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Review

A Narrative Review of the Role of Immunotherapy in Metastatic Carcinoma of the Colon Harboring a BRAF Mutation

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Abstract. *Patients affected by metastatic carcinoma of the colon/rectum (mCRC) harboring mutations in the BRAF gene (MBRAF) respond poorly to conventional therapy and have a prognosis worse than that of patients without mutations. Despite the promising outcomes of targeted therapy utilizing multi-targeted inhibition of the mitogen-activated protein kinase (MAPK) signaling system, the therapeutic efficacy, especially for the microsatellite stable/DNA proficient mismatch repair (MSS/PMMR) subtype, remains inadequate. Patients with MBRAF/mCRC and high microsatellite instability or DNA deficient mismatch repair (MSI-H/DMMR) exhibit a substantial tumor mutation burden, suggesting a high probability of response to immunotherapy. It is widely acknowledged that MSS/pMMR/mCRC is an immunologically "cold" malignancy that exhibits resistance to immunotherapy. The integration of targeted therapy and immunotherapy may enhance clinical outcomes in patients with MBRAF/mCRC. Efforts to enhance outcomes are exclusively focused on MSS/DMMR-BRAF mutant cancers, which constitute the largest proportion. This review evaluates the clinical efficacy and advancement of novel immune checkpoint blockade therapies for MSI-H/DMMR and MSS/PMMR BRAF mutant mCRC. We examine potential indicators in the tumor immune milieu for forecasting immunotherapeutic response in BRAF mutant mCRC.*

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Colorectal cancer (CRC) ranks as the third most common malignancy globally and the second most lethal cancer (1). The increase in the incidence of CRC in younger patients registered in the last decades may be linked to familiar, genetic, habits, alimentary, and environmental factors (2). Despite the efficacy of screening programs in diminishing mortality and morbidity associated with CRC, 20-35% of patients are first diagnosed with metastatic colorectal cancer (mCRC), which has a five-year overall survival (OS) rate of approximately 13% (3-5). In this setting, molecular profiling plays a pivotal role. The NCCN guidelines strongly support the performance of genetic analysis before starting treatment, including mutation of K- and *NRAS*, *BRAF*, and mismatch repair (MMR) genes (6). Wild-type RAS (wRAS) mCRC represent 40-45% of cases, while high microsatellite instability/DNA deficient mismatch repair (MSI/DMRR) represent approximately 4% of metastatic CRC. Only 12% of mCRC have a mutation in the codon 600 of the *BRAF* gene (MBRAF), *i.e.*, the V600E, in which glutamine substitutes for valine (7, 8). Mutated *BRAF* mCRC cases have a median OS shorter than 15 months (9).

Even today, chemotherapy (CT) alone or in combination with targeted agents is the therapeutic cornerstone for mCRC.

The FOLFOXIRI regimen, a combination of fluorouracil, leucovorin, oxaliplatin, and irinotecan with or without bevacizumab (BEV), is the recommended first-line therapy for fit patients with MBRAF/mCRC (10). Alternatively, a doublet regimen of CT plus BEV may be used if toxicity is a concern. However, progression-free survival (PFS) and median OS are far from being satisfactory (11).

Unlike other neoplasms, patients with MBRAF/mCRC do not benefit from single-agent BRAF inhibitors because of the intracellular activation of PI3K/Akt and Wnt/β signaling pathways, which support the development of resistance (8, 12-17).

In pretreated MBRAF/mCRC, the combination of targeted medicines, including BRAF and EGFR inhibitors, had favorable outcomes (18-20). Encorafenib (ENC) in conjunction with cetuximab (CET) enhanced the objective overall response rate (ORR), progression-free survival (PFS), and overall survival (OS) in previously treated patients with metastatic colorectal cancer (mCRC) compared to irinotecan-CET based chemotherapy. Notwithstanding the intriguing clinical efficacy, the combination of ENC, binimetinib, and CET (ANCHOR study) yielded inferior outcomes compared to CT in first-line MBRAF/mCRC treatment (21). Immune checkpoint inhibitors (ICIs) activate the immune system eliciting a potent anticancer effect and have also been tested in mCRC (22, 23). ICIs employed in clinical practice are monoclonal antibodies targeting cytotoxic T lymphocyte-associated antigen 4 and programmed cell death 1 receptor (PD-1), approved for the treatment of several malignancies (23). ICIs have significant activity in mCRC with high microsatellite instability (MSI-H) and a high cancer mutation burden (24). mCRC commonly displays BRAF mutations associated with MSI-H (30%) or DNA deficient mismatch repair (DMMR) (25). ICIs have recently been shown to achieve extended responses in patients with mCRC and high microsatellite instability/DNA deficient mismatch repair (MSI-H/DMMR) (26). However, ICI monotherapy is not very effective in MBRAF/mCRC with microsatellite stability/DNA proficient mismatch repair (MSS/PMMR) (27). In such setting, ICIs are being investigated in combination with other anticancer drugs, including BRAF inhibitors (28). This narrative review addresses the immunological microenvironment and the role of ICIs in BRAF-mutated metastatic CRC.

Pathogenesis of BRAF-mutant mCRC

The several diverse mechanisms leading to the development of CRC include the serrated and traditional adenoma-carcinoma routes, as shown in Figure 1 (29). Many serrated premalignant lesions result from precocious *BRAF* mutations (30, 31). BRAF is fundamental for tumor proliferation, differentiation, angiogenesis, apoptosis, and metastatic diffusion (32). Mutated BRAF kinase is constitutively phosphorylated, inducing the prolonged stimulation of MAPK pathway leading to cancer growth (12, 33). Metastatic CRC is often correlated with *BRAF* mutations, especially the BRAF V600E variant, which may be associated with the hypermethylation of the MLH1 promoter and the CpG island. Biomolecular investigations facilitated the categorization of MBRAF CRC into three classes: class I endowed with elevated kinase activity (V600 mutation), class II with non-V600 alterations, and class III characterized by insufficient kinase activity (33-37). The serrated pathway is strongly correlated to class 1, which is the most frequent and the worst prognostically, while class III CRCs are likely to have a prognosis close to that of wCRC (37). Three distinct molecular mechanisms are responsible for CRC: microsatellite instability (MSI), chromosomal instability (CIN), and the CpG (cytosine preceding guanine) pathway of methylation phenotype (CIMP) (38, 39). The microsatellites are part of a broad class of 1-6 kb DNA motifs that have significant levels of sequence variation that are present in all genomes (40). The dMMR status, which is primarily seen at microsatellites, results in many mutations or gene silence (41, 42). BRAF mutations are seen in less than 10% of MSS/mCRC cases (25, 35, 43). Classification of MBRAF/CRC may predict best treatment for patients (44). Molecular subtypes consensus (CMS) reported 70% of MBRAF/CRC belonging to the MSI immune-group (CMS1), which has higher number of immunologic cells and best OS (45, 46). Moreover, approximately 15% of MBRAF/mCRC are CMS4 (47). Moreover, genetic profiling differentiates some subtypes of BRAF V600E mutation, independently of sex, tumor side, and MSI or PI3K status (48). BRAF V600E Mutation 1 (BM1) has an overall more robust immunological profile than BRAF V600E Mutation 2 (BM2) mediated by pathways including the stimulation of IL2/STAT5, TNFα, IL6/JAK/STAT3 pathways, and allograft rejection. Patients with BM1 receiving EGFR inhibitors had better ORR, median PFS, and median OS than BM2 ones (49).

Immune Checkpoint Inhibitors in MBRAF/mCRC

MSI-H/dMMR subgroup. The use of ICIs in MSI-H/mCRC has led to considerable progress. The Keynote 164 phase II trial (NCT02460198) studied the PD-1 inhibitor pembrolizumab in previously untreated patients with MSI-H/DMMR mCRC. Single-agent pembrolizumab induced an 25% and 55% ORR in patients with MBRAF already treated with 1 or 2 lines of CT, respectively (50). The phase II multicenter, open-label Checkmate 142 trial (NCT02060188) is currently investigating nivolumab (NIV), another PD-1 inhibitor, alone or in combination with the CTLA-4 inhibitor ipilimumab. The investigator-assessed ORR was 31.1% [95% confidence interval (CI)=20.8-42.9%] in 74 patients with MSI-H/mCRC who had undergone prior treatment with at least one regimen, whereas the ORR in the metastatic BRAF cohort was 25% (51). In a separate trial, the combination of NIV with low-dose

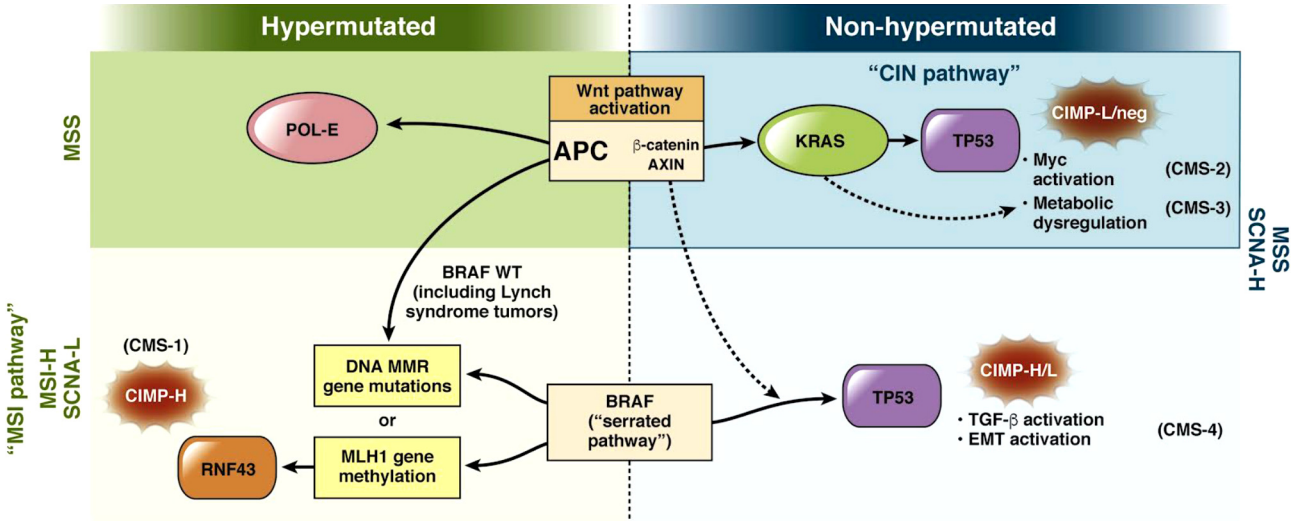

Figure 1. *Pathways of colorectal carcinogenesis. Activation of the Wnt pathway (primarily via APC mutation) or a mutation in BRAF can initiate colorectal tumorigenesis. BRAF mutations promote tumorigenesis via the serrated neoplasia pathway, leading to MSI with or without hypermutation (indicated in the Figure). Colorectal tumor classifications include CIN, MSI, and the serrated pathway (se CMS). L: Low; H: high; EMT: epithelial to mesenchymal transition (reproduced with permission from Elsevier through Copyright Clearance Center's RightsLink® service).*

ipilimumab (IPI) produced an ORR of 65% (95%CI=55-73%) and a disease control rate (DCR) of 81% (95%CI=72-87%) in previously treated patients (52). The overall response rate (ORR) was 70% in patients with MBRAF/mCRC, accompanied by a tolerable safety profile (52). No statistically significant difference was seen in the ORR with ICI between wild type and MBRAF/mCRC with MSI-H/DMMR (OR=1.04; 95%CI=0.48-2.25), despite the possibility that ICIs could provide MBRAF/mCRC patients with MSI-H/DMMR with a potential clinical response advantage in subsequent lines of therapy (9). The Checkmate 142 trial tested NIV in combination with a low-dose IPI regimen as first-line therapy achieving a 76.5% 24-month PFS rate and 76% investigator-assessed ORR in patients with MBRAF/CRC (53). The Keynote 177 phase III study compared single-agent pembrolizumab (PEM) to CT in treatment-naïve, patients with MSI-H/DMMR mCRC (26, 54). Although results showed no difference in median OS between the study arms (HR=0.74, 95%CI=0.53-1.03, p=0.036), the final analysis reported a significant prolongation in median PFS in favor of PEM (16.5 *vs*. 8.2 months; HR=0.59, 95%CI=0.45-0.79, p=0.0002). A subgroup analysis of MBRAF V600E cases showed lack of OS benefit (HR=0.72, 95%CI=0.35-1.47) even though there was a statistically significant difference in PFS (HR=0.48, 95%CI=0.27-0.86). With 60% of patients getting anti-PD-1 or anti-PD-L1 therapy following first-line CT, the lack of appreciable OS benefit could be related to the crossover design of the study. However, PEM improved the health-related quality of life (HRQOL) with fewer adverse events (54, 55). In the group of patients with MSI-H/DMMR mCRC, there was no difference in clinical benefit between the MBRAF and the non-MBRAF subgroups. The effectiveness of ICIs with conventional therapies in the subset of patients with MBRAF/mCRC should be compared in direct prospective trials. However, a recent real-world retrospective investigation reported a negative impact of the BRAF V600E mutation on the response to ICIs in MSI-H/mCRC (56). Patients with MBRAF/CRC and those with wild type showed a 44.4% *vs*. 74.2% ORR respectively, without a statistically significant difference (p=0.120) and shorter 12- and 24-month PFS rates than those of BRAF wild-type patients (12-months PFS rate of 40.0% *vs*. 73.3%, p<0.001; 24- months PFS rate 26.7% *vs*. 73.3%, p<0.001). Still, there were few BRAF V600E individuals in this subgroup analysis, and the treatment timing was unclear. A fraction of MSI-H/dMMR mCRC have a de novo or acquire resistance to ICIs showing no response to treatment.

New biomarkers to assess potential responders among patient populations and feasible treatment strategies require more research. Strategies for combined therapy have been proposed to address immunotherapy resistance. The phase III SEAMARK study is currently ongoing with the aim of comparing pembrolizumab as single agent or in combination with the EGFR inhibitor CET and the BRAF inhibitor ENC as the first-line treatment of MBRAF/mCRC with MSI-H/DMMR. In conclusion, ICIs may achieve long-lasting responses and improve prognosis, although some patients are refractory to therapy. The CA209-8HW trial randomized previously untreated patients to receive NIV-IPI (202 patients) or chemotherapy (101 patients) as first-line therapy. Of these

patients, 171 patients in the NIV-IPI arm and 84 patients in the CT arm had centrally confirmed MSI-H/dMMR. With 24.3 months of median follow-up, immunotherapy demonstrated clinically meaningful and statistically significant improvement in PFS *versus* CT, with a 79% reduction in the risk of disease progression or death [HR=0.21 (95%CI=0.14-0.32); $p<0.0001$]. These results support fist-line NIV-IPI as a standard-of-care option for patients with MSI-H/DMMR mCRC (57).

MSS subgroup. While treating MSS/PMMR mCRC with ICI alone demonstrated less benefit, the Keynote 016 trial revealed a substantial advancement in creating an immunological therapy strategy for MSI-H/DMMR mCRC (5, 27, 58, 59). Intrinsic resistance renders patients with MSS/PMMR mCRC resistant to single-agent ICI (60). Nevertheless, combination therapies, including ICIs, still need to be investigated further for MSS/PMMR mCRC, selecting pertinent patient populations with predictive biomarkers and overcoming intrinsic resistance, particularly for patients with MBRAF who have poor prognoses and no satisfying therapy options.

ICI plus anti-VEGF agents. Angiogenesis is an essential step in solid tumor growth. Cancers with the BRAF V600E mutation have a significant increase in proangiogenic factors (61). In the neoplastic tissue, aberrant and leaky blood vessels emerge because of the angiogenic vascular endothelial growth factor (VEGF) (62). Preclinical research suggests that VEGF may directly influence immune cells, such as regulatory T cells, dendritic cells, tumor-associated macrophages (TAMs), and myeloid-derived suppressor cells, all of which have been shown to exert immunosuppressive effects (63, 64). Therefore, inhibiting the VEGF signaling pathway may increase the efficacy of ICIs by normalizing the tumor vasculature and altering the immune environment.

There are currently few study data on patients with MBRAF/mCRC. In patients with MSS gastrointestinal malignancies, the phase Ib/II REGONIVO trial examined the safety and effectiveness of NIV plus the VEGFR2 inhibitor regorafenib, a multikinase inhibitor (65). The 25 patients in the MSS/PMMR mCRC cohort had a median PFS of 7.5 months and an ORR of 36%.The phase II NIVACOR study examined the efficacy of the FOLFOXIRI regimen in combination with BEV and NIV in 52 mCRC patients with a mutation in the RAS/BRAF gene (66). With a median duration of 7.59 (95%CI=6.21-11.43) months, the ORR was 78.9%. The median PFS and DCR for the MSS patient subgroup were 9.8 (95%CI=8.18-15.24) months and 96.2%, respectively. The outcome for patients with MBRAF/mCRC remains uncertain. In the meantime, a case report revealed that an MSS/PMMR mCRC patient with MBRAF had a PFS of more than 17 months when treated with the combination NIV and BEV (67).

ICIs in addition to MAPK pathway-blocking drugs. Immunotherapy may be aided by changing the tumor microenvironment, immune cell ratio, and MAPK pathway inhibition, according to growing research (68). In MBRAF metastatic melanoma, ICIs in conjunction with inhibitors of the MAPK pathway are effective (69-72). Research on these combinations has also been carried out in MBRAF/mCRC. In 73 patients with MBRAF/mCRC, a phase II trial evaluated patients with BRAF V600E mutant mCRC treated with the BRAF inhibitor dabrafenib in addition to the MEK inhibitors trametinib and spartalizumab (73, 74). The DCR was 70.3%, and the ORR was 24.3%. The median PFS of the 28 patients with MSS/mCRC who had not received prior treatment was 5.6 months, and the ORR and DCR were 25% (7/28) and 75% (21/28), respectively. A comparison between the combination of the PD-1 inhibitor and the dabrafenib plus trametinib treatment shown good efficacy (75). Following therapy, T cells and other immune cell types invaded tumor biopsies more heavily than in pre-treatment biopsies (73).

In patients with MSS and MBRAF/mCRC, a phase I/II trial assessed the dose, safety, and effectiveness of ENC when combined with CET and NIV (76). The trial is still actively recruiting, and the results showed an extraordinarily high ORR of 96% (95%CI=78-100%) and 48% (95%CI=27-69%). The average response time was 7.7 months (95%CI=4.5-NA). Notably, compared to targeted treatment, the median PFS and OS were 7.4 months (95%CI=7.7-NA) and 15.1 months (95%CI=5.6-NA), respectively, indicating greater efficacy. Patients tolerated the combination treatment well in terms of safety profile; just 19% (5/26) experienced grade 3 or 4 adverse events. A second randomized phase II trial (NCT05308446) investigates the advantages of combining NIV with CET and ENC in patients with BRAF V600E mutation-positive MSS mCRC who have already received treatment. In conclusion, the modest clinical trial implies that the combination of ICIs and MAPK inhibition may be beneficial for individuals with *BRAF*-mutated MSS/PMMR mCRC (77).

New Immunotherapy Response Biomarkers

Immune system cells. The tumor microenvironment (TME) is a dynamic system of many cell types, such as fibroblasts, stromal cells, immune cells, and extracellular matrix. This important factor affects how well immunotherapy works (78, 79). The development of tumors and their resistance to immune therapy depend on the immune microenvironment of a tumor (TIME), which is made up of immune cells, such as CD8+T cells, CD4+T cells, myeloid-derived suppressor cells (MDSCs), anti-inflammatory macrophages, natural killer (NK) cells, and related non-cellular components (80). It is imperative to comprehend the characteristics of TIME and effectively handle the involvement of immunosuppressive factors in TME to improve ICI efficacy in patients with mBRAF/mCRC.

Tumor-infiltrating lymphocytes (TILs). TILs including CD8+ cytotoxic T cells, CD4+ helper T cells, regulatory T cells, and B cells, are pivotal for the antineoplastic immune response (81, 82). Intra-tumoral TIL density may be a predictive marker for clinical outcome and response to ICI in a range of malignancies (81-83). Unluckily, no solid data on TIL density's predictive ability to evaluate ICI's effectiveness in MBRAF/mCRC are available. However, regardless of the presence of a *BRAF* mutation, a larger density of TILs was associated with a better prognosis (84, 85). Information on TIL density in MBRAF CRC is limited and somehow contradictory (86-88). A study including 24 patients with MBRAF/mCRC showed that *BRAF* mutation status was not significantly linked to the density of CD8+ (86). A different study found that the number of CD8+, CD3+, and FOXP3+ regulatory T cells was not substantially connected with *BRAF* mutation (87), while CD45RO+ T cells were more significant in 114 patients with MBRAF/mCRC (p=0.0006). When anti-PD1 therapy is used, TIL density around the periphery of tumor infiltration has been more strongly associated with anti-PD1 response than TIL density in the center of infiltration. The infiltrative borders of MBRAF/mCRC specimens exhibited a much higher density of FOXP3+ T cells compared to the core, which displayed lower densities of CD4+ and FOXP3+ T cells (p<0.001) (88).

Cen *et al*. conducted a thorough evaluation of the immunological milieu in MBRAF/mCRC (89). The expression of CD8+ T lymphocytes was significantly elevated in 43 patients with MBRAF/mCRC compared to patients WBRAF (p<0.001) (89). The proportion of CD8+ T cells was markedly elevated (p<0.01) in MBRAF/CRC tissues compared to WBRAF tissues, based on data from The Cancer Genome Atlas (TCGA) datasets, which comprised 59 patients with MBRAF and 337 patients with WBRAF (89). In contrast, the percentage of CD8+ T cells exhibited no significant differences between groups as per the Gene Expression Omnibus (GEO) datasets (89). CRCs with a BRAF mutation exhibit diminished tumor purity (p=0.0003), an elevated stromal score (p=0.02), a heightened immunological score (p<0.0001), and an increased ESTIMATE score (p=0.0001) compared to non-mutated counterparts. The assessment of TIL levels may be inadequate to fully delineate the intricate dynamics of BRAF mutant colorectal cancer. Consequently, this matter requires reevaluation, considering other factors, including CMS categorization and MSI status. Digiacomo *et al*. evaluated the tumor-infiltrating lymphocytes (TILs) in 22 microsatellite instability (MSI) and 37 microsatellites stable (MSS) metastatic BRAF-mutant colorectal cancer (MBRAF/mCRC) cases, revealing differences in TILs among various disease subtypes (90). The findings demonstrated that CD8+ and CD3+ lymphocytes, intra-tumoral lymphatic invasion (ILI), and peritumoral lymphatic invasion (PLI) were significantly more common in MSI tumors compared to MSS tumors (CD8, p=0.0001 and p<0.0001; CD3, p=0.003 and p=0.0003; ILI and PLI, respectively). Class 2 MBRAF/mCRC cases exhibited a higher infiltration of CD3+ and CD8+ lymphocytes compared to class 3 (p=0.033) (34).

Tumor-associated macrophages (TAMs). TAMs are an important component of the TME, contributing to tumor immunosuppression, development, invasion, metastasis, angiogenesis, and drug resistance through interactions with other immune cells (91). This population comprises M1 and M2 macrophages (MAC). M1 macrophages are classically activated, typically by lipopolysaccharides or IFN-γ, and are involved in producing proinflammatory cytokines, phagocytosing microbes, and initiating immune responses. The latter, however, produce reactive oxygen intermediates or nitric oxide to combat bacteria and viruses. M2 macrophages are alternatively activated by cytokines like IL-4, IL-10, or IL-13 and play a role in tissue repair and immune regulation. M2 macrophages can produce either proline to stimulate collagen synthesis or polyamines to promote cell proliferation. M1 MAC have an anticancer and proinflammatory effect, while M2 MAC may affect immunosuppression and cancer behavior (92). Initially recognized as M2-like MAC, TAMs were unreliable predictors of different cancer outcomes (92). There is limited data in the medical literature regarding the function of macrophages in MBRAF/mCRC. However, one study found that CD163+ M2 macrophages were significantly more abundant in MBRAF/mCRC tumors compared to wild-type tumors (mean 5.93 ± 3.02 *vs*. 3.67 ± 3.02, respectively, p=0.040) (93-95). In contrast, CD68+ M1 MAC levels were not different between wild-type and MBRAF patients (mean ± SD, 18.43 ± 13.53 *vs*. 20.96 ± 15.34, respectively, p=0.664). Additionally, patients with MBRAF have more M1 MAC than patients with WBRAF (p<0.05). However, a study that included 110 patients with MBRAF CRC and 798 patients with WBRAF CRC from the TCGA and the GEO datasets revealed no significant variations in M2 MAC between these groups (89). In another investigation, considering a subgroup of patients with unresectable mCRC based on BRAF status, high tumor infiltration by CD68+ MAC had no prognostic value for MBRAF/mCRC (92). However, WBRAF/mCRC patients with more tumor infiltration by CD68+ MAC had a better median OS (p=0.002) (92). Further studies are needed to clarify the features of TAMs in this subgroup of CRC and their impact on immunotherapy and prognosis (95).

PD-L1 expression. The transmembrane protein PD-L1, sometimes referred to as CD274 or B7 homolog 1 (B7-H1), is a membrane protein in cancer cells that inhibits the immune system by binding to PD-1 on T cells, facilitating escape from immunosuppression. ICIs targeting PD-L1, or PD-1 demonstrate efficacy in several malignancies outside CRC. Patients with CRC exhibit PD-L1 expression in approximately

9-15% of instances, and this expression serves as a predictor for responsiveness to ICIs, even though anti-PD-1/PD-L1 therapy proves unsuccessful for several patients with mCRC (96-98). The MSI-H status forecasts the effectiveness of ICI therapy in mCRC (58). Notably, MSI colon cancer exhibited elevated PD-L1 expression levels compared to the MSS cohort (99-101). Consequently, PD-L1 expression may serve as a significant biomarker for forecasting the efficacy of ICIs in mCRC.

Certain characteristics of serrated carcinoma, including poor differentiation, solid/medullary histology, MSI-H or DMMR, and BRAF mutation, are associated with the activation of PD-L1 (100, 102-104). Cen et al. indicated that 43 patients with MBRAF/mCRC exhibited markedly elevated expression levels of PD-L1, PD-1, CTLA4, and LAG3 (89). The V600 mutation is associated with elevated PD-L1 expression in MSS/CRC cell lines, indicating a linkage between BRAF mutation and PD-L1 expression. This indicates that PD-L1 expression is not limited to MSI-H, MBRAF CRC. Despite the absence of evidence for the MBRAF patient subset, a definitive association between PD-L1 expression and the efficacy of ICIs is lacking (51, 52). Additional immune-independent roles of PD-L1 expression in MBRAF/mCRC are present. Feng et al. demonstrated that the oncogenic BRAF V600E mutation can transcriptionally enhance intrinsic PD-L1 expression in colon cancers, hence improving CT-induced apoptosis through the up-regulation of BIM and BIK proteins (105, 106). They also showed that the c-JUN and YAP genes enhance PD-L1 expression in MBRAF/CRC (106).

Tertiary Lymphoid Structures (TLS)

Tertiary lymphoid structures (TLS) occur mainly in infectious diseases, inflammatory disorders, autoimmune syndromes, as well as tumors (107). As a part of the tumor microenvironment, TLS play a role in the growth and dissemination of malignancies (108). TLS may also be a predictive biomarker for ICI activity and a prognostic biomarker for human cancers (107, 108). Posch *et al*. reported that CRC with BRAF and MSI-H mutations is linked to greater TLS density (median: 0.61 *vs*. 0.45, p=0.03). TLS is primarily seen in the periphery of CRC tumor tissues (97%) (109).

Overall, several CRC clinical trials, including limited numbers of patients, have suggested that additional immunotherapeutic biomarkers, aside from MSI, are also connected with patient survival and ICI efficacy. As such, these findings require confirmation in larger investigations.

Conclusion

Patients with MBRAF/mCRC exhibit a limited response to standard treatment, resulting in a bleak prognosis (110). Patients with mCRC exhibiting BRAF V600E mutations may derive benefits from a combination of a BRAF inhibitor and an EGFR inhibitor ± MEK inhibitor; nonetheless, the overall response rate (ORR) is approximately 20%, but improved outcomes may occur in those with MSS and RNF43 mutations (18, 111).

ICI has achieved sustained responses in patients with MSI-H/DMMR mCRC. Subgroup analysis of clinical studies indicates a benefit for MSI-H patients with MBRAF/mCRC, while additional confirming data is required. Nonetheless, not all patients with MSI-H/DMMR exhibit a response to ICIs. A meta-analysis of 16 trials including 1503 patients with CRC showed a 42% ORR in the MBRAF and 19% in the RAS mutant group. The ORR was 14% in the PD-L1 positive subgroup and 32% in the PD-L1 negative subgroup suggesting that low expression of PD-L1 can be potential predictive marker for positive response and outcome (112).

Given that mBRAF/mCRC is a heterogeneous tumor subtype with diverse responses to ICI, additional studies into supplementary predictive biomarkers for identifying immunotherapy-sensitive patients beyond MSI-H is warranted. Patients with MSS/PMMR mCRC exhibit no response to ICIs when used alone. In the Enco-CTX-Nivo and Dabra-Trame-Sparta studies, ICIs appear to enhance the efficacy of BRAF inhibitors. Notable data have been documented with immunotherapy and MAPK pathway inhibition in mBRAF/mCRC patients with MSS/PMMR; nevertheless, confirmatory phase III clinical trials are necessary. Moreover, certain combinatorial therapies, including ICIs with radiation, CT, and tumor vaccines, may enhance the host immune response and surmount resistance to singular targeted therapies. Although MSI appears to mitigate the unfavorable prognosis associated with BRAF mutation, other biomarkers are necessary to enhance patient selection and create more precise biomarkers. The plasmatic BRAF-V600E allelic fraction holds prognostic relevance in mCRC undergoing anti-BRAF combinatorial therapies, effectively identifying individuals likely to benefit from ENC plus CET (113, 114). Ultimately, enhanced outcomes may be achieved by integrating BRAF inhibitors with Wnt pathway inhibitors and ICIs. Newer studies focused on other potential diagnostic and prognostic biomarkers. Collagen triple helix repeat containing 1 (CTHRC1), which may act as a negative regulator of collagen matrix deposition, may have potential use in CRC. A high expression of CTHRC1 was associated with mBRAF and with poor prognosis and worse clinicopathological features and with decreased sensitivity to anti-MBRAF drugs (115).

In conclusion although remarkable progresses have been achieved in the last decade, further studies and confirming phase III trials are required.

Conflicts of Interest

The Authors declare no conflicts of interest in relation to this study.

Authors' Contributions

All Authors contributed to the medical literature search and evaluation. AC and VG wrote the manuscript draft and edited it. All Authors read and approved the final version of the manuscript.

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