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The Progress, Achievement and Challenge of Immunotherapy in Colorectal Cancer

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Abstract

Colorectal Cancer (CRC) is currently one of the most common malignant tumors worldwide, and its incidence and mortality are increasing year by year. In 2023, colorectal cancer has become the second most common cancer in China. Fortunately, the advent of immunotherapy has changed the therapeutic prospects of various solid tumors and has gradually become the main treatment for metastatic or recurrent solid tumors in recent years, including non-small cell lung cancer, liver cancer and malignant melanoma. Among them, encouraging progress has been made in the use of Immune Checkpoint Inhibitor (ICI)-based immunotherapy in patients with Colorectal Cancer (CRC), and various immunotherapeutic agents such as pembrolizumab, nivolumab, and ipilimumab have been approved for the treatment of advanced colorectal cancer. In this review, we summarize the past advances in immunotherapy in colorectal cancer and briefly introduce biomarkers that may play a key role in efficacy prediction in colorectal cancer immunotherapy.

Keywords: Colorectal cancer; Immunotherapy; Immune checkpoint inhibitor

Introduction

Colorectal cancer, a widespread malignant tumor, is the third most frequent cancer and the second factor of the causes of cancer-related death globally [1]. According to the latest cancer burden data released by the World Health Organization's International Agency Research on cancer, more than 1.9 million new colorectal cancer cases and 935,000 deaths were estimated to occur in 2020, representing about one in 10 cancer cases and deaths [2].

The treatment of colorectal cancer includes surgery, chemotherapy and radiotherapy. However, because early colorectal cancer lacks typical clinical symptoms, although screening has reduced the incidence and mortality, most patients have reached the stage of progression at the time of diagnosis. Moreover, up to 50% of patients with locally advanced disease eventually develop metastases [3]. Locally Advanced Colorectal Cancer (LACRC) is defined as CRC stage II (cT3–4, N0)/stage III (any cT, N+) [4]. During recent years, advancements in standardized surgery and subsequent improvements in neoadjuvant therapy have improved outcomes. The advantages of neoadjuvant therapy are that it can reduce tumor stage, improve the rate of R0 resection, decrease the rate of local recurrence, and enable some patients to achieve a clinical Complete Response (cCR) or even a pathological Complete Response (pCR). However, distant metastases, surgical morbidities, and Adverse Effects (AEs) caused by neoadjuvant radiotherapy or chemotherapy remain significant problems. Among the metastatic CRC (mCRC) patients, small fraction of these patients can undergo curative resection and the overall survival of the patients with metastatic tumors reaches only 30 months [5]. While the benefit of classical chemotherapy regimens plateaued, we urgently need to develop a new effective treatment strategy to improve the survival prognosis of colorectal cancer.

Immunotherapy, aiming to boost natural defenses to eliminate malignant cells, is a monumental breakthrough for cancer treatment and has revolutionized the field of oncology [6]. The history of immunotherapy could be traced back to century ago, which unleashing the host immune system to combat cancer. After a century of development, particularly the last decade, with the discovery of Immune Checkpoint Inhibitors Immunotherapy (ICIs), immunotherapy has revolutionized cancer treatment and rejuvenated the field of tumor immunology [7,8]. ICIs can reinvigorate antitumor immune responses by interrupting coinhibitory signaling pathways and to promote immune-mediated elimination of malignant cells [9,10], which is recognized as a very effective therapy for patients with CRC that is Mismatch-Repair-deficient (dMMR) or Microsatellite Instability-High (MSI-H) (termed dMMR/MSI-H CRC) [11]. In 2020, KEYNOTE-177 Confirmed Clinical

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Benefit of Pembrolizumab as First-Line Treatment for MSI-H/dMMR mCRC, at the same year, FDA approves Pembrolizumab as First-Line Treatment for MSI-H/dMMR mCRC. At the same time, MSI-H or dMMR populations have also achieved striking efficacy in the field of neoadjuvant immunotherapy [12,13]. However, the application of colorectal cancer neoadjuvant immunotherapy still faces many adjustments. In this review, we summarize the types of immunotherapies that have been clinically applied so far, briefly introduce the current status of the application of neoadjuvant immunotherapy, and the controversies and challenges faced by neoadjuvant immunotherapy for CRC. And we also discuss the development direction of neoadjuvant immunotherapy research in the future.

Current status of neoadjuvant immunotherapy

The activation of Major Histocompatibility Complex (MHC)-T Cell Receptor (TCR)-dependent signaling has been demonstrated to be a critical pathway for the immune system to kill tumor cells [14,15]. However, TCR recognition peptides and class I MHC molecular complexes alone are not sufficient to activate T cells, and the TCR-MHC signaling pathway is regulated by signals released from tumor cells and can act as costimulatory or coinhibitory signals. Cancer cells engage with inhibitory ligands (against PD-1 and CTLA-4) to prevent cytotoxic killing of tumor cells [10,16,17], targeting this mechanism, a new class of monoclonal Antibodies (mAbs), Immune Checkpoint Inhibitors (ICIs), have become one of the most important immunotherapies [18]. ICI inhibitory receptors and ICIs can bind selectively to inhibitory ligands against PD-1 and CTLA-4 in CD8+ T-cell, and block inhibitory receptors, which allows active T cell function towards cancer cells. Nowadays, the most widely used targets for ICIs are Cytotoxic T Lymphocyte-Associated molecule-4 (CTLA-4), Programmed Cell Death receptor-1 (PD-1), and Programmed Cell Death Ligand-1 (PD-L1).

Rizvi et al. research has shown that immunotherapy efficacy is associated with Tumor Mutational Burden (TMB), and class I MHC molecules with High Tumor Mutational Burden (TMB-H) can produce more peptide neoantigens and are recognized as "non-self", which trigger T cell activation and kill tumor cells [19]. The immunogenic subtype of CRC is related to a defective Mismatch Repair (dMMR) system and associated with High-frequency Microsatellite Instability (MSI-H). The dMMR/MSI-H cancers have long been associated with strong lymphocytic infiltration in and around the tumor [20,21]. The dMMR tumors have a high tumor mutational burden and an abundance of neoantigens [22], which is usually caused by mutations in genes encoding Mismatch Repair (MMR) proteins (MLH1, MSH2, MSH6, and PMS2) responsible for recognizing and correcting mismatched nucleotides, or by methylation of the MLH1 gene promoter [23]. If the MMR system fails, mismatches accumulate and cause changes in microsatellite sequence length or base composition, called Microsatellite Instability (MSI), and lead to a highly mutated phenotype [24,25]. Therefore, the defects in the MMR gene result in loss of repair protein expression or function, which results in the production of proteins containing Mutated Neoantigens (MANA), and then, MANA/MHC complexes bind to T cells [26], the latter contributing to an activated immune cell response and anti-tumor activity.

In 2010, Julie R. Brahmer et al. first applied immunotherapy to patients with metastatic colorectal cancer and found that one of the mCRC patients achieved prolonged complete remission (3 years) after immunotherapy [27]. After that, in 2015, Le et al. first presented the KEYNOTE-016 study at the ASCO annual meeting. In this study, pembrolizumab, an anti-PD-1 agent, was found to have a significantly higher response rate in CRC patients with DNA Mismatch Repair deficient (dMMR)/Microsatellite Instability-High (MSI-H) molecular type, suggesting that CRC patients with dMMR tumors may benefit from anti-PD-1 agents [11,28]. This opens a new era of colorectal cancer immunotherapy.

Beyond the above clinical studies, in recent years, many clinical studies have demonstrated the therapeutic potential of ICIs for CRC patients. In 2017, the CheckMate 142 trial demonstrated the clinical benefit of nivolumab over second-line treatment in patients with MSI-H/dMMR mCRC [29]. The CheckMate 142 study included 76% of the population after second-line and above chemotherapy and/or targeted therapy, and the ORR and Disease Control Rate (DCR) remained as high as 69% and 84%, respectively, with a complete response rate of 13%. The same year, KEYNOTE-164 trial and KEYNOTE-158 trial demonstrated a significantly greater survival benefit with pembrolizumab compared with second-line therapy for patients with advanced MSI-H solid tumors [30,31]. In KEYNOTE-164 trial, 63 patients were included, the objective response rate was 32%, and the median progression-free survival was 4.1 months. The median overall survival is yet to be reached. The 1-year progression-free survival and overall survival rates were 41% and 76%, respectively.

A phase 2 trial (NCT03667170) evaluated the efficacy of envafolimab in patients with treated dMMR/MSI-H mCRC. Among the 65 CRC patients included, the objective response rate was 32%, and the median progression-free survival was 4.1 months. The median overall survival is yet to be reached. The 1-year progression-free survival and overall survival rates were 41% and 76%, respectively [32].

CheckMate 142 and KEYNOTE 177 investigated the value of posterior line and first-line treatment of dMMR or MSI 177 metastatic colorectal cancer, respectively. The results of the KEYNOTE 177 study at a median follow-up of 32.4 months showed that the pembrolizumab monotherapy group was superior to the chemotherapy group in Progression-Free Survival (PFS) (16.5 *vs.* 8.2 months) as well as quality of life. Accordingly, the US FDA approved pembrolizumab as a first-line treatment for MSI-H advanced colorectal cancer [33] (Figure 1).

The application of neoadjuvant immunotherapy in CRC

The aim of neoadjuvant therapy is to shrink the tumor, remove tumor micrometastases, reduce the difficulty of subsequent radical surgery, reduce the risk of postoperative tumor recurrence, and improve patient outcomes. Nowadays, neoadjuvant therapy for advanced colorectal cancer is based on traditional chemoradiotherapy, especially rectal cancer. According to the data of neoadjuvant therapy for rectal cancer in our hospital between 2010 and 2021, neoadjuvant short-course radiotherapy combined with consolidation chemotherapy had similar pCR rates (19.4% *vs.* 20.5%) to classical long-course concurrent chemoradiotherapy; meanwhile, neoadjuvant chemotherapy alone had inferior pCR rates (7.9%) to neoadjuvant chemoradiotherapy. Neoadjuvant therapy for colon cancer is rarely studied and applied, and chemotherapy and targeted therapy are currently the main treatment. According to CAO/ARO/AIO-94 trial, of 799 eligible patients, 404 were randomly assigned to preoperative and 395 to postoperative Chemoradiotherapy (CRT)

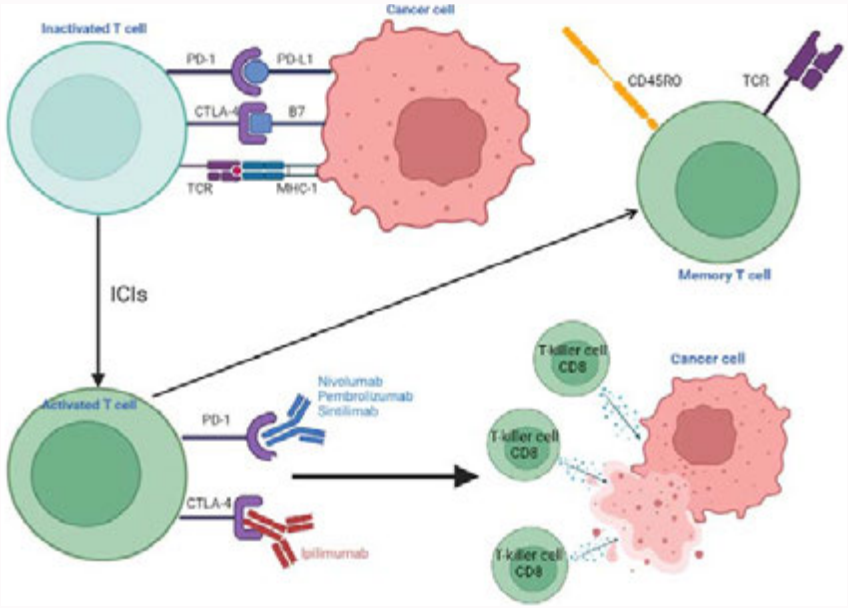

Figure 1: Mechanisms of immune checkpoint inhibitors in colorectal cancer patients.

[34]. The result shown that overall survival at 10 years was 59.6% in the preoperative arm and 59.9% in the postoperative arm (P=0.85). The 10-year cumulative incidence of local relapse was 7.1% and 10.1% in the pre- and post-operative arms, respectively (P=0.048). No significant differences were detected for 10-year cumulative incidence of distant metastases (29.8% and 29.6%; P=0.9) and disease-free survival. Another multicenter, open-label, phase III trial, FOWARC study enrolled 495 patients from June 2010 to February 2015; 475 were evaluable (fluorouracil-radiotherapy, n=155; mFOLFOX6-radiotherapy, n=157; mFOLFOX6, n=163). In the fluorouracil-radiotherapy, mFOLFOX6-radiotherapy, and mFOLFOX6 groups, the rate of pathologic Complete Response (pCR) was 14.0%, 27.5%, and 6.6%, and downstaging (ypStage 0 to 1) was achieved by 37.1%, 56.4%, and 35.5% of patients, respectively. Higher toxicity and more post-operative complications were observed in patients who received radiotherapy [35].

The NICHE study: The single-arm NICHE study from the Netherlands was published in Nature Medicine during 2020. This study examined 40 patients (21 patients with dMMR status and 19 with pMMR status) who had stage I~III colon cancer. The double immune neoadjuvant therapy consisted of nivolumab (anti-PD-1 antibody) combined with ipilimumab (anti-CTLA-4 antibody). Remarkably, all patients in the dMMR group survived without disease, with a median follow-up time of 8.1 months. These results indicated that the nivolumab + ipilimumab regimen was a suitable nIT for patients with dMMR non-mCRC, and suggested that immunotherapy could provide a lasting curative effect after the initial benefit. Moreover, the nIT was safe, feasible, and well tolerated. Thus, it is likely that nIT would not adversely affect the outcome of the subsequent operation, in that there should be no unexpected or redundant post-surgical complications. The most likely reason for the significant reduction in toxicity was the use of a lower dose of ipilimumab and the shorter duration of the nIT. The NICHE study pioneered the use of nIT for CRC, and therefore provided hope to patients with MSI-H/dMMR LACRC [12].

The PICC trial: The PICC trial was a single-center, open-label, parallel-group, non-comparative, randomized, phase 2 study. In this study, 53 patients were screened, of whom 34 were randomly assigned to either the toripalimab plus celecoxib group (n=17) or the toripalimab monotherapy group (n=17). All 34 patients had an R0 resection (>1 mm resection margin). The results shown that 15 of 17 patients (88% [95% CI 64-99]) in the toripalimab plus celecoxib group and 11 of 17 patients (65% [38-86]) in the toripalimab monotherapy group had a pathological complete response. All patients continued to receive adjuvant toripalimab with or without celecoxib for a total perioperative duration of 6 months and were alive and free of recurrence at data cutoff. During neoadjuvant treatment, ten (59%) patients in the toripalimab plus celecoxib group and ten (59%) in the toripalimab monotherapy group had grade 1 to 2 treatment-related adverse events. Only one (3%) of 34 patients, who was in the toripalimab plus celecoxib group, had a grade 3 or higher treatment-related adverse event during the neoadjuvant phase, which was grade 3 increased aspartate aminotransferase levels. In the adjuvant phase, only one (3%) of 34 patients, who was in the toripalimab monotherapy group, had a grade 3 or higher treatment-related adverse events, which was grade 3 increased aspartate aminotransferase and alanine aminotransferase levels [13].

The VOLTAGE-A study: The sort-term results of the exploratory VOLTAGE-A phase II study from Japan were published in the Journal of Clinical Oncology during 2020 [12]. This study, which compared LACRC patients in an MSS group and an MSI-H group, examined the effect of an initial long-term nCRT, followed by nIT, surgical resection, and then adjuvant chemotherapy. Both groups achieved Major Pathologic Response (MPR) (Table 1). As of January 2020, the median follow-up time was 22.5 months for the MSS group and 6.6 months for the MSI-H group. In the MSS group, 2 patients had local recurrence and 2 had distant metastases; however, no patients in the MSI-H group had recurrence. The FOWARC study [13,36] found that the pCR rate of mFOLFOX6 combined with preoperative radiotherapy was 28%.

However, this improvement of pCR was not accompanied by improvements in final survival. The pCR rate in the VOLTAGE

study was 30%, however, whether this treatment can also provide a survival benefit likely depends on whether the nIT after nCRT was able to activate the immune system and remove small residual lesions. A long-term follow-up of patient survival is needed. Although the sample size of the VOLTAGE study was small, it was the first to compare the application of nIT combined with nCRT for patients who had MSI-H and MSS LACRC. Moreover, the results suggested a better combination biomarker for predicting the efficacy of nIT - PD-L1 positivity and a high ratio of CD8+/Treg cells. This combination biomarker has potential for use in subsequent studies [36].

The NCT04165772 study: A total of 12 patients have completed treatment with dostarlimab and have undergone at least 6 months of follow up. All 12 patients (100%; 95% confidence interval, 74 to 100) had a clinical complete response, with no evidence of tumor on magnetic resonance imaging, 18F-fluorodeoxyglucose-positron emission tomography, endoscopic evaluation, digital rectal examination, or biopsy. At the time of this report, no patients had received chemoradiotherapy or undergone surgery, and no cases of progression or recurrence had been reported during follow-up (range, 6 to 25 months). No adverse events of grade 3 or higher have been reported [37].

The REGONIVO trial: REGONIVO trial, phase Ib trial of regorafenib plus nivolumab for gastric and colorectal cancer, which enrolled Fifty patients (25 each with gastric and colorectal cancer). All patients had received ≥ 2 previous lines of chemotherapy, including anti-angiogenetic inhibitors in 96% of patients. Objective tumor response was observed in 20 patients (40%), including 11 with gastric cancer (44%) and 9 with colorectal cancer (36%). Median progression-free survival was 5.6 and 7.9 months in patients with gastric and colorectal cancer, respectively [38].

The NCT04304209 study: Sintilimab is a fully human IgG4 monoclonal antibody (PD-1) [39], which becomes the first PD-1 monoclonal antibody independently developed in China. Sintilimab has been widely used for first-line treatment of liver cancer and NSCLC. However, the efficacy in colorectal cancer remains unclear. NCT04304209 trial, an open-label, single-arm, phase 2 study was done at the Sun Yat-sen University Cancer Center, Guangzhou. This trial enrolled patients who aged 18 to 75 years with mismatch-repair deficient or microsatellite instability-high locally advanced rectal cancer. All patients received neoadjuvant sintilimab monotherapy (200 mg by intravenous infusion) every 21 days. A complete response was thus noted for 12 (75%; 95% CI 47-92) of 16 patients. The median follow-up was 17.2 (IQR 8.2-28.5) months. After that, all patients were alive and none had disease recurrence [40].

Controversies and challenges faced by neoadjuvant immunotherapy for CRC

Although neoadjuvant immunotherapy for colorectal cancer has achieved great results, it is not so suitable for all colorectal cancer patients. Before ICIs immunotherapy, the predictive of efficacy biomarkers should be screened first. Here, we summarize predictive biomarkers currently used to assess the efficacy of colorectal cancer immunotherapy.

dMMR/MSI-H: The DNA Mismatch Repair (MMR) system, which exists extensively in organisms from prokaryotes to eukaryotes, is a highly conserved repair mechanism in cellular evolution. MMR was first found as a causative germline alteration in patients with Lynch syndrome in 1993 and was termed a microsatellite [26,41-43]. The MMR system plays key roles in identifying and repairing mismatched nucleotides during genetic recombination or as a result of damage caused by external physical or chemical insults. MMR guarantees genomic integrity and stability and avoids insertions and deletions of abnormal DNA at microsatellites. The post studies have demonstrated that defects in the DNA Mismatch Repair (MMR) pathway underlie the development of MSI in CRC. After the inactivation of the DNA MMR pathway, misincorporations, insertions and deletions introduced by DNA polymerase slippage are not properly recognized and corrected. It has been established that defects in the DNA Mismatch Repair (MMR) pathway underlie the development of MSI in CRC [44]. After the inactivation of the DNA MMR pathway, misincorporations, insertions and deletions introduced by DNA polymerase slippage are not properly recognized and corrected. Previous studies have shown the strong correlation between the development of MSI CRC and LS: Almost all CRC derived from Lynch syndrome patients have MSI. Beyond Lynch syndrome patients, MSI is encountered in approximately 15% to 20% of the CRC derived from sporadic CRC patients [45,46]. Because of the high correlation between dMMR/MSI-H and colorectal cancer mutation burden, the recent updated CSCO Guidelines for the Diagnosis and Treatment of Colorectal Cancer in 2022 expand the sample types recommended for MSI testing to all sample types, that is, MSI testing is recommended for all colorectal cancer patients [47].

POLE/POLD1: POLE and POLD1 are crucial for polymerase ε and δ encoding, respectively, which are essential for proofreading and fidelity in DNA replication [48,49]. The somatic or germline mutations in POLE and POLD1 lead to the pathogenesis of CRC *via* a DNA hypermutated phenotype [50,51]. Nearly 7.4% of CRCs harbor mutations in either POLE or POLD1 and 74% of tumors with POLE or POLD1 mutations were MSS or MSI-L [52]. Among pMMR CRCs, POLE-mutant CRCs show prominently higher CD8+ lymphocyte infiltration, expression of cytotoxic T-cell markers and effector cytokines than POLE wild-type CRCs, with upregulated expression levels of PD-L1, PD-1 and CTLA-4, etc. [53]. Considering the enhanced immunogenicity, POLE may become another acceptable effective biomarker similar MMR/MSI to in the near future. NCT03435107, NCT03827044, and NCT03150706 are underway to investigate the benefit of ICIs in POLE-mutant CRC [54-56].

Tumor mutational burden: Tumor Mutational Burden (TMB) refers to the total number of somatic mutations per coding area of a tumor genome, the more tumor mutations, the more neoantigens are produced, and the higher the chance that one or more of these self-neoantigens are immunogenic and trigger T cell response [57,58]. TMB has been proved to be an independent predictor of therapeutic efficacy of ICIs in several solid tumors including CRC [59-61]. As known by now, there is a strong association between MSI-H and TMB-H, with previous studies indicating that approximately 83% of MSI-H tumors also have TMB-H [62]. Notably, a high TMB value could emerge not only with MSI-H, but also in MSS tumors [63,64]. The efficacy of immunotherapy was preliminarily confirmed in MSS CRC patients with a high TMB value. In the exploratory analysis of REGONIVO trial, TMB was evaluated in 23 patients with CRC. ORRs were 50% and 35.3% in the TMB high and low group, respectively, and the median PFSs were 12.5 *vs*. 7.9 months [38]. Regardless of the type of treatment, higher TMB may serve as a prognostic factor for better outcomes [65].

TPS/CPS: With the widespread use of PD-L1, a simple and

reliable biomarker is urgently needed to identify patients who may respond to drugs. The most commonly used PD-L1 immunostaining score is the Tumor Proportion Score (TPS), which assesses whether a patient is tolerant to PD-L1 immunotherapy by calculating the percentage of tumor cells that express PD-L1 [66,67]. Previous studies have shown that the higher the expression of PD-L1 in tumor cells (higher the TPS), the better the prognosis of immunotherapy [4]. This scoring method is most commonly used in Non-Small Cell Lung Cancer (NSCLC) [68-70]. However, with the application of colorectal cancer immunotherapy, TPS has also become an important indicator to assess the efficacy of colorectal cancer immunotherapy. It is calculated by measuring number of PD-L1 stained tumor and inflammatory cells (Lymphocytes and macrophages)/Number of tumor cells) × 100.

Another biomarker CPS (Combined Positive Score), which considers the expression of PD-L1 in both tumor and inflammatory cells, exhibited a better correlation with immunotherapy response [71,72].

CMS: The International Consortium published the Consensus Molecular Subtypes (CMS), which broadly defines disease into four groups with clinical relevance [73-76]. Among them, CMS1 comprised the more significant number of MSI tumors that demonstrated the hypermethylation status, which leads to its being the most suitable CMS subtype for immunotherapy [28,77]. Hence, testing for specific molecular traits and measuring immune cell infiltration have become important for both prognostic and predictive purposes in CRC [78-80]. For patients with localized CRC (stages I–III) with dMMR (usually around 15-20 percent of all CRCs), overall prognosis is better than that for patients with proficient Mismatch Repair (pMMR) tumors [81]. However, dMMR CRC with metastasis has a very poor prognosis. Metastatic tumors with MSI/dMMR are most of-ten driven by associated BRAF mutation [82], and such metastatic dMMR tumors have poor response to chemotherapy and an overall worse prognosis. Therefore, molecular features such as MSI and KRAS and BRAF mutations have clear clinical implications and have become essential predictors beyond regular image-based tumor staging [73].

Although both CMS1 and CMS4 showed significant overexpression of lymphocyte and monocyte markers, CMS1 showed overexpression of cytotoxic lymphocyte-specific genes, while CMS4 showed inflammatory changes, angiogenesis, and immunosuppression. Therefore, CMS4 type is called immune exemption type [83] (Figure 2).

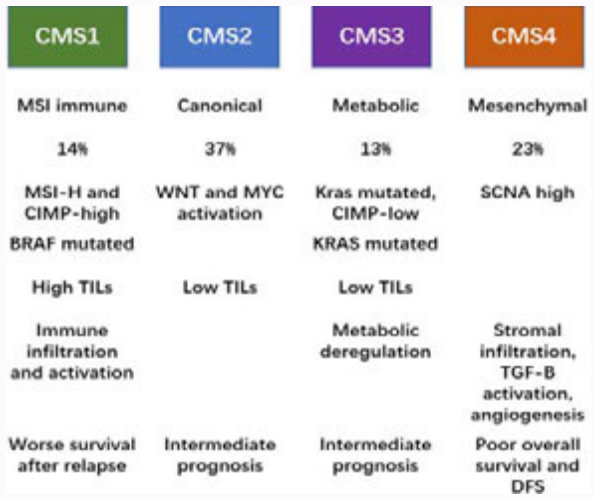

Figure 2: Specific contents of four consensus molecular subtypes.

Future directions of neoadjuvant immunotherapy for colorectal cancer

Neoadjuvant immunotherapy in pMMR/MSI-L CRC: Although neoadjuvant therapy for colorectal cancer has achieved good efficacy in the dMMR/MSI-H population, pMMR-MSI-L tumors, which contribute to 95% of all mCRC cases, harbor a much lower mutation burden and poor recruitment of immune cells, leading to an unsatisfactory response to ICIs. Therefore, immunotherapy that can be effectively used in dMMR/MSI-L patients will become the main research direction in the next few years. Here, we summarize the current research progress on immunotherapy for dMMR/MSI-L patients.

Combination of ICIs: The NICHE study from the Netherlands focused on patients with early colon cancer who received double immunization combined with neoadjuvant therapy, CTLA-4 monoclonal antibody ipilimumab (day 1) and PD-1 monoclonal antibody nivolumab (days 1, 15) before surgery, followed by surgery within 6 weeks, with the primary study endpoints of safety and feasibility [12]. The NICHE study included both dMMR and pMMR patients, and the results showed that neoadjuvant immunotherapy was safe and did not show surgical delay; all 20 dMMR patients had pathological response, of which 19 (95%) achieved Pathological Major Response (MPR) with <10% residual active cancer cells on postoperative pathological examination and 12 (60%) achieved pathological Complete Response (pCR) [3]. Of the 15 pMMR patients studied by NICHE, 4 (27%) also showed pathological responses, 3 of whom achieved MPR. Subsequent translational research analyses identified CD8+ and PD-1-positive T-cell infiltration as predictors of response to immunotherapy in patients with pMMR.

Immunotherapy combined with radiotherapy neoadjuvant therapy: Previous studies have shown that radiotherapy combined with PD-L1 can enhance the infiltration of CD8+ T cells, while reducing the local accumulation of bone marrow-derived suppressor cells and regulatory T cells, thereby enhancing host anti-tumor immunity and improving anti-tumor efficacy [84]. Recent preclinical data also suggest that preexistent intratumoral T cells can survive radiation and contribute to its therapeutic effect [85]. Therefore, radiation therapy is considered to have immunogenic potential for solid tumors and increases the sensitivity of solid tumors to response to ICIs [86-88]. A recent single-arm, non-randomized, Phase 2 trial (NCT03104439) combining radiation, ipilimumab and nivolumab in patients with metastatic MSS CRC (n=40) and PDAC (n=25) with an ECOG performance status of 0 or 1. The primary endpoint was Disease Control Rate (DCR) by intention to treat. DCR was 25% for CRC (10/40; 95% CI: 13-41%) and 20% for PDAC (5/25; 95% CI: 7-41%). In the per-protocol analysis, defined as receipt of radiation, DCR was 37% (10/27; 95% CI: 19-58%) in CRC and 29% (5/17; 95% CI: 10-56%) in PDAC. In the CRC cohort, the Adverse Events (AEs) related to immunotherapy Grade ≥ 3 were reported in 70% of patients while in the PDAC cohort this number decreased to 56%. One CRC and one PDAC case had a Complete Response (CR) by imaging criteria [89,90]. Recently, the latest results of the study demonstrated that the disease control rate and objective response rate of 40 patients with MSS mCRC were 25% (10/40) and 10% (4/10),

respectively, and the median progression-free survival and median overall survival time were 2.4 and 7.1 months, respectively. Twenty-seven patients who received radiotherapy had good disease control and objective response rates of 37% (10/27) and 15% (4/27), and median progression-free survival and median overall survival times of 2.5 and 10.9 months [91].

Immunotherapy combined with targeted drug neoadjuvant therapy: Vascular Endothelial Growth Factor (VEGF) affects multiple steps of tumor immune circulation and can lead to tumor immune escape [92]. Anti-angiogenic targeted drugs can normalize blood vessels, reduce interstitial pressure, improve drug delivery, while reducing the activity of inhibitory Treg cells and improving the immune microenvironment, and immunotherapy combined with anti-angiogenic targeted drugs has shown good synergistic effects in tumors such as liver cancer and gastric cancer [93-95]. A phase IB study of regorafenib combined with nivolumab followed by line therapy in metastatic gastric cancer and colorectal cancer (REGONIVO study) showed that in metastatic colorectal cancer with MSS or pMMR, the response rate of line therapy after immunization combined with targeted drugs was 33.3%, and the median PFS was 7.9 months [38]. Although subsequent similar studies have failed to replicate the same response rate, some patients still benefit from this combination. Therefore, some studies have also explored whether immunization combined with targeted drugs has further improved efficacy in neoadjuvant therapy for locally advanced colorectal cancer. A multicenter single-arm phase II study (REGINA study) was conducted in Belgium to include 60 patients with intermediate-risk stage II-III rectal cancer treated with regorafenib (days 1 to 14, days 28 to 49) combined with neoadjuvant nivolumab (days 1, 14, 28, 42, and 56) and short-course radiotherapy (days 21 to 25), and the primary study endpoint was the pCR rate, which remains to be demonstrated by the results of this study [96].

Adoptive cell therapy: In addition to ICIs, another highly anticipated new immunotherapy therapy is Adoptive Cell Therapy (ACT). ACT selects either host antitumor activity or host cells exhibiting cells engineered with Chimeric Antigens Receptors (CARs) or antitumor T Cell Receptors (TCR) to augment the host antitumor immune response [97].

The levels of CEA are low or absent in normal cells, but abundant in CRC [98]. Based on this, several trials targeted CEA for ACT. In a phase I trial, CAR T-cells therapy targeting CEA was firstly tested in 3 patients with mCRC [99]. Obvious decreases in serum CEA was observed in all of patients, and one patient received objective response of lung and liver metastasis. Unfortunately, all 3 patients experienced severe colitis. Another phase I trial (NCT02349724) was conducted to evaluate the safety and efficacy of anti-CEA CAR-modified T cells in CEA positive refractory mCRC patients [100]. Seven of 10 patients obtained stable disease, without significant CAR-related toxicity.

The shed Natural Killer Group 2D (NKG2D) ligands from tumor cells may downregulate NKG2D expression on NK and T cells, contributing to tumor immune escape [101-103]. A novel attempt to further augment the host antitumor immune response is to genetically modify CAR T cells to express proteins such as PD-L1 and NKG2D receptor. The safety and efficacy of this "armored" CRATs remain to be investigated.

Encouraging results on TILs were shown in a case report [104]. Researchers identified a polyclonal CD8+ T-cell response against mutant KRAS G12D in TILs and transferred the TILs into the patient. The result showed that all 7 metastatic lung lesions regressed at the first follow-up of 40 days, and the patient had a 9-month partial response until one lesion had progression. The patient remained 4 months clinically disease-free after the lung resection.

Cancer vaccines: Although existing for more than a century, cancer vaccines have barely received response in patients with CRC. Recently, cancer vaccines have elicited renewed interest owing to the convinced efficacy of immunotherapy. Unlike immune checkpoint inhibitors and cell-penetrating therapies, cancer vaccines target genetically modified genes or neoantigens to enhance the immune response, use an autoimmune function to kill tumor cells, provide antitumor effects, and can be continuously monitored to prevent their regrowth [105]. Multiple trials aiming to find the right antigenic stimulants are under investigation. In 1973, Professors Zanvil Cohn and Ralph Steinman discovered DCs in the mouse spleen, a new type of immune cell that plays a crucial role in bridging innate and adaptive immunity [106]. In 2010, Sipuleucel-T, an autoimmune therapy-based vaccine, was the first to be approved by the FDA for the treatment of refractory prostate cancer. DC vaccine therapy enhances the function of the patient's immune system by identifying tumor cell-specific antigens to target and focus on cancer cells, effectively inhibiting the proliferation of residual lesions [107].

Several prospective trials have investigated the role of autologous tumor lysate DC vaccines against CRC. For example, in two early phase 2 trials, results showed promising efficacy and beneficial survival outcomes in support of autologous tumor lysate DC vaccine for mCRC [108,109]. In addition, a recent phase 2 clinical study compared the efficacy of autologous tumor lysate DC vaccines *vs.* placebo in mCRC patients. In this trial, it was observed that the vaccine produced a tumor-specific immune response, but the study was terminated early because the results showed no benefit in median progression-free survival and median overall survival of 28 patients in the DC vaccine group [110]. In the immune microenvironment, the maturity of DCs, tumor tolerance, and the expression of costimulatory ligands are the main factors affecting the immune response of DC vaccines [111]. This may explain the contradicting results of the above studies. Potentially, DC vaccines combined with other cancer therapies, such as chemotherapy, radiotherapy, and ICIs, could activate antigen-specific effector T cells and may be ideal for addressing tumor immunosuppression.

Conclusion

With the emergence and application of more and more immunotherapeutic drugs, immunotherapy, the revolutionary cancer treatment, has changed the treatment concept of traditional solid tumors. Current progress most prominent with ICIs, and also CTLA-4 inhibitors and various forms of cellular therapy that are actively being investigated, is beginning to make an impact and will provide better knowledge for how these modalities may improve upon current strategies for intent-to-cure neo-adjuvant therapies for these diseases. However, not all patients with dMMR/MSI-H colorectal cancer could get response from current ICIs therapy, which express the response rate from 30% to 60% and 10% to 25% of patients had progressive disease [112], This suggests that for patients with dMMR/MSI-H CRC, commonly used biomarkers such as PD-L1, KARS, and BRAF are insufficient for the evaluation of the efficacy and prognosis of ICIs. Therefore, in the next study, finding more representative biomarkers to more accurately predict the efficacy and prognosis of

ICIs will become the key to colorectal cancer immunotherapy in the future. In addition, MSS/pMMR CRCs with low immunogenicity account for the majority of CRCs. They are less sensitive to current immunotherapies and have limited responses to single-agent ICIs. Hence, the key goal of current colorectal cancer immunotherapy is to assess various immunotherapy combinations at different stages of clinical development to find the ideal combination for the treatment of MSS/pMMR CRC.

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