The clinical significance of PD-L1 in colorectal cancer (Review)

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Abstract. Colorectal cancer (CRC) is one of the most frequently encountered neoplasms and has a high rate of morbidity and mortality. Recent findings showing that tumor immune evasion is an important mechanism underlying propagation of a cancer have changed the landscape of medical oncology through identification of Programmed-Death receptor 1 and its ligand (PD-1 and PD-L1) as novel targets for oncological immune therapies. PD-1 is primarily expressed on peritumoral lymphocytes and when activated, it suppresses its immune functions. Conversely, PD-L1 is primarily expressed on the tumor infiltrating front with the purpose of deregulating physiological cytotoxic immune responses. Numerous studies have linked PD-L1 overexpression to specific adverse clinicopathological features, such as poor differentiation, lymphovascular invasion and worse overall survival in CRC patients. Nevertheless, there is no concrete evidence showing which patients may exhibit the maximal beneficial effects of PD-1/PD-L1 blockade therapy, and how these novel molecular targets may be optimally integrated into therapeutic regimens for management of CRC patients with resectable and generalized disease.

Contents

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1. Introduction

Colorectal cancer (CRC) is one of the most frequently diagnosed types of cancer globally and one of the most prevalent causes of cancer-related death (1). CRC has a high percentage of morbidity and mortality (2), and has been shown to be associated with age, bacterial and viral infections, smoking, alcohol consumption, ulcerative colitis, genetic mutations, sedentary lifestyles and immunosuppression (3).

Surgery, chemotherapy, biological agents and/or radiotherapy comprise the existing modes of treatment for CRC. For second-line therapy, immunotherapy is garnering increasing attention and use, with anti-programmed cell death protein 1 (PD-1)/programmed death ligand-1 (PD-L1) immunotherapy showing promising results in melanoma and other types of solid tumors (4).

Since 2011, six antibodies against PD-1 or PD-L1 have been used as anti-cancer treatments following approval by the FDA (5): Atezolizumab (PD-L1 inhibitor), nivolumab (PD-1 inhibitor), durvalumab (PD-L1 inhibitor), avelumab (PD-L1 inhibitor), pembrolizumab (PD-1 inhibitor) and cemiplimab (PD-1 inhibitor). Pembrolizumab has been in use since 2017 for the treatment of microsatellite instability (MSI)-high metastatic CRC if the disease progresses following treatment with 5-FU, oxaliplatin or irinotecan based regimens, with promising results (6). For refractory MSI-high metastatic CRC, the CheckMate 142 phase II clinical trial showed that nivolumab may adequately control the disease (7).

It is possible that these novel molecular targets may revolutionize the management of solid tumors by adding an immunomodulatory component to the existing cytotoxic arsenal. In this context, the present review aims to summarize the current body of knowledge regarding targeting of PD-1/PD-L1 in the treatment of colorectal neoplasms, and to explore their significance in changing the landscape of medical oncology.

2. Molecular biology

PD-1 is a checkpoint protein and a member of the CD28 family that was discovered and named by Ishida *et al* (8) in 1992. It is expressed on activated T cells and interacts with its two ligands, PD-L1 (B7-H1/CD274) and programmed death ligand 2 (PD-L2/B7-DC), inhibiting the activation of T-cells and the production of cytokines (9) (Fig. 1).

PD-1 and its respective ligand molecule are immune checkpoint molecules that deliver co-inhibitory signals that suppress exaggerated immune responses (10). In solid tumors, overactivation of the intracellular signaling cascades involving MAPK and PI3K-Akt pathways, as well as increased activity of the STAT3 transcription factors all lead to increased expression of PD-L1 on the cellular membranes of cancer cells (11). Moreover, the presence of inflammatory mediators, such as interferon-γ and interleukin-6 further enhances PD-L1 transcription (12). Consequently, the presence of PD-L1 on the propagating tumor front binds to PD-1 molecules present on the surrounding populations of $CD8^+$ T-cells suppressing their clonal expansion and thus evading the suppressive innate immune response (13).

In CRC, PD-L1 is primarily expressed on tumor-infiltrating cells- such as B and T lymphocytes, macrophages, dendritic cells, other bone marrow-derived innate immune cells and vascular endothelial cells, providing valuable targets for the development of immune-oriented therapies (14-17). In a recent study, PD-L1 expression was more prevalent in liver and lung metastatic foci compared to the primary tumor (18).

In CRC, high densities of tumor-infiltrating lymphocytes (TILs) have been detected (19), and an association between the density of T cell subpopulations ($CD8^+$, $CD45RO^+$ and $FOXP3^+$) and a favorable clinical outcome has been previously shown (20). Within the microenvironment of the tumor, accumulation of $CD8^+$TILs (termed a 'hot' tumor microenvironment) has been shown to be correlated with MSI-high tumors, absence of perineural invasion, lymph node metastases, and high grade and proximal colon tumors. Tumors that are $PD\text{-}L1^+/TIL^+$ may benefit more from PD-1/PD-L1 blockade immunotherapy combined with conventional chemotherapy (20). Subsequently, TILs may possibly be used as a prognostic biomarker and a means of tumor classification (21). Galon *et al* (22) showed that CRC patients that had low densities of $CD3^+$ cells and $CD45RO^+$ memory T cells in both the center of the tumor and the invasive margin had a similarly poor prognosis to patients that had synchronous distant metastases.

It is well known that MSI-high tumors are commonly high grade proximal tumors that produce mucin, and exhibit increased inflammatory reactions and an exophytic pattern of growth (23-25). These tumors are also associated with a decreased risk of metastasis and improved prognosis (26,27). Patients with MSI-high or MMR deficient CRC exhibit improved responses to PD-1/PD-L1 immunotherapy and improved survival rates, suggestive of upregulation of tumor CD274 expression due to a high mutation burden in the tumor (28,29). Wyss *et al* (30) showed that increased PD-L1 expression was primarily associated with right-sided CRC tumors, females and elder patients. Interestingly, PD-L1 expression was significantly higher in MSI-high tumors, particularly in those harboring a BRAF mutation.

The expression status of PTEN in comparison with PD-L1 expression and clinical variables in CRC was studied by Song *et al* (31). Increased PD-L1 expression was correlated with distant metastases ($P<0.01$) and less favorable pathological features ($P<0.01$). Furthermore, PD-L1 expression was correlated with PTEN expression, whereas PTEN expression was not correlated with staging variables or overall and metastasis-free survival. A possible interplay between PTEN and PD-L1 expression is hinted by the results of their study, and possibly a trend towards increased rates of tumor dissemination in patients with PD-L1 positive CRC can be inferred, although the lack of prospective clinically relevant data is a major caveat.

3. PD-1/PD-L1 expression profiles in human neoplasias

Several types of cancer have been analyzed with regard to their PD-1 or PD-L1 expression profiles, and the effects of the subsequent therapy with anti-PD-1/anti-PD-L1 antibodies have been demonstrated in patients with melanoma, non-small cell lung and renal cancer (32-38).

Nonetheless, evidence now suggests improved patient outcomes may be achieved using PD-1/PD-L1 blockade therapy in several gastrointestinal malignancies, such as esophageal, gastric, liver and CRC (39). Results from the KEYNOTE-158 Phase II Clinical Study showed pembrolizumab administration improved outcomes in patients with non-resectable MSI-high non-CRC following failure of standard therapy (40). Similarly, in gastric cancer, it is evident that PD-L1 positive staining (>5% on tumor cells) is indicative of a poorer prognosis. When PD-L1 expression is combined with the MSI status or the extent of $CD8^+$TIL invasion, the PD-L1(+)/non-MSI and PD-L1(+)/CD8-low subgroups were correlated with poor prognosis, with higher HR compared with the PD-L1(+)-alone subgroup, emphasizing the potential of these biomarkers in distinguishing aggressive disease subtypes (41).

Other neoplasms that can also be treated with anti-PD-1/PD-L1 immunotherapy are: Hodgkin's lymphoma, head and neck squamous cell carcinoma, hepatocellular carcinoma, mediastinal lymphoma (large B-cell), cancer of the urinary bladder, Merkel cell carcinoma and MSI-high or MMR-deficient solid tumors (17).

In a meta-analysis by Huang *et al* (42), positive expression of PD-L1 was observed in ~50% of the patients that had gastrointestinal malignancies, and it was shown to be correlated with significantly poorer overall survival compared with malignancies that did not express PD-L1. A major drawback of this meta-analysis was the considerable clinical and statistical heterogeneity of the included studies, as is reflected by the I^2 Higgin's statistic of 89%, and the fact that the analyzed studies used different cut-off points to determine PD-L1 positivity. Despite these downsides, this study effectively demonstrated that PD-L1 positivity is indeed correlated with a worse overall survival, although the true magnitude of the effect could not be accurately estimated.

4. Implications of PD-1/PD-L1 in CRC according to stage

Taking into account the biomechanics of the interaction between PD-1 and PD-L1, analysis of two different expression profiles is mandated to evaluate a relationship between expression of

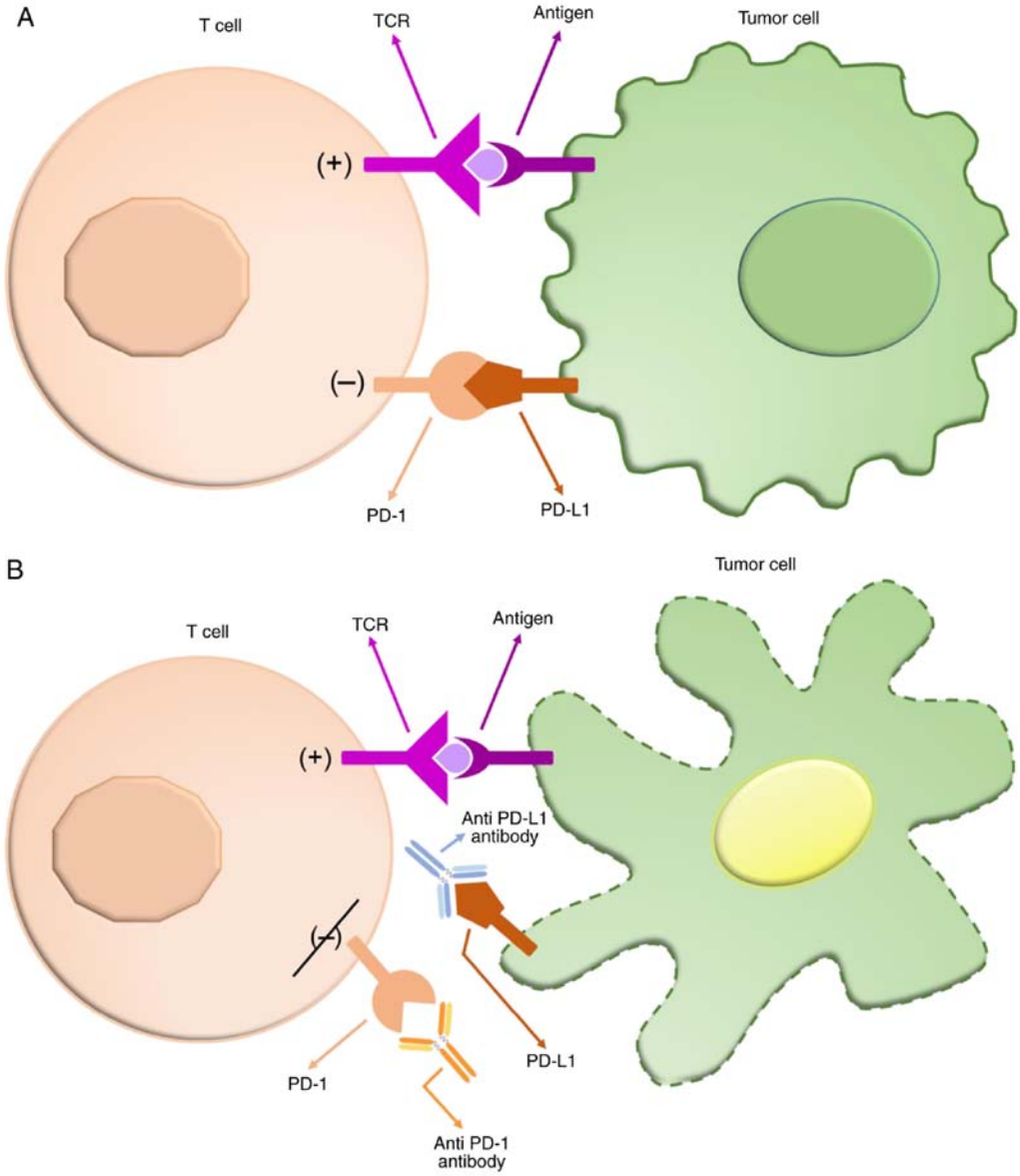

Figure 1. Schematic presentation of PD-1, PD-L1 and the interactions of targeting antibodies. (A) PD-1 and PD-L1 interaction inhibits the activation of T-cells and the production of cytokines, preventing the immune system from attacking the tumor cells. (B) Antibodies against PD-1 or PD-L1 induce killing of tumor cells by activating T cells. PD-1, programmed death receptor-1; PD-L1, programmed death ligand-1; TCR, T-cell receptor.

these biomarkers and patient survival. Specifically, PD-L1 overexpression gains significance when it is observed in the tumor infiltrating front, whereas expression of its molecular receptor is primarily relevant in the surrounding inflammatory cells of the innate immune system (TILs) (43). Based on this paradigm, increased expression of PD-L1 leads to immune evasion and suppressed expression of PD-1 results in enhanced penetration of the tumor by cytotoxic immune cells.

Taking the above into consideration, Droeser *et al* (44) showed that PD-L1 expression is associated with enhanced survival in MMR-proficient colorectal tumors, with median overall survival being 32 months in cases with high PD-L1 expression and 23 months for MMR-proficient tumors with absent or low PD-L1 expression. Expanding on these results, Li *et al* (45) studied 356 CRC patients from The Cancer Genome Atlas (TCGA) database and 276 CRC patients from the Fudan University Shanghai Cancer Center. MSI status was assessed by exome sequencing analysis in the TCGA cohort, classifying 243 patients as microsatellite stable (MSS) and 113 patients as MSI. They found a positive correlation between increased expression of PD-1 and PD-L1 with improved overall survival, both in TCGA patients and in the cohort of recruited patients. PD-1 expression in TILs and PD-L1 expression in tumor cells were independent prognostic biomarkers for overall survival and disease free survival (DFS), particularly for patients with a MMR-proficient status. This result highlighted the importance of the interplay between the lymphocytes and tumors in tumor spread. These results appear conflicting; plausibly, the observed enhanced overall survival in PD-1/PD-L1 positive tumors may be attributed to the presence of baseline clinical confounding factors, such as tumor stage and use of adjuvant chemotherapy, that were not accounted for in these two studies.

In a recent study by Enkhbat *et al* (46), 116 patients diagnosed with stage II or III CRC underwent curative resection, with stage III patients receiving adjuvant chemotherapy. The expression of PD-1 and PD-L1 was assessed on immune cells

and tumor cells, respectively and the results of the study showed no significant differences in DFS (5-years DFS rate, 77.9% in patients with PD expression vs. 78.1% in patients without PD-1 expression). As for OS rates, the 5-year OS was 74% in patients with PD-1 positivity vs. 92.4% for the PD-1 negative group, and 76.7% for PD-L1(+) patients vs. 93.2% for PD-L1(-) patients. As a result, PD-L1 positivity may be an independent risk factor of a poor prognosis, and both PD-1 and PD-L1 were independent prognostic biomarkers for overall survival but not for DFS, leading to the conclusion that PD-1 and PD-L1 are associated with metastases-related deaths rather than recurrence related deaths. Additionally, similar to other types of cancer, PD-L1 positivity is correlated with increased tumor aggressiveness and a greater metastatic potential (47,48).

A group of 572 patients diagnosed with stage II CRC were studied with the aim of identifying patients that may benefit from adjuvant therapy (49). The 5% cut-off of PD-L1 positive staining on tumor cells was used, as in previous studies (50-52). Overall, 6% of the colon tumors were classed as high PD-L1, and high PD-L1 expression was significantly associated with females, higher grade cancer, right sided tumors and MSI (high PD-L1 in 18% of MSI vs. 1% in MSS tumors), but not with survival.

As mentioned above, the relationship between PD-L1 and $CD8^+$TILs has been studied with regard to their prognostic value (20), to predict survival responses in patients with stage II or III CRC. Tumor differentiation and perineural invasion were shown to be associated with PD-L1 expression. However, there was no correlation between PD-L1 expression and MMR-deficiency status.

When reviewed in tandem, the previously discussed studies exhibited a greater degree of variability with regard to the effect of PD-1/PD-L1 expression on stage-stratified CRC patient survival. Although a correlation was identified, the retrospective nature of these studies does not allow for the establishment of a direct causal relationship between PD-1/PD-L1 expression and poor survival, and hence, no concrete conclusions can be drawn from the current body of literature. PD-1 and PD-L1 overexpression may likely be another facet of the aggressive neoplastic phenotype that encompasses perineural and microscopic lymphovascular invasion, and consequently, may not be directly implicated in the biological process of disease propagation and metastatic dissemination.

5. Prognostic significance

PD-L1 expression can also be utilized as an independent factor for assessing the suitability of patients for immunotherapy, as PD-L1 expression is commonly expressed in colorectal metastases in comparison with the primary tumors (81.8% in metastatic nodes vs. 40.9% in primary CRC) (18). This difference can be attributed to genomic alterations that accrue during the metastatic spread of the tumor (53,54) and post-translational modifications (phosphorylation, glycosylation and/or ubiquitination) that upregulate the expression of PD-L1 (55).

In a meta-analysis by Wu *et al* (56), several studies were incorporated to investigate PD-L1 expression and overall survival in solid tumors, with two studies pertaining to CRC (57,58). In CRC, PD-L1 expression was associated with a worse outcome with regard to the 5-year survival rate. This highlights the potential of PD-L1 as a prognostic tool, and that the combination of PD-L1 and PD-1 expression may contribute to the therapeutic response.

A meta-analysis of 10 studies and 3,481 patients by Shen *et al* (59) studied the clinicopathological parameters associated with PD-L1 expression in patients with colorectal tumors. Increased PD-L1 expression was found to be correlated with lymphatic invasion and advanced disease stage, but not with sex, MSI status or the location of the tumor. Therefore, PD-L1 expression may be used as an independent prognostic marker in colorectal tumors, taking into consideration the heterogeneity of the included studies and the subgroup analysis performed. Lymphatic invasion occurring due to epithelial to mesenchymal transition (EMT), as well as immunosuppression promoted by PD-L1 expression in tumor cells has been demonstrated; both of these phenomena lead to tumor growth and metastatic spread (60). Ultimately, there is a parallel interconnection between lymphatic invasion and PD-L1 expression, thus patients with lymphatic invasion or advanced cancer stage are more likely to express PD-L1. Nevertheless, there is no compelling evidence showing whether PD-L1 upregulation precedes lymphatic dissemination or if it ensues due to tumor propagation and dedifferentiation.

A meta-analysis by Cao *et al* (61), including 15 studies and 3,078 patients, revealed that PD-L1 overexpression was associated with less favorable overall survival and poor recurrence free survival.

Finally, in a meta-analysis by Li *et al* (62) from 2019 of 10 studies and 1,131 CRC patients, increased PD-L1 expression was associated with right-sided colonic neoplasms exhibiting poor differentiation. With regard to OS and DFS, both were significantly reduced in the highly positive PD-L1 patients, a finding consistent with previous meta-analyses.

Of note, cancer patients had a worse prognosis when treated with anti-PD-1/PD-L1 if they suffered from disorders of the intestinal flora (63), revealing a modulating role of the gut microbiota in the responses to immune checkpoint blockade (64). Further studies are required to determine the role of the intestinal flora in relation to immunotherapy.

6. Serrated neoplasms

Serrated adenomas and hyperplastic polyps have been considered as precursors of colorectal carcinomas that appear in the right colon and are MSI-high (65,66). However, a study by Tuppurainen *et al* (67) showed there was no significant difference in MSI status between serrated and non-serrated carcinomas. The prognostic importance of PD-L1 expression in serrated adenocarcinomas was studied by Zhu *et al* (68); the 3-year overall survival was 58.4% in serrated adenocarcinomas with high PD-L1 expression compared with 72.5% in serrated adenocarcinomas with low PD-L1 expression, although the difference was not significant.

7. Rectal cancer

Rectal cancer, is a separate subtype of CRC, and the rate of local recurrence is reduced by neoadjuvant chemotherapy, radiotherapy and total mesorectal excision (69-71). Studies

have attempted to survey the difference in the expression of PD-L1 in patients with rectal cancer before and after neoadjuvant chemo/radiotherapy, and to assess the potential use of PD-L1 as predictive marker or therapeutic target (72-74).

Jomrich *et al* (72) studied 29 patients that underwent surgery following neoadjuvant chemoradiation. Although the clinical and pathological responses to therapy were notable, PD-L1 expression was not detectable in the tumor cells, either before or after therapy. Conversely, weak PD-L1 expression was detected on stromal immune cells in five post-therapy specimens, 2 of 3 cases with complete pathological response, 2 of 22 with partial response and 1 of 4 patients with no response.

Conversely, Hecht *et al* (73) showed that low PD-L1 expression in cancer and inflammatory cells was a negative prognostic marker for overall survival, after studying the effect of neoadjuvant therapy on pre- and post-therapy specimens in 63 patients that underwent surgery for rectal cancer following treatment with neoadjuvant therapy.

The relationship between PD-L1 positive staining on tumor cells and clinical as well as pathological variables in rectal cancer following neoadjuvant therapy was retrospectively studied by Saigusa *et al* (74). High PD-L1 expression was associated with tumor recurrence and vascular invasion. Additionally, high PD-L1 expression was associated with poor recurrence free and overall survival rates. Thus, PD-L1 inhibition may reduce tumor recurrence and improve outcomes in patients with rectal cancer who undergo surgical resection following neoadjuvant therapy.

These results are in agreement with studies on other types of cancer. Which showed that chemo/radiotherapy results in upregulation of PD-L1 expression (75-78). Further studies are required for patients with rectal cancer to confirm this hypothesis. Existing studies suggest that different patient survival profiles are expected dependent on whether PD-L1 expression is upregulated in the tumor itself or in the surrounding reactive immune front. Based on existing studies, the former is associated with a worse survival outcomes (74), whereas the latter is associated with increased survival and improved outcomes following surgery (73). Ultimately, PD-L1 profiling may serve as a valuable therapeutic tool in patients with rectal cancer and low PD-L1 expression that may potentially be eligible for organ sparing surgery.

8. Special considerations

In a study by Koganemaru *et al* (51), 235 patients diagnosed with CRC (stage III) were included, with the aim of assessing of the potential effect of PD-L1 expression in tumor cells in the tumor microenvironment on the clinical and pathological characteristics of stage III CRC. Patients with high PD-L1 expression had significantly shorter disease-free survival times compared with patients with low PD-L1 expression. Conversely, patients with high PD-L1 expression on tumor-infiltrating mononuclear cells had longer disease-free survival periods compared with those with low expression. These results suggest that PD-L1 expression status can predict recurrence for stage III CRC, and at the same time it stresses the importance of separately assessing the expression of PD-L1 both in the tumor and in tumor-infiltrating immune cells.

Patients with low PD-L1 expression in tumor cells and high intratumoral $CD8^+$TIL infiltration had the best prognosis, whereas patients with high PD-L1 expression on tumor cells and low $CD8^+$TIL infiltration had the worst prognosis. These results indicate that the integration of PD-L1 and $CD8^+$TILs may be used as a suitable prognostic marker for stage II/III CRC patients (20).

In a study by Hamada *et al* (79), 617 CRC patients were studied to assess whether aspirin administration could improve survival in patients exhibiting low PD-L1 expression. The results suggested that aspirin administration following diagnosis of CRC improved overall survival (80). Aspirin administration following diagnosis was also correlated with longer cancer-specific survival in patients with low expression of PD-L1 in tumor cells compared with those with high expression, showing that aspirin as an adjuvant may increase the therapeutic benefit of immune checkpoint blockade in selected patients with CRC.

9. Treatment

There are six anti-PD-1 and anti-PD-L1 therapeutic antibodies currently in therapeutic use. Several studies have assessed the efficacy of these agents as a monotherapy or in combination with chemotherapy (81), radiotherapy (82), targeted therapies (83), antiviral drugs, neoantigen tumor vaccines (84) or with other immune-modulatory drugs (85).

Nivolumab, is an antibody that blocks PD-1 and has been used for treating patients with advanced or metastatic melanoma and metastatic squamous-cell lung cancer (86,87), with potential effectiveness for treatment of Hodgkin's lymphoma (88) and hepatocellular carcinoma (89). Several studies that included patients with CRC showed that nivolumab is well tolerated and that PD-L1 expression on tumor cells may serve as a predictive biomarker of response to therapy (32,90,91).

Overman *et al* (7) showed that nivolumab treatment exhibited favorable responses and disease control in patients with MSI-H/MMR-deficient metastatic.

CRC in a phase II study. In this study, 74 patients were enrolled. A median follow-up of 12 months was assessed and 51 of the patients had favorable disease control for ≥12 weeks (12-month overall survival, 73%). A study assessing co-administration of two immunotherapy drugs, nivolumab and ipilimumab (anti-CTLA-4 antibody), was also performed by Overman *et al* (92). Progression-free survival was 76% after 9 months and 71% after 12 months. Overall survival was estimated to be 87 and 85% respectively, highlighting a promising combination treatment for MSI-H/MMR-deficient patients.

Pembrolizumab, is an anti-PD-1 antibody that is currently used for treating patients diagnosed with advanced stage melanoma (93), urothelial cancer (advanced or metastatic) (94), advanced non-small cell lung cancer (95), Hodgkin's lymphoma (96) and squamous cell carcinoma of the head and neck (97). In the multicohort KEYNOTE-028 trial, patients with advanced CRC were treated with pembrolizumab, regardless of their MSI status (98). Median progression free survival was 1.8 months, with 6 and 12-month progression free survival rates of 17.4 and 4.3%, respectively. Median overall survival was 5.3 months, with 6 and 12-month overall survival rates

of 43.5 and 29.8%, respectively. No serious side effects were reported, highlighting the safety profile of the drug.

Le *et al* (6) assessed the clinical effectiveness of pembrolizumab in 41 patients with metastatic carcinoma, regardless of their MMR status. They stratified the patients into 3 groups: MMR-deficient CRC patients, MMR-proficient CRC patients and MMR-deficient non-CRC patients. They reported an objective response rate of 40% in the MMR-deficient cancer patients and 0% in the MMR-proficient cancer patients, with a progression free survival of 78 and 11% respectively, suggesting that MMR tumor status could be used to predict the efficacy of pembrolizumab therapy.

Recently, Le *et al* (99), assessed pembrolizumab therapy in 124 patients with treatment-refractory MSI-high/MMR-deficient metastatic CRC in a phase-II study, showing objective response rates of 33% in 31.3 months and 33% in 24.2 months. Progression free survival was 2.3 months and 4.1 months, respectively.

There are three anti-PD-L1 antibodies currently used for treatment of other types of cancer other than CRC: Atezolizumab (100-103), avelumab (104,105) and duravulumab (106,107). Atezolizumab may show promising results when administered to patients with MMR-deficient colorectal tumors, particularly when co-administered with chemotherapy and/or targeted therapy (108).

Several clinical trials are in progress assessing the efficacy of anti-PD-L1 antibodies, particularly in patients with MSI-high colorectal tumors, and they are showing encouraging results (109), even though the MSI-high/MMR-deficient patient subgroup constitutes only ~5% of those diagnosed with metastatic cancer.

PD-1/PD-L1 interaction is a remarkable regulatory immune system mechanism, with PD-L1 being primarily expressed on tumor cells in several types of tumor, including CRC (57). Subsequently, inhibition of the interaction between PD-1 and PD-L1 can induce an anti-tumor effect. Additionally, integration of these novel immune therapies may radically change the landscape of preoperative therapy, particularly in cases of rectal cancer, in which organ preserving surgery is recommended.

10. Conclusions

At present, there are several studies in progress assessing the safety and efficacy of drugs targeting immune checkpoints in association with PD-L1 and PD-1 expression in CRC. The primary issue that needs to be answered before these drugs can be used clinically are: What group of patients will benefit most from these therapies with regard to their MSI/MMR profile, the extent of $CD8^+$TIL infiltration and the status of PD-1/PD-L1 expression. Combinatorial approaches should be taken into consideration, taking into account the safety of the discussed therapeutic regimens. We are now in a new era of cancer therapies with the hope of improved survival and quality of life.

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Availability of data and materials

The datasets generated and analysed during the current study are all available in the PUBMED repository.

Authors' contributions

VN, KS and EP conceived the study. VN, DP, PF and IP designed the study. DP, AP, VN and IA acquired and analyzed the data. DP, PF, KS and VN interpreted the data. VN, DP, PF and EP drafted the manuscript. IA designed and executed the schematic presentation of Figure 1. All authors revised, read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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