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Prognostic and predictive role of immune microenvironment in colorectal cancer

Kuznetsova O *et al.* Immune microenvironment in CRC

Abstract

Colorectal cancer (CRC) represents a molecularly heterogeneous disease and one of the most frequent causes of cancer-related death worldwide. The traditional classification of CRC is based on pathomorphological and molecular characteristics of tumor cells (mucinous, ring-cell carcinomas, *etc.*), analysis of mechanisms of carcinogenesis involved (chromosomal instability, microsatellite instability, CpG island methylator phenotype) and mutational statuses of commonly altered genes (KRAS, NRAS, BRAF, APC, *etc.*), as well as expression signatures (CMS 1-4). It is also suggested that the tumor microenvironment is a key player in tumor progression and metastasis in CRC. According to the latest data, the immune microenvironment can also be predictive of immune checkpoint inhibitors response. In this review, we highlight how the immune environment influences CRC prognosis and sensitivity to systemic therapy.

Key Words: Immunoscore; Immune microenvironment; Colorectal cancer

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Core Tip: Here we describe the current data and perspectives of integrating the immune microenvironment in risk assessment and treatment strategies of colorectal cancer. The identification of tumors with immunoscore showing sensitivity to immunotherapy gave us insights to planning future clinical research.

INTRODUCTION

American Joint Committee on Cancer/Union for International Cancer Control is the most common staging system used for malignant tumors. It allows ranking patients by risk of progression depending on the size of the primary tumor (T), lymph node

involvement (N), and the presence of distant metastases (M)^[1]. However, the disease prognosis can vary significantly even within the same stage group^[2]. This variability raises the need for further personalization of staging and treatment approaches. Thus, for certain tumors a list of molecular predictive and prognostic biomarkers has been proposed to choose the optimal treatment strategy^[3,4].

The traditional classification of colorectal cancer (CRC) is based on pathomorphological and molecular characteristics of tumor cells (mucinous, ring-cell carcinomas, *etc.*), analysis of mechanisms of carcinogenesis involved [chromosomal instability (CIN), microsatellite instability (MSI), CpG island methylator phenotype (CIMP)] and mutational statuses of commonly altered genes (KRAS, NRAS, BRAF, APC, *etc.*), as well as expression signatures (CMS 1-4). The analysis of tumor microenvironment (TME) has been proposed as an alternative approach. This review highlights the influence of the immune environment on CRC prognosis and sensitivity to systemic therapy.

Immunological aspects of CRC

Immune evasion, or antigenic escape, occurs through various mechanisms. The production of cytokines that activate suppressor T lymphocytes and myeloid suppressor cell (MDSC) is a common mechanism of immune system evasion. It deactivates cytotoxic CD8+, CD3+, CD4+ lymphocytes and reduces the recognition of nonshared antigens. The other mechanism is the loss of major histocompatibility complex on tumor cells or programmed cell death-ligand 1 (PD-L1) activation. It leads to the depletion of peripheral T-cells and evasion of apoptosis, one of the hallmarks of cancer^[5,6].

It is recognized that CRC has low immunogenicity, and the use of immunotherapy, specifically immune checkpoint inhibitors (ICIs), can only be effective for a small subgroup of patients with this type of tumor. Specifically, immunotherapy is effective for tumors with impaired mechanisms of DNA mismatch repair (dMMR), which are characterized by high lymphocytic infiltration and high immunogenicity. The

deficiency of MMR proteins (MLH1, PMS2, MSH2, MSH6), which are responsible for correcting mismatch errors during replication, leads to the accumulation of mutations in microsatellites (short non-coding nucleotide sequences), causing MSI. This tumor phenotype is characterized by multiple neoantigens that are recognized by the immune system. Some studies have demonstrated a positive correlation between MSI status and CD8+ T-cells infiltration^[7-10]. However, MSI tumors also have peculiar mechanisms for evading the immune system, which is reflected in an increased expression of PD-L1, CTLA-4, LAG-3 and IDO ligands on myeloid cells along the tumor invasion margin (IM)^[11]. Thus, PD-L1 is not only a component of the PD-1/PD-L1 system, but also is a marker of a more complex interaction between the tumor and microenvironment^[12]. Droeser *et al*^[13] demonstrated that high PD-L1 expression is more common for microsatellite stable/MMR proficient (MSS/pMMR) (37%) than for MSI/dMMR (29%) tumors. A univariate analysis found that PD-L1 expression in MSS/pMMR tumors was associated with a lower depth of invasion, absence of regional lymph node involvement and vascular invasion.

In a meta-analysis by Fridman *et al*^[14], it was demonstrated that ⁴ high densities of cytotoxic CD3+, CD8+ and memory CD45RO+ T cells were associated with longer disease-free survival (DFS) after surgical resection of the primary tumor, and/or overall survival (OS) in melanoma, lung, pancreatic and gastric cancers. However, no impact on survival was found for other immune cells [B-lymphocytes, natural killer cells, macrophages, T helper subsets (Th2, Th17, and Treg), MDSC]. The systematic review of 200 published trials describing the role of immune cell subsets in 20 different disease entities demonstrated that the infiltration of cytotoxic CD8+ lymphocytes was associated with a favorable prognosis in 97% of studies^[15]. Additionally, Pagès *et al*^[16] found out that high infiltration densities of effector and memory T cells were associated with a low risk of lymphovascular and perineural invasion, as well as regional lymph node involvement among patients with localized CRC. By using immunohistochemistry (IHC), the authors identified a cluster of disease characteristics associated with a negative correlation of recurrence risk^[17]. Thus, the density of CD3+, CD8+ positive

cells, associated cytotoxic molecule granzyme B, CD45RO+ memory cells, assessed in the tumor center (TC) and the IM, made it possible to stratify patients into risk groups. In a multivariate analysis, the density of CD3 (TC)/CD3 (IM) lymphocytes was an independent prognostic factor [hazard ratio (HR) = 2.379; $P = 1.4 \times 10^{-6}$] in terms of DFS, and the only parameter associated with OS (HR = 1.89; $P = 1.2 \times 10^{-5}$) after adjustment for tumor size (T), degree of differentiation, and lymph node status (N). Overall, the studies have shown that various immunological infiltrates can correlate with prognosis, yet these findings require further validation.

The role of the microenvironment in resectable colon cancer

The prognostic role of the TME, as well as its assessment, has long been a question of debate. To standardize the assessment of TME role, Pagès *et al*^[16] created a prognostic scoring system, immunoscore (IS). This method is based on a quantitative IHC of CD3+ and CD8+ lymphocytes populations in the TC and IM with the use of digital pathology for accurate assessment. This scale grades the distribution of CD3+ and CD8+ lymphocytes into five categories, with IS 0 (I0) corresponding to a low density of both cell types in the TC and IM and IS 4 (I4) corresponding to a high density. Noteworthy, a higher IS value is associated with better patient survival.

The prognostic role of the IS system was validated in an international consortium for patients with stage I-III CRC based on the assessment of over 2500 patients^[18]. Additionally, IS analysis showed a high level of reproducibility between centers and pathologists ($r = 0.97$ for primary tumor and $r = 0.97$ for IM; $P < 0.0001$). Formalin-fixed, paraffin-embedded blocks containing TC (at least 5% of the tissue) and IM (with surrounding tissues) are needed for the analysis^[19]. In order to be applied in a research setting, two adjacent tumor slides are then stained with antibodies against CD3 and CD8 using the automated BenchMark XT immunostainer (Ventana Medical System). The slides are then scanned with a Hamamatsu NanoZoomer (Hamamatsu Photonics, Japan) to convert the physical slides into digital images. This instrument applies a 20 scanning resolution mode (0.45 $\mu\text{m}/\text{pixel}$) on a single focus plane. The images are

further uploaded into the software, which allows for the automatic detection of the tissue and its histological structure. After image processing, densities of CD3+ and CD8+ positive lymphocytes in the two regions of interests, TC or IM, are reported. According to an automated calculation, the density level of each marker in each region is translated to the density percentile defined previously by Pagès *et al*[16]. The mean density percentile is then calculated, categorizing IS into five classes (from 0 to 4). IS classified from 0 to 1 corresponds to low (IS low), IS-2 - to a moderate (IS Int) and IS-3-4 to a high CD3+ and CD8+ lymphocyte infiltrate (IS high).

According to the published data, the accuracy of predicting relapse-free survival (integrated area under the curve) for IS is comparable to staging based on T and N status. Multivariate analysis found that IS value, T and N status had a significant impact on patient survival, while the degree of tumor differentiation, perineural/lymphovascular invasion, MSI status and gender did not. The previous studies demonstrated that the prognosis of patients with MSI/dMMR locally advanced tumors depend on the degree of immune cell infiltration rather than on genomic (KRAS, NRAS, BRAF mutation status) or transcriptomic (CMS 1-4) signatures[20-22]. In a subgroup analysis of a phase III study, 600 patients with stage III CRC receiving adjuvant oxaliplatin-containing therapy were analyzed according to their IS statuses. In a group of low-risk patients (T1-3, N1), low IS was associated with poor 5-year PFS [77.5% *vs* 91.8%; HR = 1.70; 95% confidence interval (CI): 1.03-2.79; *P* = 0.037]. A similar trend was observed for high-risk patients (T4/N2) (5-years PFS for low IS 55.3% *vs* high IS 70.3%, HR = 1.65; 95%CI: 1.11-2.47; *P* = 0.013). When comparing low-risk patients with low IS and high-risk patients with high IS, long-term outcomes were similar (*P* = 0.174). In line with the previous results, in the meta-analysis of 11 studies and 5718 patients, Padayao J *et al* confirmed the prognostic role of IS in a group of patients with localized CRC. The authors demonstrated that the patients with low IS had worse PFS compared to patients with the high IS (HR = 1.75, 95%CI: 1.53-2.49) and OS (HR = 1.87, 95%CI: 1.45-2.13)[23]. Taken together, these findings raise the question about the role of

adjuvant chemotherapy (ACT) depending on the tumor immunogenicity^[24]. Overall, the results of multiple studies have validated the prognostic role of IS.

Despite the proven prognostic role of IS in early and locally advanced CRC, its impact on real clinical practice remains controversial. For instance, evidence for adjuvant treatment decisions based on IS value is still lacking. Given the disputable role of ACT in stage II CRC patients, more precise criteria are needed to determine the risk of recurrence for these patients. This question was addressed in several studies. In the analysis of quantitative infiltration of CD3+ and CD8+, evaluated in 113 patients with stage II CRC and immune cell infiltration, Wang *et al*^[25] demonstrated that it translated into a low (8%), intermediate (55%) or high (38%) IS value. The authors confirmed that ACT in patients with intermediate and low IS improved DFS compared with no systemic treatment after surgery (HR = 0.3; 95%CI: 0.1-0.92; *P* = 0.026)^[25]. A small American study of the ACT prescribing practices for stage II CRC patients demonstrated clinicians' willingness to integrate IS data into clinical practice. The authors asked 25 practicing medical oncologists to review the clinical data of 10 patients and decide whether the ACT recommendation was needed. An educational session was subsequently conducted, and the same patients' profiles were represented with added IS results. Except for a single participant (96%), all clinicians decided to change their management recommendation in more than a single case. Specifically, for the IS-high cases, recommendations for ACT decreased from 60% to 31%^[26]. Several works provide conflicting data on the necessity, duration and specific regimen of ACT based on IS value^[27-30] (Table 1). Therefore, the results of randomized trials are needed to further establish whether IS is ready to be introduced into routine clinical practice.

The assessment of IS using biopsy samples is crucial for timely decisions. Considering the prognostic significance of IS, a modified IS method has been developed to assess biopsy samples (ISb). ISb method excludes the need to assess TC and allows to consider the risk of locally advanced rectal cancer progression. Pagès *et al*^[31] confirmed a positive correlation between ISb value and the degree of pathological response to neoadjuvant chemoradiotherapy (CRT). A lower risk of relapse after CRT and surgery was also

6 demonstrated for patients with high rather than low ISb (HR = 0.21; 95%CI: 0.06-0.78; $P = 0.009$). The prognostic role of ISb for DFS was demonstrated in a multivariate analysis; its value was more reliable than pre- and post-neoadjuvant radiological assessment^[31]. The use of ISb was optimized to determine a cohort of patients suitable for Watch-and-Wait (WW) strategy. The patients with ISb-high had the lowest risk of recurrence when WW was chosen; 5-year DFS in groups of high, intermediate, low ISb were 97%, 61% and 56%, respectively. In a multivariate analysis, ISb was independent of age, gender, cTNM stage and was the only parameter that correlated with the time to recurrence^[32]. In summary, in addition to magnetic resonance imaging data following neoadjuvant computed tomography, consideration of ISb has been demonstrated to be effective in identifying candidates for a WW strategy among patients with locally advanced rectal cancer. Therefore, the assessment of ISb might be potentially implemented into routine clinical practice.

Taken together, these data have influenced the recommendations of international societies for medical oncologists. IS has already been mentioned in European Society for Medical Oncology consensus and Pan-Asian guidelines adaptation^[33,34]. It is proposed as an additional tool to TNM staging to determine prognosis and guide decision-making in ACT for low-risk stage II and stage III patients. In NCCN guidelines, IS is also discussed as a prognostic, but not predictive factor in terms of the effectiveness of ACT. For this reason, as well as considering the financial costs, experts do not advise the use of this test in routine practice when assessing the risk of recurrence or when considering ACT^[35].

Despite its extensive validation, the place of IS in patient management remains controversial. Moreover, the role of IS seems even more controversial due to the implementation of minimal residual disease assessment using circulating tumor DNA (ctDNA) analysis. In GALAXY, this approach was evaluated, leading to the conclusion that the most significant risk factor for recurrence was postsurgical ctDNA positivity (at 4 wk after surgery) for stage II-III patients (HR = 10.82, $P < 0.001$). Furthermore, postsurgical ctDNA positivity was identified in patients with stage II or III CRC who

derived benefit from ACT (HR = 6.59, $P < 0.0001$)^[36]. The subgroup analysis demonstrated that in addition to the ctDNA status, its dynamics should be considered, as the conversion of ctDNA from positive to negative from 4 to 12 wk after surgery might determine a more favorable prognosis (HR = 52.3, 95%CI: 7.2-380.5; $P < 0.001$)^[37]. Polyanskaya *et al*^[38] also confirmed the prognostic significance of ctDNA positivity after surgery in patients with stages I-III CRC. One-year PFS in the groups of positive and negative ctDNA status was 62% and 100%, respectively ($P < 0.001$), and in the group of stage II patients with negative ctDNA after surgery, disease progression was not observed in 100% of cases. Tie *et al*^[39] demonstrated that compared to the standard-management group, a lower percentage of patients in the ctDNA-guided group received ACT (15% vs 28%; risk ratio = 1.82; 95%CI: 1.25-2.65). Three-year recurrence-free survival was 86.4% among ctDNA-positive patients who received ACT and 92.5% among ctDNA-negative patients who did not. Thus, the importance of ctDNA as a factor for de-escalation of ACT was emphasized.

Despite the convincing evidence of the importance of ctDNA, clinical and morphological aspects cannot be ignored when considering ACT. Samaille *et al*^[40] have demonstrated that carcinoembryonic antigen (CEA) of more than 2 ng/mL is an important prognostic factor in terms of PFS regardless of the ctDNA status and the disease stage. The authors proposed the classification using the characteristics identified in a multivariate analysis (ctDNA, CEA and stage), which, assuming the most accurate prediction of PFS, identifies patients who would benefit the most from 6 months of ACT^[40]. Taken together, the question regarding adding IS to previously reported factors still needs answering. Wang *et al*^[25] analyzed the correlation between IS and ctDNA in patients with stage II CRC. The authors confirmed that IS-high patients have the lowest risk of recurrence: Among 43 patients (15% - high-risk patients with T4/risk factors), there was no progression during 3 years of follow-up independent of ACT recommendation. In intermediate- and low-IS patients, there was a statistically significant difference in 3-year DFS (85% with ACT, 62% - without ACT, HR = 0.3; 95%CI: 0.1-0.92; $P = 0.026$). In 49 patients, ctDNA analysis was performed after surgery;

positive status was associated with a higher risk of relapse (40% *vs* 2%, $P = 0.024$). A trend towards a higher rate of ctDNA detection in the case of low IS (ctDNA positivity results in the high, intermediate and low IS was detected in 5%, 12%, 25% respectively, $P = 0.339$) was observed. The authors explain the lack of statistical significance by small sample size and propose the assessment of both IS and ctDNA to optimize approaches to the ACT. However, it is currently unclear how to implement both IS and ctDNA analysis into patient management, and whether it is needed.

Microenvironment as a predictor of immunotherapy effectiveness

The predictive role of TME has been widely studied. TME plays an essential role in the efficacy of ICI therapy. Tumor infiltrating lymphocytes (TILs) are the main effectors of antitumor activity and are considered as predictive for immunotherapy response^[41]. Although immunotherapy results in long-lasting anti-cancer responses in patients with advanced melanoma, lung cancer, bladder cancer, its effectiveness is limited to a specific patient cohort^[42]. Unfortunately, there is currently no universal predictive biomarker to identify such patients, as the effectiveness of immunotherapy can be influenced by different microenvironment cell types. For example, Wang *et al*^[43] confirmed that high CD4⁺ and CD8⁺ infiltration was associated with melanoma response to ipilimumab, and the decreased CD8⁺ value in biopsies was associated with increased risk of relapse. In another prospective phase II study, the increase of TILs following ipilimumab treatment in metastatic melanoma was associated with a more pronounced response^[44]. Tumeh *et al*^[5] confirmed that high CD8⁺ infiltration in IM before treatment was associated with PD-1/PD-L1 expression and predicted a response to ICI in advanced melanoma patients. In 2015, based on data for melanoma and non-small cell lung cancer, a new classification of cancers was proposed considering the presence of TIL and PD-L1 expression^[45]. The detection of TILs and PD-L1 expression in TME can be associated with a greater sensitivity to ICI. Conversely, patients with PD-L1 negative tumors without TILs usually have a poor prognosis due to low immunogenicity. However, given that the TME is a complex system, density, location of

cell distribution and lymphocyte subpopulations must also be considered. In a pan-cancer study no correlation was observed between TILs levels prior to treatment and response to nivolumab therapy^[46]. Despite convincing evidence supporting the predictive role of TME, its complexity is a limiting factor and requires standardization.

The role of TME is especially critical in dMMR/MSI disease. Immunotherapy plays a major role in advanced dMMR/MSI CRC patients^[47,48]. Despite the high immunogenicity of these tumors, about 30%-50% are resistant to treatment with anti-PD1 ± anti-CTLA-4 antibodies^[47-49]. In pMMR/MSS tumors, the lack of response to ICI was demonstrated in several studies. In phase II study among 18 pretreated CRC patients pembrolizumab monotherapy resulted in an objective response rate (ORR) of 0% and mPFS of 2.2 months. In dMMR/MSI group ORR was 40%, mPFS and mOS were not reached^[50]. Dual blockade with durvalumab and tremelimumab for 166 patients with the pMMR/MSS CRC resulted in mPFS of 1.8 months in the treatment group and 1.9 months in the best supportive care group^[51]. In a study of botensilimab and balstilimab, the ORR among 41 patients with pMMR/MSS tumors was 24%, which was higher (42%) in the absence of liver disease. Thus, this data suggests that the location of metastases has the potential to influence the response^[52].

Differences in microenvironment provide a biological explanation for the unequal effectiveness of ICI in CRC among patients with MSI and MSS phenotypes. For example, pMMR/MSS tumors possess a greater number of tumor-associated macrophages, which was associated with a poor prognosis in most studies^[53]. The another study demonstrated that increased activation of β -catenin by melanoma cells leads to a decrease in the population of CD8 α ⁺ and CD103⁺ dendritic cells, which leads to decline in recruitment of cytotoxic T-lymphocytes into the TME^[54]. β -catenin is a known activator of Wnt pathway signaling in CRC. Furthermore, the APC gene, an important regulator of β -catenin, is mutated in over 70% of pMMR/MSS CRC cases^[55]. Additionally, mutations altering the APC gene occur in 20% of dMMR/MSI CRC cases, which can partially influence the resistance to ICI. The increased Wnt/ β -catenin activity in CRC is thought to be correlated with the absence of T-lymphocyte infiltration in the

TME^[56]. The activation of the transforming growth factor- β (TGF- β) pathway can provide an additional potential explanation of ICI resistance, as it promotes epithelial-to-mesenchymal transition. The tumors with this activated pathway belong to CMS4 (mesenchymal subtype)^[57]. The role of TGF- β in the microenvironment regulation in *in vitro* studies was associated with an increase in the number of regulatory T-cells and suppression of antitumor immunity^[58]. In particular, TGF- β activation was observed in liver metastases from CRC and was associated with the suppression of CD4+ and CD8+ lymphocytes^[59]. Preclinical studies evaluating TGF- β tyrosine kinase inhibitors demonstrated a decrease in the incidence of CRC metastasis to the liver^[60,61]. Despite the presence of other factors that explain the ineffectiveness of ICI in the pMMR/MSS CRC^[62], the accumulated data on the role of the microenvironment provide another promising area of application of IS in terms of the selection of candidates for ICI.

For instance, in CheckMate 9X8 the authors demonstrated no improvement in PFS [median 11.9 *vs* 11.9 months (HR = 0.81, 95%CI: 0.53-1.21) *P* = 0.3] or OS [median 29.1 months *vs* ND (HR = 1.03, 95%CI: 0.64-1.66)] while adding nivolumab to the first line FOLFOX + bevacizumab treatment. However, in subgroup analysis a level of CD8+ cells $\geq 2\%$ in the TME identified patients with longer survival rates with the addition of ICI (mPFS 13.2 *vs* 11.8 months with the addition of nivolumab for CD8 $\geq 2\%$ and CD8 $< 2\%$, respectively)^[63].

In a randomized AtezoTRIBE trial comparing FOLFOXIRI, bevacizumab with FOLFOXIRI, bevacizumab and atezolizumab, an increase in PFS was demonstrated [median 13.1 *vs* 11.5 months (HR = 0.69, 95%CI: 0.56-0.85, *P* = 0.012)] but not in OS (33.0 *vs* 27.2 months, HR = 0.81, 95%CI: 0.63-1.04, *P* = 0.136) for the entire patient cohort^[64,65]. The aim of the further analysis of AtezoTRIBE study was to characterize tumor immune cell infiltrate by assessing the TMB (in 65% of patients), TILs (83%), PD-L1 TPS expression (74%), IS (35%), IS IC (72%). The IS assessment methodology was based on a technique similar to aforementioned one. For IS-IC, surgically resected specimens or biopsies from either primary tumor or metastatic sites were used, and IHC was performed with antibodies to PD-L1 and CD8. Stained slides were then scanned with a

high-resolution scanner (NanoZoomer XR, Hamamatsu Photonics, Hamamatsu, Japan) to obtain 20 × digital images. The density of PD-L1+ and CD8+ cells in the tumor core were quantified by digital pathology using the HALO platform (Indica Labs, Corrales, New Mexico, United States). Five parameters, measured as linear values, were selected for inclusion into the IS-IC score density of total CD8, density of CD8-free (without PD-L1+ cell in proximity), density of CD8-cluster (CD8 cells in proximity of less than 20 micrometers of another CD8), density of PD-L1 cells, and distance between CD8-positive and PD-L1-positive cells). Depending on the described parameters, patients were divided into low risk (high CD8, PD-L1, density and small distance between them) and high-risk groups^[66]. Overall, the predictive role of TME on the effect of immunotherapy has been recognized, despite a major limitation for its use in clinical practice due to the heterogeneity in its measurement.

There is some data in regards to the agreement of IS and immunogenic signatures. For instance, a poor agreement was observed between TILs and IS or IS-IC (K of Cohen < 0.20). The disadvantage of describing TILs is only a rough assessment of lymphocytes in a sample without defining T-cell populations by their function, which may incorrectly reflect the immunogenicity of the TME. Discordance of 48% between IS and TILs density was previously reported^[17]. These differences may be attributed to the fact that IS and IS-IC are based on the assessment of predefined T-cell subtypes and describe their spatial distribution in specific tumor regions. TILs evaluation provides a semi-quantitative method for assessing unselected cell populations in randomly selected areas and is an operator-dependent technique. In a study by Boquet *et al*^[67] visual scoring of CD3+ and CD8+ T-cell densities to IS performed by different pathologists were compared. The disagreement of the results with the reference IS was observed in almost half of the cases (48%). The agreement among pathologists was minimal with a Kappa of 0.34 and 0.57 before and after training, respectively. The standardized IS assay outperformed expert pathologist assessment in the clinical setting^[67]. In AtezoTRIBE, among patients with pMMR tumors, there was no difference in PFS between patients with high and low TILs levels ($P = 0.36$), or with high and low PD-L1 expression ($P =$

1.0). However, regarding the impact of IS-IC, patients with a high value achieved longer PFS compared with patients with a low score (-16.4 *vs* 12.2 months; HR = 0.55, 95%CI: 0.30-0.99 *P* = 0.049). Although the data on OS was immature, it was described that IS/IS-IC had no prognostic value in the case of treating patients without the use of ICI. However, these immunogenicity criteria were proven to be predictive of response to ICI. Thus, the IS-IC has the potential to identify approximately 30% of pMMR/MSS CRC patients with an activated immune microenvironment who can potentially exhibit longer-lasting response with the addition of immunotherapy. Nonetheless, this requires further validation *via* prospective randomized studies, one of which has been planned by the authors of AtezoTRIBE and IS for 2024. A few studies have indicated that this approach to selecting patients for ICI treatment can be universal for a number of other tumors^[68] (Table 2).

CONCLUSION

TME plays an important role in disease progression and response to antitumor treatment. Based on accumulated knowledge about TME properties, the developed IS methodology and its modifications (could) might be implemented in clinical practice in the future. This test stratifies patients with CRC into risk groups depending on the tumor infiltration by immune cells, which correlates with the prognosis in a locally advanced disease. But introducing the IS test into routine practice to aid in ACT decision-making seems premature, despite its confirmed prognostic role. Another application of immune environment data might help identifying patients who are likely to respond to immunotherapy. Data from post-hoc analyses can provide valuable information to plan further studies to identify candidates for ICI treatment, even in pMMR/MSS tumors. Thus, supporting evidence from prospective studies designed with the participation of clinicians and pathologists is currently warranted to understand the possibility of incorporating characteristics of the TME into the treatment of the patients with locally advanced and metastatic disease.

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