Score (CPS) and the Tumor Proportion Score (TPS) and their related diagnostic pitfalls [5]. Since then, an alternative scoring system, namely the Tumor Area Positivity (TAP), has been proposed and may represent a significant methodological advancement [11]. Unlike other scoring systems, the TAP score relies on a visual estimation of the percentage of the total tumor area occupied by PD-L1-positive cells, including both PD-L1-positive tumor cells and PD-L1-expressing immune cells within the tumor microenvironment. Overall, the TAP scoring system aligns more closely with the visual assessment practices of pathologists and has demonstrated high interobserver reproducibility and concordance with CPS, particularly at lower values [11]. Accordingly, TAP score is increasingly used in recent clinical trials testing ICIs [12,13], as in the Matterhorn [14] and EDGE-gastric trials [15,16]. Furthermore, CPS and TAP can be interchangeably used according to most recent National Comprehensive Cancer Network's (NCCN) Clinical Practice Guidelines [17,18]. Despite the advantages introduced by the TAP scoring system, practical challenges may still occur in PD-L1 IHC evaluation, such as accurately identifying the total tumor area.

Based on these considerations, this review aims to provide an updated and practical approach to the histopathological IHC assessment of PD-L1 expression in GEC. We first define the clinical context to highlight the relevance of this assay and then focus on the practical challenges of the newly introduced TAP scoring system for both gastroesophageal adenocarcinomas (GEAs) and esophageal squamous cell carcinomas (ESCCs), while revisiting crucial aspects of CPS and TPS. Furthermore, we provide a standardized report template for PD-L1 protein expression assessment via IHC and concluded the review with our insights on how we expect the field to move forward.

New approaches for new drugs – Predictive biomarkers and clinical trials.

Testing for HER2, PD-L1, mismatch repair (MMR) proteins, and claudin 18.2 (CLDN18.2) is recommended (by all major guidelines) to direct first-line treatment choices in patients with advanced GEAs. For patients with advanced ESCC, PD-L1 testing alone is recommended [7,19,20].

In locally advanced and metastatic **HER2-positive GEA**, PD-L1 status is a prerequisite for the use of pembrolizumab (an anti-PD-1 ICI) associated with anti-HER2 treatment. Indeed, The KEYNOTE-811 trial met both co-primary endpoints of progression-free survival (PFS) and overall survival (OS), demonstrating statistically significant and clinically meaningful improvements in these survival outcomes, with an increased magnitude of benefit observed in patients with PD-L1 CPS ≥ 1. However, no benefit from the addition of pembrolizumab to trastuzumab and chemotherapy was observed in patients with CPS < 1. This led to the EMA approval of the new KEYNOTE-811 combination specifically for patients with CPS ≥ 1, and to the restriction of the initial FDA conditional approval, which had been based on a previous interim analysis of tumor response results [14,21]. Patients with a PD-L1 CPS ≥ 1 are now offered the addition of pembrolizumab to trastuzumab and chemotherapy, whereas the combination of trastuzumab and chemotherapy remains the standard of care for patients with HER-2 positivity but a CPS of < 1.

In locally advanced and metastatic **HER2-negative GEA**, the therapeutic landscape has become increasingly complex. Four global, phase 3 trials established the first-line use of a PD-1 inhibitor added to chemotherapy: nivolumab (CheckMate-649) [22,23], pembrolizumab (KEYNOTE-859 and KEYNOTE-590) [24-26], and tislelizumab (RATIONALE-305) [12]. These trials were quite heterogeneous regarding the chemotherapy backbone, the study design (placebo-controlled, open-label or double-blind), and, more importantly, the

PD-L1 clone/assay as well as the related scoring systems and cut-offs. Notably, PD-L1 metrics were used both as stratification factors before randomization and to identify the primary endpoint population or to guide the pre-planned subgroup analyses (Tab. I) [4,5].

Following this, regulatory approvals for different anti-PD-1 ICIs have demonstrated considerable heterogeneity. The FDA initially approved nivolumab and pembrolizumab for use in all comers, while after much debate, the same regulatory authority approved tislelizumab in patients with PD-L1 expression ≥ 1 and, more recently, nivolumab and pembrolizumab in patients with PD-L1 expression ≥ 1. In contrast, EMA approved nivolumab for patients with CPS ≥ 5 and tislelizumab for patients with TAP > 5%, reflecting the specific scoring systems noted in the drug labels and with the understanding that, in clinical trials, the benefit observed in all comers or patients with lower PD-L1 expression cut-offs was primarily driven by those with higher levels. However, EMA approved pembrolizumab for patients with CPS ≥ 1, acknowledging a modest, albeit statistically significant, benefit reported as post-hoc analysis in the subgroup with CPS between 1 and 9, even if the pre-planned analyses were conducted in patients with CPS ≥ 10, ≥ 1, and all comers. Despite these cross-trial differences, the results from the association of an anti-PD-1 agent to doublet chemotherapy were largely consistent across trials, both in all randomized patients and within PD-L1 subgroups. This consistency holds regardless of whether these subgroups were pre-planned, derived by academic investigators using statistical methods (such as KMSubtraction) [27-29], or expressly requested by regulatory authorities (EMA or the FDA oncology drugs advisory committee - ODAC). Notably, the FDA ODAC pooled analysis of CheckMate-649, KEYNOTE-859, and RATIONALE-305 revealed inconsistent benefits in patients with microsatellite stable (MSS) status and different ranges of PD-L1 expression (e.g., 1-4, or 5-9, or 1-9), and no discernible benefit in patients with PD-L1 < 1. As a consequence, current guidelines: (I) consistently and strongly recommend the use of anti-PD-1 ICIs in patients with high PD-L1 expression (at least 5, regardless of the assessment systems – either CPS or TAP), (II) do not recommend ICIs in patients with no PD-L1 expression (< 1), and (III) suggest a case-by-case evaluation with shared decision making in patients with CPS/TAP between 1 and 4, especially considering the availability of emerging alternative treatment options (such as zolbetuximab, an anti-CDN18.2 monoclonal antibody) [30,31].

Regarding metastatic ESCC, upfront PD-L1 testing with TPS, CPS, and TAP is recommended to guide first-line treatment choices for the integration of anti-PD-1 ICIs with chemotherapy. Based on randomized clinical trials (CheckMate-648, KEYNOTE-590, RATIONALE-306) [13,24,28,32], the FDA initially approved nivolumab and pembrolizumab for all comers, and tislelizumab for patients with PD-L1 expression ≥ 1. On the other hand, EMA approved nivolumab for patients with TPS ≥ 1%, pembrolizumab for patients with CPS ≥ 10 and tislelizumab for patients with TAP ≥ 5%, again considering the specific scoring system in the drug labels and the concept that the benefit in all comer patients was driven by those with higher PD-L1 levels in these clinical trials. The ESMO guidelines recommend using specific anti-PD-1 agents plus chemotherapy according to their respective drug labels and specific PD-L1 values/scores [7,19,20]. Additionally, the chemo-free combination of ipilimumab plus nivolumab is an option for patients unfit for combination chemotherapy and TPS ≥ 1%, though this has a lower strength of recommendation due to the inferior PFS when compared to chemotherapy alone.

Table I. Heterogeneity of clinical trials testing ICIs for locally advanced and metastatic HER2-negative GEA, especially in terms of PD-L1 testing.

Clinical Trial	Chemotherapy backbone	ICI tested	PD-L1 assay	PD-L1 score	PD-L1 cut-off°	Reference
CheckMate-649	capecitabine and oxaliplatin or leucovorin, fluorouracil, and oxaliplatin	Nivolumab	28–8	CPS	CPS ≥ 5	[22,23]
KEYNOTE-590	5-fluorouracil and cisplatin	Pembrolizumab	22C3	CPS	CPS ≥ 10	[24]
KEYNOTE-859	5-fluorouracil and cisplatin or capecitabine and oxaliplatin	Pembrolizumab	22C3	CPS	CPS ≥ 1 and CPS ≥ 10	[25,26]
RATIONALE-305	5-fluorouracil and cisplatin or capecitabine and oxaliplatin	Tislelizumab	SP263	TAP	TAP ≥ 5%	[12]

SP263: VENTANA PD-L1 (SP263) Assay; 22C3: Dako PD-L1 IHC 22C3 pharmDx Assay; 28-8: Dako PD-L1 IHC 28-8 pharmDx Assay.
°For primary endpoint

The NCCN Clinical Practice Guidelines in Oncology – which are based on the FDA ODAC evaluation – recommend using chemoimmunotherapy in patients with PD-L1 expression ≥ 1 without specific restrictions on the scoring system or the specific agent intended to be used [17,18].

Histopathologic assessment of PD-L1: problems, pitfalls, and standardization

Preanalytical variables – what the pathologist needs to know (and report)

As can be readily inferred from well-known literature data and daily routine experience, several preanalytical variables significantly influence the accuracy and reliability of PD-L1 IHC staining and evaluation [33-38]. Key factors include cold ischemia time, length of fixation times, and the aging of tissue blocks and tissue sections.

Prolonged cold ischemia, particularly exceeding 3 hours, has been demonstrated to result in a higher proportion of suboptimal staining and false-negative results, with varying tolerance levels among different antibody clones [36]. Similarly, both under- and over-fixation in formalin can detrimentally affect PD-L1 immunoreactivity, as observed with fixation times beyond 72 hours [35]. The aging of tissue slides and tissue blocks also plays a crucial role [33,34,37], with longer storage times (e.g., tissue blocks older than 24-60 months) correlating with reduced PD-L1 values [34,39]. Other preanalytical variables that can generally affect IHC yield, such as tissue decalcification, also compromise PD-L1 IHC staining, but are rarely, if ever, involved with GEC samples [38,40].

These preanalytical challenges can contribute to inter-institutional variability in PD-L1 assessment, as recently highlighted by a nationwide survey of PD-L1 testing in pathology units [39,41]. Based on these considerations, dedicated protocols and preventive measures should be employed to optimize PD-L1 IHC evaluation, such as the use of on-slide positive controls and participation in external quality assessment schemes [42-44].

Analytical variables – differences between available clones and related benchmark/assays

Quite unique to targeted therapies and related predictive biomarkers is the requirement of companion diagnostic assays, which call for a particular antibody clone and staining platform to grant patient access to a specific drug/compound. The most common commercially available companion diagnostic assays for PD-L1 are four: the VENTANA PD-L1 (SP263) Assay, the VENTANA PD-L1 (SP142) Assay, the Dako PD-L1 IHC 22C3 pharmDx Assay, and the Dako PD-L1 IHC 28-8 pharmDx Assay. Notably, it is worth reminding that SP142 is not used for GEC samples. The companion diagnostic approach would ideally require pathology laboratories to utilize different platforms and clones depending on the ICI a patient may need to access. Unfortunately, practical drawbacks make this impossible in routine practice, as most pathology units rarely possess the resources to have all required platforms. Moreover, each assay requires dedicated staining protocols, reagents, equipment, and IHC interpretation cut-offs, leading to (I) a potential source of confusion for both oncologists and pathologists, and (II) a non-negligible increase in the turn-around-time of daily pathology practice. Based on these considerations, several studies have investigated the potential interchangeability of PD-L1 IHC assays for GEC samples. In this paragraph, we summarize the most relevant evidence from these studies.

The first topic worth addressing is the PD-L1 epitopes targeted by the different IHC antibodies. Clones SP263 and SP142 bind the very same epitope located in the cytoplasmic domain at the extreme C-terminus, whereas 22C3 and 28-8 bind to distinct epitopes, both located in the extracellular domain [45]. While it may be argued that different epitopes could lead to IHC staining variability, conflicting PD-L1 IHC yields have been observed with clones binding the same rather than different epitopes (i.e., SP142 showed discordant IHC yield compared to SP263, 22C3 and 28-8). This observation suggests that epitope differences are unlikely to cause PD-L1 IHC staining differences [45].

One of the earliest well-structured studies addressing clone and assay interchangeability was performed by Ahn and colleagues [46]. They analyzed 55 gastric cancer cases, performing 22C3 and 28-8 assays on the same tissue blocks. Pathologists' agreement was then compared at different, clinically relevant CPS cut-offs. At a CPS cut-off of 1, the overall agreement was 96.4% with excellent concordance rates considering both a CPS cut-off of 1 (kappa value = 0.927) and 10 (kappa value = 0.899). As expected, the overall agreement reached 100% with a CPS cut-off of 50. Interestingly, the authors described a non-negligible unspecific background staining with the 28-8 assay alone, which advised caution with its interpretation. Regardless, the results of both 22C3 and 28-8 assays correlated with the clinical outcomes, and the Authors concluded that 22C3 and 28-8 can be considered interchangeable in gastric cancer samples [46].

Similar results have been recently reported by Klempner and colleagues [47]. They collected 100 surgically

resected GEC cases, performed 28-8, 22C3, and SP263 assays, and compared the results provided by three trained pathologists using CPS and TAP scoring systems. Moreover, digital image analysis was also performed to simulate CPS and TAP. Interestingly, the three assays showed moderate to good correlations with both CPS and TAP. The worst correlations were observed between SP263 and 28-8 with both CPS (intraclass correlation coefficients – ICC: 0.62) and TAP (ICC: 0.60), whereas the best correlations were observed between SP263 and 22C3 (CPS, ICC: 0.83; TAP, ICC: 0.80).

The data published by Park and colleagues [48] are in line with this trend. The authors developed tissue microarrays by combining cores of the center and invasive margin of 379 GEC cases. They evaluated PD-L1 immunostaining expression with 22C3 and SP263 using both CPS and TPS and described an overall percent agreement greater than 90% with both CPS (≥ 1 and ≥ 10 cut-offs) and TPS (≥ 1% and ≥ 10% cut-offs). In particular, the overall percent agreement between the two assays was better with a CPS cut-off ≥ 10 (99.2%) than ≥ 1 (94.7%).

Contrasting results and considerations were reported by Yeong and colleagues [49]. They investigated the interchangeability between the 22C3, 28–8, and SP142 assays via multiplex IHC on TMAs developed from 362 GEC samples. The authors described scoring with 28–8 assay in a consistently higher proportion of GEC samples compared to 22C3 at CPS ≥ 1 (70.3 vs 49.4%, $p < 0.001$), at CPS ≥ 5 (29.1 vs 13.4%, $p < 0.001$) and at CPS ≥ 10 (13.7 vs 7.0%, $p = 0.004$). Furthermore, the mean CPS score obtained from the 28–8 assay was significantly higher than both the 22C3 and SP142 assays, whereas no significant difference between 22C3 and SP142 was observed. Despite the limitations represented by using TMA and multiplex IHC, the authors advised against the interchangeability of assays, pending further confirmation [49].

As international multi-institutional initiatives are currently ongoing [50] and despite some notable exceptions [49], the overall impression from the current literature data suggests that PD-L1 assays can be used interchangeably for GEC samples [46-48]. This provides pathology units with evidence to choose different assays in combination with different scoring algorithms, potentially solving some of the practical challenges associated with maintaining availability to multiple companion diagnostic assay platforms.

Understanding PD-L1 Scoring Systems in Gastroesophageal Cancer – welcome to the jungle!

PD-L1 is a transmembrane protein that acts as an immune checkpoint molecule, inhibiting immune system activation and preventing excessive immune response and autoimmunity in non-neoplastic conditions. Tumor cells harness PD-L1 expression and downstream pathway as an adaptive immune resistance mechanism to induce T-cell exhaustion and inhibit the peri-tumor immune microenvironment cytotoxic response [51]. PD-L1 was initially identified in 1999 by Dong and colleagues as B7-H1 – a novel member of the B7 family of proteins [52] – but its critical role in immune evasion and its potential as a predictive biomarker for ICIs began to emerge more clearly in the early 2000s, particularly with preclinical studies demonstrating that blocking PD-1/PD-L1 interaction could reactivate anti-tumor immune responses [53,54]. Clinical implementation of ICIs largely relies on PD-L1 protein expression assessment via IHC assay. PD-L1 can be expressed on a variety of immune (e.g., T cells, B cells, macrophages, dendritic cells) [55,56] and non-immune cells (e.g., placental trophoblastic cells, tonsil crypt reticulated epithelial cells) [57-60]. Therefore, in GEC tissue samples, PD-L1 protein expression can be observed in both immune and tumor cells, and tonsil tissue samples are generally used as on-slide positive control. As mentioned above, three scoring systems – CPS, TPS, and TAP – exist to quantify PD-L1 protein expression on tumor tissue slides, each with its own methodology and clinical application. In the following paragraphs, we will detail and discuss each score individually.

The **Combined Positive Score - CPS** was introduced by Dako/Agilent Technologies alongside the PD-L1 IHC 22C3 pharmDx assay in the context of pembrolizumab testing for melanoma and non-small cell lung cancer [61,62]. One of its first significant applications in GEC clinical trials was in the KEYNOTE-059 trial [63,64]. CPS is calculated as the number of PD-L1-staining cells (tumor cells, lymphocytes, and macrophages) divided by the total number of viable tumor cells, multiplied by 100. Notably, the CPS is not a percentage, but an absolute numeric value, ranging from 0 to 100. Its maximal value can exceed 100 but, all values that exceed this figure should nevertheless be reported as 100. For the tumor component, only invasive viable tumor cells exhibiting complete or incomplete membrane (but not cytoplasmic) staining of any intensity are considered positive. For the immune component, immune cells are considered positive if they show membrane or cytoplasmic staining of any intensity but should be located within a 20X objective field from the invasive viable tumor cells [4]. This score provides a broad assessment of PD-L1 expression within the tumor microenvironment and reflects the interaction between tumor cells and immune cells. Within the context of GEC samples, the CPS has been widely adopted and

has been a primary biomarker in several pivotal GEC ICIs trials (including Checkmate-648, Checkmate-649, KEYNOTE-059, KEYNOTE-859, KEYNOTE-811, and KEYNOTE-590) for both GEA and ESCC [22-26,28,29,63,64]. Unfortunately, CPS is not exempt from drawbacks. The calculation can be challenging due to the need to (I) differentiate infiltrative tumor cells from pre-invasive/dysplastic tumor cells (the latter should not be considered in the CPS calculation), (II) identify the immune cells of interest (i.e., lymphocytes and macrophages) from those that should not be considered (e.g., neutrophils, eosinophils, plasma cells) in areas with dense immune infiltrates, (III) exclude positive immune cells involved in ulcers and other non-neoplastic inflammatory processes of the gastroesophageal mucosa rather than peritumoral immune response, and (IV) the subjectivity involved in assessing scattered PD-L1 positive cells [4,5]. Altogether, these issues can lead to non-negligible inter-observer variability, as discussed in a dedicated section of this review.

The **Tumor Proportion Score - TPS** was initially developed and applied in the context of non-small cell lung cancer with the PD-L1 IHC 22C3 pharmDx assay [65,66]. Its initial use in GEC clinical trials, though less predominant than CPS, was often seen in earlier studies exploring PD-L1 expression in these tumors (e.g., KEYNOTE-12) [67,68]. TPS is calculated as the percentage of viable tumor cells showing partial or complete membrane staining for PD-L1, relative to all viable tumor cells. Immune cells are not included in this score. TPS is conceptually simpler to calculate than CPS as it focuses solely on tumor cells, potentially reducing some drawbacks and inter-observer variability related to immune cell identification. On the other hand, TPS may underestimate the overall PD-L1 immune landscape, particularly in tumors where immune cells play a significant role in the response to ICIs, but tumor cell expression is low or absent. This phenomenon – PD-L1 expression more prominent in immune cells compared to tumor cells – is generally observed in GEA [69] as opposed to ESCC [24]. Its utility as a standalone biomarker in GEC is limited compared to CPS.

The **Tumor Area Positivity - TAP** scoring system is a more recent development aimed at providing a more intuitive and potentially reproducible method for PD-L1 assessment [11]. TAP is based on the visual estimation of the percentage of the total tumor area occupied by PD-L1-positive cells, including both PD-L1-positive tumor cells and PD-L1-expressing immune cells within the tumor microenvironment [11]. TAP aligns more closely with the routine visual assessment practices of pathologists, potentially leading to higher interobserver reproducibility compared to cell-by-cell-based enumeration scores. Early data suggest good concordance with CPS, particularly at lower values [11,47,70]. In the study that first described the TAP score [11], excellent overall percentage agreement (> 90%) between TAP and CPS was observed with both 1% (TAP) vs 1 (CPS) cutoffs and 5% (TAP) vs 1 (CPS) cutoffs. Furthermore, the average time spent on tumor slide scoring was significantly reduced with TAP (5 minutes) compared with CPS (30 minutes). Similarly, Klempner and colleagues demonstrated analytical comparability of three major PD-L1 assays (28-8, 22C3, and SP263)

Table II. Comparative reproducibility studies demonstrated excellent agreement between recently introduced TAP and previous scores.

Study	Sample Size	Assay	Scoring systems	Agreement/analytical comparability
Liu et al. [11]	52 GEA and ESCC samples	SP263	TAP (1% and 5% cut-offs) and CPS (1 cut-off)	96.2% (OPA, TAP 1% vs CPS 1) and 92.3% (OPA, TAP 5% vs CPS 1)
Klempner et al. [47]	100 samples (28 GEJ, 72 stomach)	28-8, 22C3, and SP263	TAP and CPS (same level cut-offs comparison)	1.00, 0.98, 0.77 (K, TAP 1%, 5%, and 10% vs CPS 1, 5, 10, respectively; both SP263); 0.47, 0.77, 0.82 (K, TAP 1%, 5%, and 10% vs CPS 1, 5, 10, respectively; TAP SP263, CPS 28-8); 0.51, 0.77, 0.85 (K, TAP 1%, 5%, and 10% vs CPS 1, 5, 10, respectively; TAP SP263, CPS 22C3)
Moehler et al. [70]	1866 samples from three clinical trials*	SP263	TAP and CPS (same level cut-offs comparison)	0.78, 0.64, 0.64 (K, TAP 1%, 5%, and 10% vs CPS 1, 5, 10, respectively; RATIONALE-305); 0.73, 0.75, 0.79 (K, TAP 1%, 5%, and 10% vs CPS 1, 5, 10, respectively; RATIONALE-302); 0.85, 0.67, 0.78 (K, TAP 1%, 5%, and 10% vs CPS 1, 5, 10, respectively; RATIONALE-306)

*: 974 samples from RATIONALE-305, 355 samples from RATIONALE-302, and 537 samples from RATIONALE-306
GEJ: gastroesophageal junction adenocarcinoma; K: Cohen's kappa; OPA: overall percent agreement

Table III. Implementation of TAP for PD-L1 IHC assessment may solve most of CPS and TPS drawbacks.

Scoring system	Definition	Advantages	Disadvantages	Clinical Predictive Value
CPS [61,62]	Number of PD-L1-staining cells (tumor cells, lymphocytes, and macrophages) divided by the total number of viable tumor cells, multiplied by 100	- Allows contemporary evaluation of both tumor and immune cells. - Widely used in both clinical trials and real-world practice.	- Cell-by-cell counting with associated challenges in distinguishing cell types and overlapping cells	69% [71], 29.4% (GEA) [72] 34.3% (ESCC) [72]
TPS [65,66]	Percentage of viable tumor cells showing partial or complete membrane staining for PD-L1, relative to all viable tumor cells	- Easier evaluation by removing immune cells evaluation. - May not necessarily result faster as discrimination between cell types still needs to be performed during assessment.	- Removal of immune cells evaluation can misrepresent some tumor histotype with prevalent PD-L1 immune cell expression (GEA). - Tumor cells assessment is still cell-by-cell-counting-based, thus not solving associated challenges.	10.1% (GEA) [72]; 47% (ESCC) [72]
TAP [11]	Percentage of the total tumor area occupied by PD-L1-positive cells, including both PD-L1-positive tumor cells and PD-L1-expressing immune cells within the tumor microenvironment	- Visual estimation of relevant cell populations replacing cell-by-cell counting. - Simpler and faster evaluation. - Maintain evaluation of both tumor and immune cells.	- Recently proposed and not fully implemented in real-world practice. - Few data available regarding advantages, pending large scale evaluations. - Some practical caveats still need explanation (see subsequent paragraph – 2.4).	OS HR: 0.57 (TAP ≥ 10%) vs 0.68 (CPS ≥ 10) [70]

OS: overall survival; HR: hazard ratio.

in gastric cancer [47]. They observed moderate to almost-perfect inter-assay agreement (Cohen›s kappa range, 0.47-0.83) and substantial to almost-perfect intra-assay agreement (kappa range, 0.77-1.00) when utilizing either CPS or TAP [47]. Finally, Moehler and colleagues investigated concordance between TAP and CPS in GEA and ESCC treated with tislelizumab, reporting significant agreement (Cohen›s kappa, 0.64-0.85) across PD-L1 cutoffs of ≥1%, ≥5%, and ≥10% [70]. They also indicated that both TAP and CPS yielded similar clinical outcomes for OS, PFS, and objective response rates, suggesting their clinical comparability. A summary of these comparative reproducibility studies is provided in Table II, whereas a summary of definitions, advantages, and disadvantages of the three scoring systems is provided in Table III. This latter topic is further discussed in the following section.

How to survive PD-L1 testing – practical suggestions for scoring system assessment

Understanding the caveats and implications of each scoring system is essential, as their clinical utility and predictive value can vary depending on the tumor type and the specific PD-1/PD-L1 inhibitor being considered. While CPS and TPS have already been widely discussed and analyzed in the literature [5], the TAP scoring system has been poorly explored so far, and few institutions have, till now, implemented it in their diagnostic daily routine [39]. As a consequence, practical interpretation questions remain largely unsolved. Based on these considerations, this section will be mostly dedicated to the TAP score.

We have already discussed the advantages of the TAP scoring system in terms of (I) ease and speed of use due to the visual estimation versus cell-by-cell counting [11], and (II) substantial interchangeability with CPS while preserving the information on PD-L1 expression of both tumor cells and immune cells [11,47,70]. However, accurately identifying the total tumor area, especially in heterogeneous tumors with extensive desmoplastic reaction or inflammation, or in biopsy samples, can still present practical challenges. In general, careful initial delineation of the tumor boundaries and area of interest is always required and can be aided by hematoxylin and eosin-stained slides comparison.

In the first study proposing the TAP score, the authors defined the tumor area as the area occupied by all viable invasive tumor cells and associated immune cells [11]. Herein, we provided a pictorial case-by-case interpretation guide. Criteria for tumor cell inclusion into the tumor area calculation are I) any intensity of staining, II) both circumferential and partial membrane but not cytoplasmic patterns of PD-L1 expression, III). viable and not necrotic tumor cells, IV). invasive (i.e.,

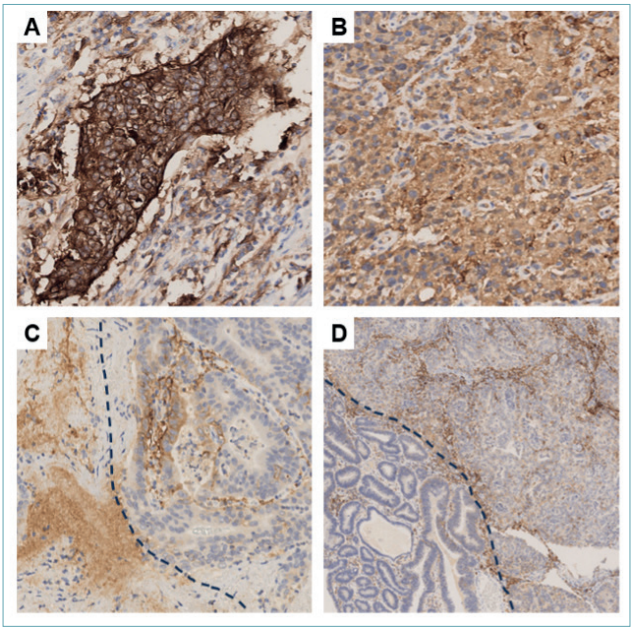

Figure 1. Criteria for tumor cell inclusion into the tumor area calculation. A, B) tumor cells with cytoplasmic staining only should not be included, while tumor cells with both membrane (partial or complete, regardless of intensity) and cytoplasmic staining should be included. C) Necrotic tumor cells and D) non-invasive neoplasia (dysplasia) should not be included.

carcinoma) tumor cells only (area of dysplasia is excluded) (Fig. 1).

Criteria for immune cell inclusion are less restrictive, and, essentially, any intensity and any pattern of staining (i.e., membranous, cytoplasmic, and punctate) are legit for inclusion in the calculation (Fig. 2).

The intercalated stroma should also be considered in the tumor area calculation, as well as mucin pools and gland lumen for GEA cases (Fig. 3) [11].

According to the authors, for the identification of tumor area boundaries, pathologists should use the "10x field" rule, especially if dealing with separate tumor nests (and poorly-cohesive GEC): any tissue (including non-immune and non-neoplastic tissue, such as muscle) within the boundaries of a 10x field that is delimited by tumor cells should be considered as part of the tumor area. Unfortunately, this definition may mislead PD-L1 IHC interpretation. A practical issue immediately emerges when considering the actual dimension of the 10x field. Depending on the specific field number (FN) of each microscope eyepiece, the area at 10x can vary greatly, ranging from 2.5 mm^2 (FN 18) to 5.5 mm^2 (FN 26.5). Additional diagnostic challenges occur depending on the tissue that is included in the calculations. In GEC tissue samples, tumor cells can frequently invade muscularis propria and involve myocytes – especially with the GEA poorly cohesive histotype (Fig. 4).

However, myocytes generally play a minor role in the peritumoral immune microenvironment and immune checkpoints regulation compared to other immune and non-immune cell populations [73-76]. The evaluation of PD-L1-positive immune cells can also be challenging. The caveat is related to identifying immune cells that are actually involved in the peritumor immune response, as opposed to immune cells associated with local inflammatory processes (such as ulcers and other non-neoplastic inflammatory processes of the gastroesophageal mucosa). This issue has also been encountered with the use of the CPS, and it is only

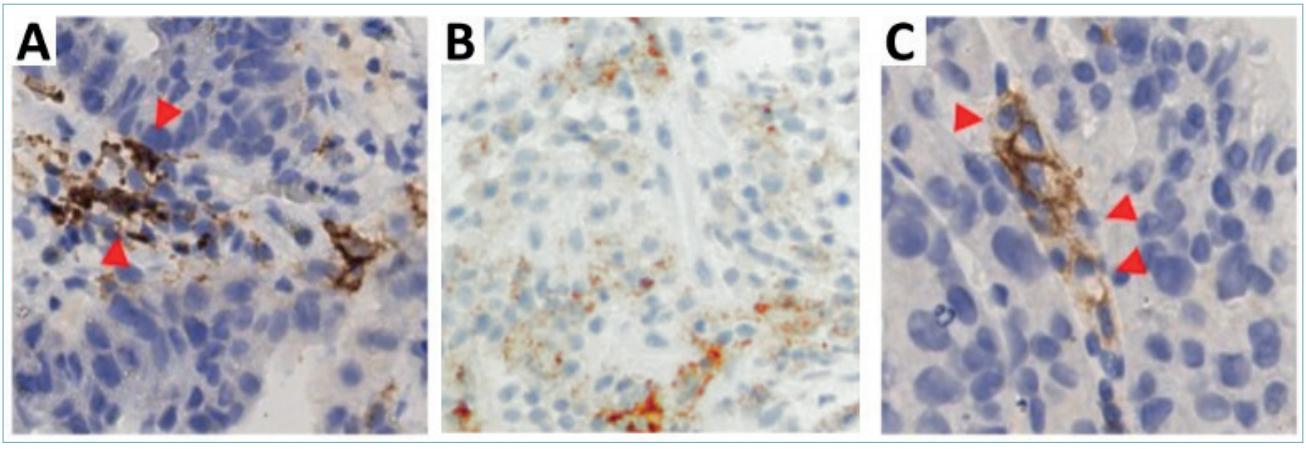

Figure 2. Criteria for immune cell inclusion. (A, B) Immune cells with cytoplasmic staining (A, red arrows), (C) with incomplete/punctuate membranous staining (red arrows) should be included.

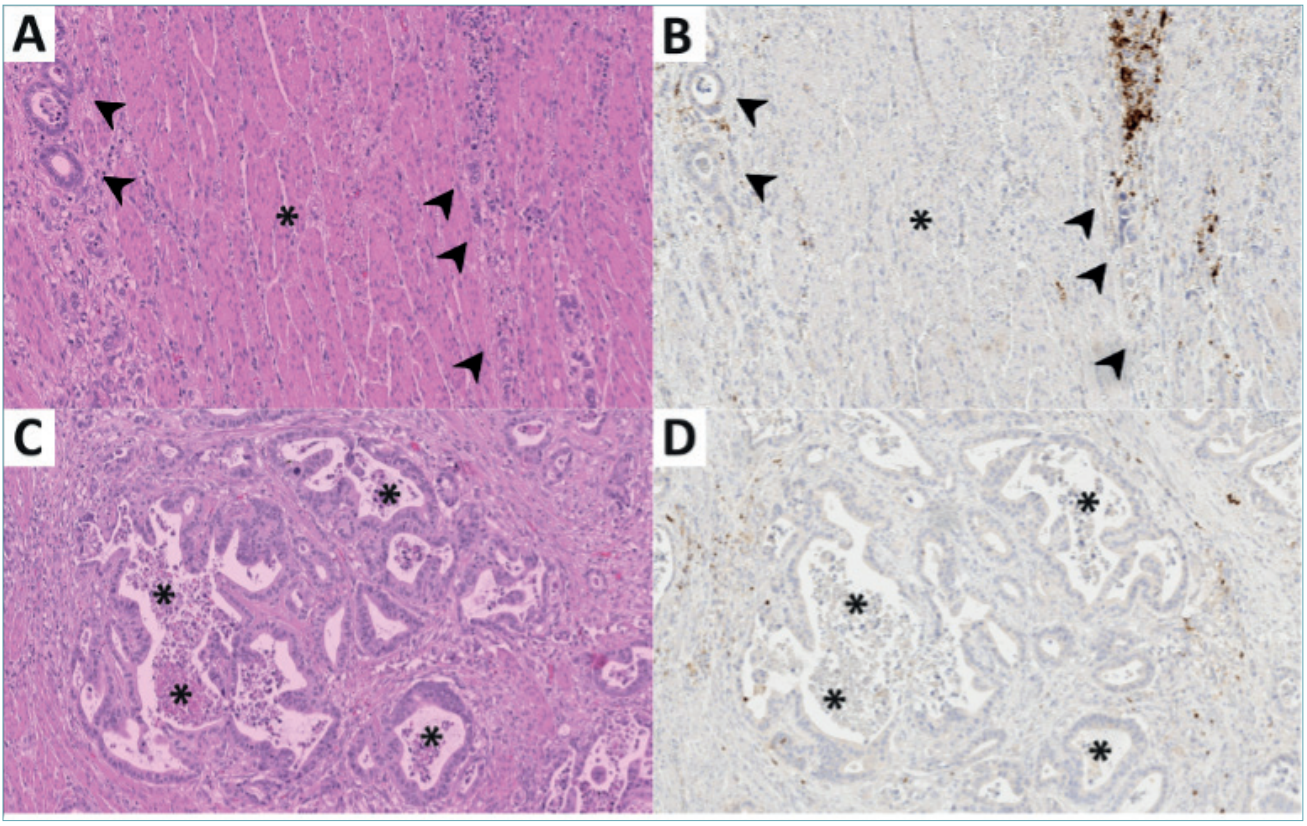

Figure 3. Inclusion criteria into the tumor area calculation. A-D) Representative image of a 10x fields in H&E (A, C) and PD-L1 (B, D). A, B) Intercalated stroma (asterisk) between tumor cells (black arrowheads) should be considered in the calculation. C, D) Gland lumen spaces (asterisks) should be included.

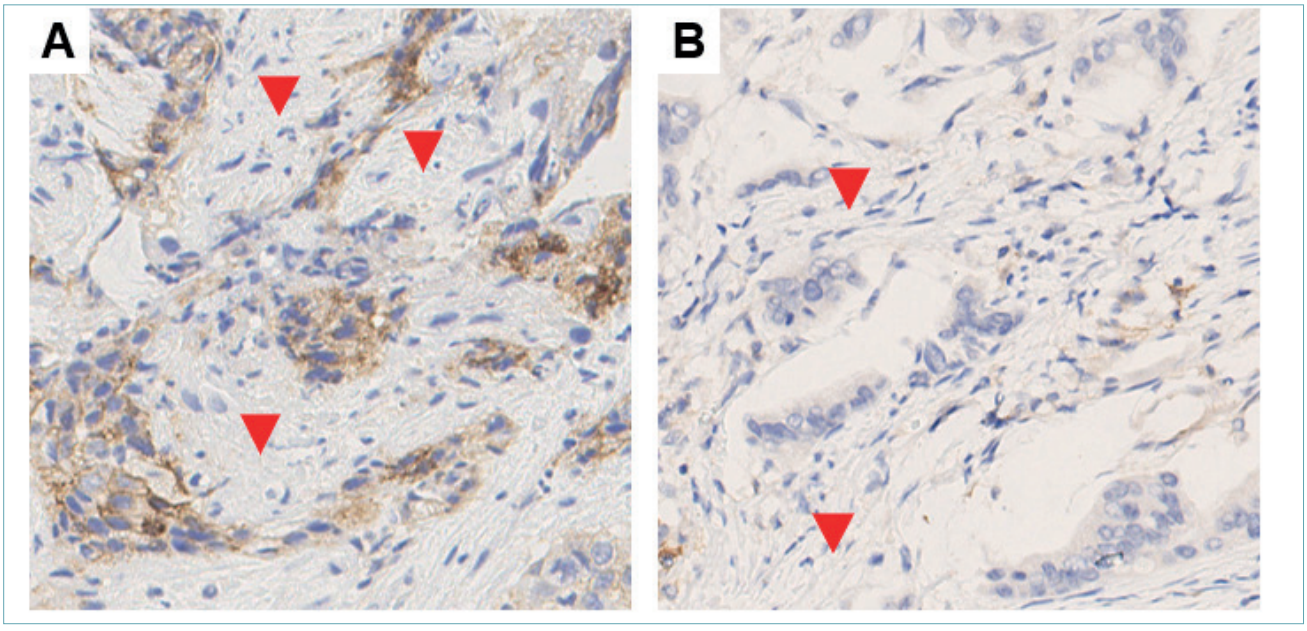

Figure 4. (A, B) PD-L1 negative muscle cells (red arrows) within tumor cell clusters should be included in the TAP score as long as the tumor nests are bordered on both sides of a 10x field.

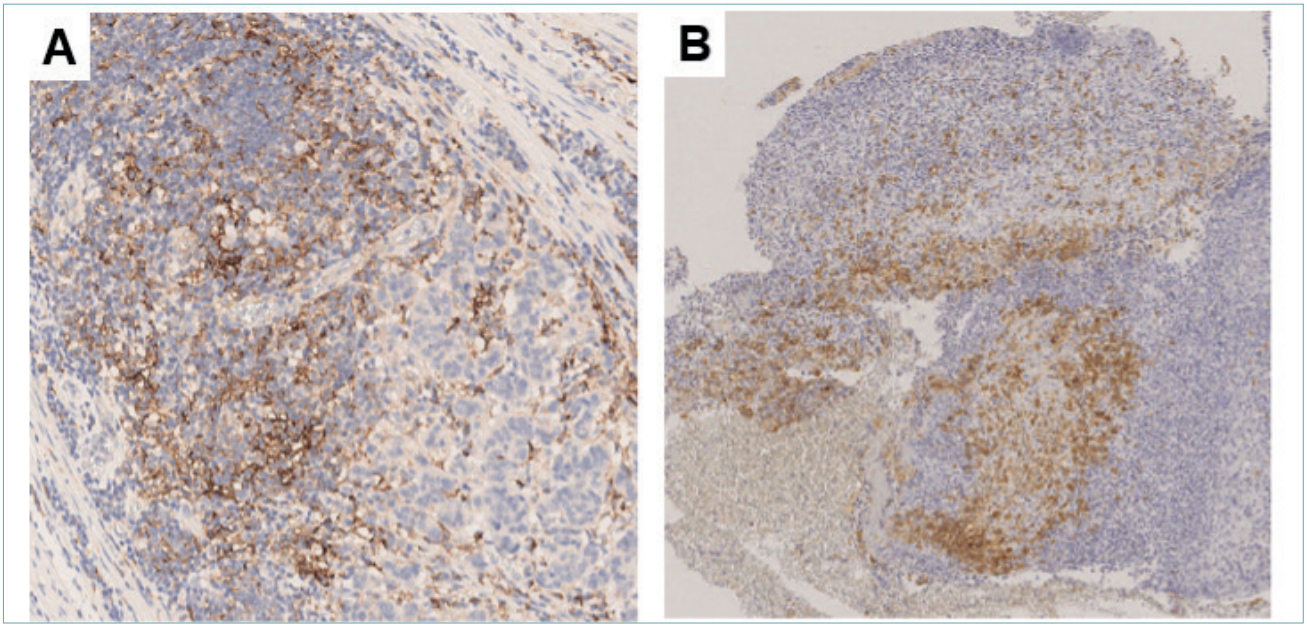

Figure 5. (A) Germinal centers of lymphoid aggregates are included in the TAP score if they are located within the tumor area. (B) Immune cells associated with local inflammatory processes (such as ulcers and other non-neoplastic inflammatory processes of the gastroesophageal mucosa) should not be included.

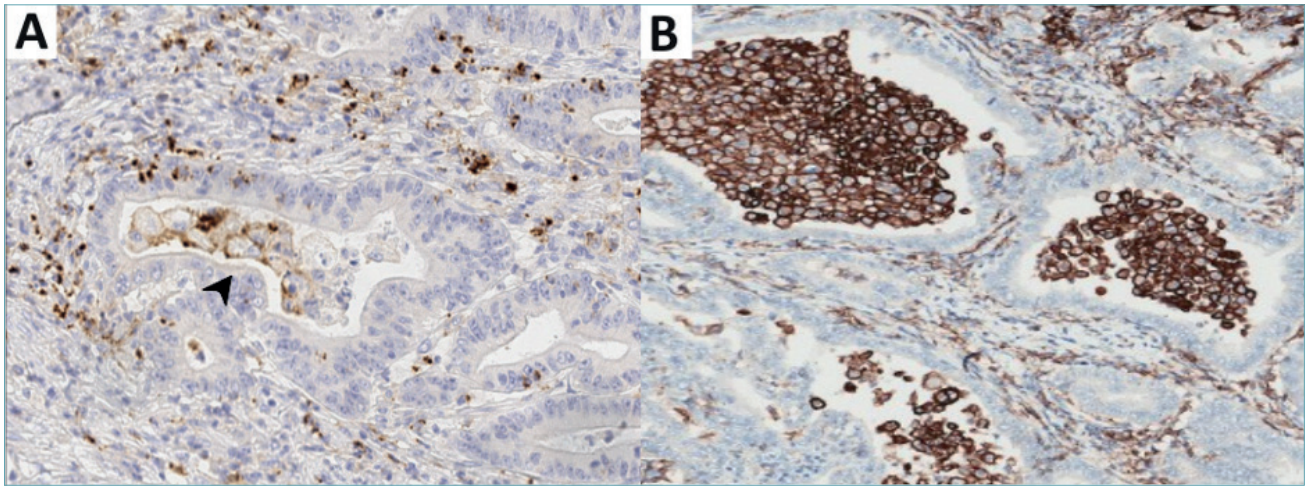

Figure 6. (A) Intra-luminal macrophages that do not fill the luminal space (black arrowhead) should not be included in the TAP score, while (B) intra-luminal macrophages that completely fill the luminal space and are in direct contact with the tumor cells should be included in the TAP score.

partially solved by the 10x field rule (Fig. 5).

Another debated area is represented by PD-L1-positive intraluminal macrophages in GEA samples. According to the authors, intraluminal macrophages should not be included in the TAP score unless the macrophages completely fill the tumor gland lumen and are in direct contact with the tumor cells [11]. Again, this definition may lead to heterogeneous interpretations, as the same tumor glands may present variable macrophage "packing" depending on the tissue section (e.g., H&E and the PD-L1 IHC; Fig. 6).

As shown, PD-L1 IHC assessment can present several caveats and diagnostic challenges. Therefore, we strongly recommend that a pathologist, possibly with

dedicated GI training, should perform PD-L1 IHC assessment and reporting and, preferably, the pathologist that reported the original diagnosis.

Real-world versus clinical trials in PD-L1 testing and related percentages

Real-world practice can substantially vary from clinical trial. Clinical trials are structured to optimize and standardize sample management – including their pre-analytical and analytical phases – by implementing sample centralization and adopting a dedicated IHC workflow. Therefore, direct comparison of clinical trials and real-world practice can be challenging, and literature data about this topic are particularly scarce for GEC PD-L1 IHC assessment [41]. A recent initiative from the Italian Group of Gastrointestinal Pathologists (GIPAD) – the RELIABL study group – provides a much-needed comparison between PD-L1 percentage reported by clinical trials and those collected from 28 Italian pathology units via a dedicated survey [39].

Results from this survey have demonstrated concordance with published clinical trial data regarding the overall percentage of PD-L1 positive cases, particularly with the CPS. The observed 76.1% CPS ≥ 1 positivity rate in GEA cases from the survey is similar to the 78-85% range that can be inferred from clinical trials [21,22,25,26]. Notably, TPS and TAP results from the survey were comparatively higher than clinical trials, warranting a dedicated study to identify potential causes and implications for clinical practice. A detailed comparison between the RELIABL study and clinical trials is reported in Table IV.

Potential causes of this discrepancy are related to pre-analytical and analytical variables that can vary across pathology units (as described in the study), but interobserver variability should also be considered. There is contrasting evidence in the literature on this latter topic [47,81-83]. Kulangara and colleagues evaluated the reproducibility of the CPS using the 22C3 pharmDx assay on 68 GEC samples and demonstrated a high degree of inter- (96.6%) and intra-observer (97.2%) overall percent agreement [81]. Similar data were presented by Nuti and colleagues [83]. They evaluated several tumor types from different anatomical locations, including GEC, using the 22C3 assay and CPS system. Within the GEC subgroup, both the inter- and intra-observer overall percent agreement were excellent, ranging from 90.2% (intra-observer for gastric and gastroesophageal cancer) to 93.7% (inter-observer agreement for esophageal cancer). In contrast, a more recent international study by Robert and colleagues investigated inter-observer agreement for CPS using both the 28-8 and 22C3 pharmDx assays on whole slide images [82]. Despite a dedicated training session, inter-observer variability for CPS was notably high, with only fair agreement observed among pathologists both pre-training (ICC: 0.45-0.55) and post-training (ICC: 0.56-0.57). Similar results were observed for specific subgroup analysis, including the total number of viable tumor cells (ICC: 0.09), PD-L1-positive immune cells (ICC: 0.19), PD-L1-positive tumor cells (ICC; 0.54), and the calculated CPS on biopsy samples (ICC: 0.14). Interestingly, the TPS demonstrated better metrics (ICC: 0.82). To the best of our knowledge, only one study has analyzed inter-observer agreement with the TAP score [47]. Klempner and colleagues collected 100 GEC samples and tested concordance rates using 28-8, 22C3, and SP263 assays, and both CPS and TAP scoring systems. They reported an excellent level of agreement (ICC:

Table IV. Real-world percentage of PD-L1 IHC expression are substantially in-line with clinical trials data. Real-world percentages are obtained from our recent publication [39].

PD-L1 scoring system	GEA – Real-world	GEA - Clinical trial	Reference - Clinical trial	ESCC – Real-world	ESCC - Clinical trial	Reference - Clinical trial
CPS						
< 1	23.9	-		15.0	-	
≥ 1	76.1	78-85	21,22,25,26	85.0	-	
≥ 5	55.5	60-61	22,77	70.8	-	
≥ 10	35.8	35	25,26	50.4	43-53.6	24,78
TPS						
< 1%	56.9	-		27.8	-	
≥ 1%	43.1	16	22,79	72.2	51	80
TAP						
< 1%	19.7	-		10.3	-	
≥ 1%	80.3	-		89.6	-	
≥ 5%	56.3	55	12	79.3	-	
≥ 10%	40.7	-		72.4	30.7-34.4	13,32

0.92- 0.99) for all three assays across both CPS and TAP scoring algorithms. Indeed, due to its area-based visual estimation, the TAP scoring method is expected to further enhance overall inter-observer agreement in routine diagnostic practice, offering a potentially more robust and efficient alternative for PD-L1 assessment.

Standardized report for PD-L1 protein expression assessment via IHC – Minimum requirements for the pathology reports

As part of this narrative review, we have produced a standardized report template for PD-L1 and other biomarkers (i.e., HER-2, MMR, and Claudin 18.2) protein expression assessment via IHC, in both English (Tab. V) and Italian (Supplementary Material I). These suggested templates, developed based on our personal experience, incorporate the minimal data and information required for the appropriate reporting of these assays.

The template is structured as a "checklist" that details most of the areas examined in this review. We deemed it necessary to include features pertaining to: sample type (Section 1), adequacy of the assay (Section 2), and overall IHC procedure (Section 3), in addition to

Table V. Pathology report template for PD-L1 IHC assessment - English version. This pathology report template is intended for PD-L1 IHC assessment on GI samples. It lists the minimal data and information required for appropriate reporting and multidisciplinary discussion. Required information is in bold and organized as bullet-points. Following each entry, there are alternatives that can be selected. Further indications or comments on the specific entry are italicized.

1. Tissue sample features	
1.a. Sample type	Endoscopic biopsy, specify site[1]
	Hepatic core needle biopsy
	Surgical biopsy, specify site[2]
	Surgical sample, specify site[1]
	Post-neoadjuvant treatment sample, specify site[1]
	Endoscopic resection, specify site[1]
	Other, specify:
1.b. Sample accession	Internal sample[3]
	External/Consult[4]
1.c. Original diagnosis	*specify the histotype according to WHO*
2. Sample adequacy	
2.a. Tissue sample adequacy	Adequate: > 500 invasive tumor cells available for analysis
	Borderline: 100-500 invasive tumor cells available for analysis
	Inadequate: < 100 invasive tumor cells available for analysis
	If the sample is an endoscopic biopsy, **ALSO** report the number of fragments with invasive tumor cells over the overall number of fragments
2.b. Immunohistochemical stain adequacy	Adequate (on slide positive control)
	Inadequate, specify
2.c. Aging	Tissue slide aging[5]:
	Inclusion/tissue block aging[6]:
3. Immunohistochemical stain procedure	
3.a. PD-L1 antibody clone	SP263 Ventana
	28.8 Dako
	22C3 Dako
	Other, specify
3.b. Immuno-Stainer	Ventana
	Dako
	Other, specify
4. Histopathologic assessment of PD-L1 protein expression via immunohistochemical stain. - Attention: regardless of the scoring system applied, it is mandatory to always report the exact numeric value of PD-L1 expression; the use of range or intervals is discouraged, as the exact numeric value is required for subsequent therapeutic management.	
4.a. Combined Positive Score – CPS	CPS (exact value):
4.b. Tumor Proportion Score – TPS	TPS (exact value): … %
4.c. Tumor Area Positivity – TAP	TAP (exact value): … %
5. HER-2	

5.a. HER-2 antibody clone	CB11 Leica
	A0485 Dako
	4B5 Ventana
	Other, specify
5.b. Immuno-Stainer	Ventana
	Dako
	Leica
	Other, specify
5.c. Result	Score 0
	Score 1+
	Score 2+
	Score 3+
5.d. Pattern of staining	Homogeneous
	Heterogeneous
6. MMR Status	
6.a. MLH1 antibody clone	M1 Ventana
	Other, specify
6.b. MSH2 antibody clone	G219-1129 Ventana
	Other, specify
6.c. MSH6 antibody clone	SP93 Ventana
	Other, specify
6.d. PMS2 antibody clone	A16-4 Ventana
	Other, specify
6.e. Immuno-Stainer used	Ventana
	Other, specify
6.f. MLH1	Preserved expression
	Indeterminate expression[7]
	Complete loss of expression
6.g. MSH2	Preserved expression
	Indeterminate expression[7]
	Complete loss of expression
6.h. MSH6	Preserved expression
	Indeterminate expression[7]
	Complete loss of expression
6.i. PMS2	Preserved expression
	Indeterminate expression[7]
	Complete loss of expression
6.j. Overall assessment	Preserved MMR system (MMRp) – Low probability of microsatellite instability (i.e., Microsatellite stable (MSS))
	Deficient MMR system (MMRd) – High probability of microsatellite instability (i.e., Microsatellite instability (MSI))
7. Claudin 18.2 (CLDN18.2)	
7.a. CLDN 18.2 Clone	43-14A Ventana
	Other, specify
7.b. Immuno-Stainer	Ventana
	Other, specify
7.c. CLDN 18.2	… % (exact value) of neoplastic cells with moderate (2+) or strong (3+) staining intensity[8]
8. Quality control	
8.a. Is the Pathology Unit performing PD-L1 IHC enrolled in any external quality assessment scheme?	Yes, specify:
	No

1. esophageal, esophago-gastric junction, colorectal; 2. peritoneal, lymph node; 3. specify accession number and inclusion/tissue block selected; 4. specify the Institution that sent the specimen and where the sample was originally collected, and the related accession number and inclusion/tissue block selected; 5. specify the number of days between slide cutting to PD-L1 staining (usually, less than 1 day); 6. specify the number of months between inclusion/tissue block preparation to tissue slide sectioning for PD-L1 IHC stain; 7. expression should be considered indeterminate when detected in < 10% of tumor cells or when the staining intensity is lower than that of the internal positive control; 8. see references [30,84].

characteristics strictly related to PD-L1 expression assessment (e.g., PD-L1 scoring systems used and related score; Section 4). Considering their relevance for the clinical management of patients with GEC and to provide a complete report template, we thought it necessary to also include HER2 (Section 5) and mismatch repair system (MMR; section 6) sections. Finally, a declaration of enrollment in external quality assurance schemes is also included (Section t) to foster pathology unit participation in this type of activity. Most relevant literature references and a summary of the practical approach for available scoring systems are also provided to facilitate their use and reporting.

Conclusions and future perspectives.

The development of innovative approaches – such as the TAP scoring system – that support pathologists in their daily diagnostic activity is always gladly embraced by the pathology community. This is especially true in the field of ICIs and target therapy in general, where the role of the pathologist is to precisely assess predictive biomarkers, with a crucial role in subsequent therapeutic decisions. In this review, we aimed to provide clinical context as well as insights and practical suggestions to avoid difficulties in PD-L1 assessment and further facilitate and standardize its assessment.

Within the growing area of innovation in pathology, a major role is played by digital and computational pathology and AI-based algorithms. Despite regulatory and resource limitations, the implementation of AI tools into clinical practice has recently been implemented in specific niches, aiming to streamline and expedite the daily workload of often understaffed pathology units [85,86]. As highlighted by recent studies [87-89] and excellent dedicated reviews [90], these advanced computational tools offer significant promise for enhancing efficiency, improving the accuracy and reproducibility of biomarker scoring (including PD-L1), and ultimately reducing the workload burden on pathologists. To date, few studies focused on AI-based PD-L1 IHC scoring in GEC in contrast to other districts [90]. Initial data had relevant limitations, including the lack of cell-type discrimination and false-positive (pigment) and -negative (weak staining) issues [87]. Recent studies focused on CPS and reported more promising data, including (I) good accuracy (88%) [71], sensitivity (96%) [71], and positive predictive value (88%) [71], and (II) good agreement with dedicated pathologists (Cohen's kappa of 0.782 in an internal cohort and 0.737 in an external cohort) [91]. With this in mind, we expect to draft the next updated review on PD-L1 IHC assessment in GEC by introducing tailored AI-based diagnostic tools.

Conflicts of interest statement

FG: consulting or advisory role: MSD, GSK, Beigene; invited speaker: Pierre Fabbre, MSD, GSK, Servier, Astellas, Incyte, BMS, AstraZeneca, BeiGene, Daiichi-Sankyo, Amgen. FP: Research funding (to Institution) from Lilly, BMS, Incyte, AstraZeneca, Amgen, Agenus, Rottapharm, Johnson&Johnson, GSK. Personal honoraria as an invited speaker from BeOne, Daiichi-Sankyo, Seagen, Astellas, Ipsen, AstraZeneca, Servier, Bayer, Takeda, Johnson & Johnson, BMS, MSD, Amgen, Merck-Serono, Pierre-Fabre, Incyte, AstraZeneca. Advisory/Consultancy from BMS, MSD, Amgen, Pierre-Fabre, Johnson & Johnson, Servier, Bayer, Takeda, Astellas, GSK, Daiichi-Sankyo, Pfizer, BeiGene, Jazz Pharmaceuticals, Incyte, Rottapharm, Merck-Serono, Italfarmaco, Gilead, AstraZeneca, Agenus, Revolution Medicines. AV: consulting or advisory role (with honoraria) for Amgen, MSD Italia, Sanofi, BeOne. PP: consulting or advisory role: Amgen, Astellas, AstraZeneca, BMS, BeOne, Incyte, Servier, Daiichi-Sankyo, GSK, MSD, Diaceutics. LM: consulting or advisory role: MSD Italia, BeOne, Sanofi, Astrazeneca; invited speaker: MSD, Astrazeneca, Daiichi-Sankyo. LM is supported by the Italian Ministry of Health (Ricerca Corrente). MF: research funding (to the institution) from Roche, Thermofisher, and Diaceutics and personal honoraria from Roche, Astellas, AstraZeneca, Gilead, Lilly, Incyte, Bristol-Myers Squibb, Sanofi, Agilent, Merck Serono, Pierre Fabre, GSK, Novartis, and Amgen (speaker bureau), and Amgen, Astellas, Roche, Pfizer, Merck Serono, GSK, Novartis, and Janssen (advisory board).

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Authors' contributions

Conceptualization: FG, PP, LM, MF; writing — original draft preparation: AG, VA; writing—review and editing: all authors.

All authors have read and agreed to the published version of the manuscript.

Ethical consideration

The research was conducted ethically, with all study procedures being performed in accordance with the requirements of the World Medical Association's Declaration of Helsinki. Written informed consent was waived due to the nature of the manuscript.

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