

Chapter

Immunotherapy for Colorectal Cancer in the Era of Precision Medicine

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Abstract

Colorectal cancer (CRC) is considered the third most common cancer type and the second cause of cancer-related death worldwide, representing a significant global public health issue. Approximately 20% of patients present with metastatic disease, while up to 50% of those with early stages will eventually develop metastasis. During the last two decades, sustained efforts have been made to discover the molecular landscape of CRC and identify novel therapeutic targets. These efforts changed the treatment paradigm for CRC and improved survival significantly in metastatic disease. Immunotherapy represents a novel and exciting treatment option with promising results in gastrointestinal malignancies. The application of immunotherapy in CRC showed impressive results in a subset of patients with high microsatellite instability/deficient mismatch repair (MSI-H/dMMR) phenotype. An in-depth analysis of these particular MSI-H/dMMR tumors revealed that they are characterized by a high mutational load resulting in an increased number of neoantigens and a highly infiltrated tumor microenvironment. The Food and Drug Association (FDA) has recently approved immune checkpoint inhibitors (ICIs) pembrolizumab and nivolumab +/- ipilimumab for first-line and non-first-line therapy of MSI-H/dMMR metastatic CRC, contributing to the continuum of care in these patients. This chapter aims to overview the immune landscape and immunotherapeutic strategies in CRC.

Keywords: colorectal cancer, immunotherapy, pembrolizumab, nivolumab, ipilimumab, MSI-H/dMMR

1. Introduction

According to the GLOBOCAN database, colorectal cancer (CRC) represents the second most frequent cancer type diagnosed in women and the third in men. Globally, the highest incidence rates of CRC are seen in New Zealand, Australia, North America, and Europe [1]. In contrast, the lowest incidence is found in South-Centre Asia and Africa. The existing discrepancies among geographic regions are mainly attributed to lower screening rates in undeveloped countries, socioeconomic status, lifestyle, and dietary disparities [2]. Age is considered a risk factor for CRC. However, recent epidemiologic studies reported an increased incidence in people under 50 years old due to lifestyle changes and genetic implications [3].

Despite the sustained efforts focused on developing new treatment options for CRC, metastatic CRC (mCRC) patients still have a very poor prognosis [4]. For advanced and metastatic CRC treatment, the breakthrough was the addition of oxaliplatin and irinotecan to the original 5-fluorouracil (5-FU) regimen. The combination almost doubled the survival rates and has been the standard of care for more than 20 years. The addition of targeted agents, such as bevacizumab (anti-VEGF), panitumumab, and cetuximab (anti-EGFR), further increased the efficacy of the treatment [5]. In recent times, treatment strategies focused on altering the immune system, like immune checkpoint inhibitors (ICIs), have made their way into oncology practice after showing promising results in solid tumors like melanoma and lung cancer. These approaches have been demonstrated to be less effective in CRC patients [6]. However, a better understanding of the tumor immune contexture and CRCs' molecular subtypes demonstrated that a specific subset of patients having a hypermutated phenotype might benefit from ICIs [7]. Mainly, these tumors are distinguished by a robust immune activation and high microsatellite instability (MSI-H) due to dysfunctions of the mismatch repair (MMR) genes-dMMR. By contrast, in tumors with low microsatellite instability (MSI-L) and proficient mismatch repair (pMMR) function, ICIs are ineffective [8]. To date, many novel combinatorial approaches have been researched in order to overcome the relative resistance seen in CRCs.

This chapter aims to overview the immune landscape and immunotherapeutic strategies in CRC.

2. Immune landscape of colorectal cancer

The pathogenesis of CRC is a very complex multistep event linked to the accumulations of both the epigenetic and genetic alterations [9]. Other exogenous factors, including lifestyle, diet, and microbiota, contribute to this process [10]. Moreover, another essential aspect correlated with CRC development is the host immune dysfunction, primarily relying on escape mechanisms and immune evasion, which create a favorable environment for tumor growth [11]. The immune system can distinguish tumor antigens after their presentation via major histocompatibility complex (MHC) proteins present on antigen-presenting cells adenomatous polyposis coli to T cell receptors (TCR) found on the surface of T cells. The interaction between MHC proteins and TCR is insufficient for T cell activation. These pathways are further modulated by co-inhibitory and co-stimulatory signals, which tumor cells exploit to evade recognition and destruction [12, 13]. Among the co-stimulatory molecules that positively influence T cell activation and expansion after interaction with their ligands, we mention CD80 and CD86, found on cancer cells or APC. Other co-stimulatory molecules recently described include 4-1BB, GITR, and X40 [14].

On the other hand, co-inhibitory molecules, including cytotoxic T lymphocyte antigen 4 (CTLA4), programmed cell death protein-1 (PD-1), LAG-3, and TIM-3, antagonize the effects mentioned above upon interaction with their ligands. These signaling pathways prevent excessive immune responses and autoimmune phenomena [15]. Tumor cells often hijack these mechanisms, overexpress co-inhibitory molecules, which promote the activation of immunosuppressive regulatory T cells (Treg) instead of effector T cells (Teff), and, therefore, evade immune surveillance [16].

ICIs using anti-PD1, anti-PD-L1 (programmed cell death protein-ligand 1), and anti-CTLA4 molecules have been successfully used in various cancer types to

promote an effective antitumor immune response and overcome immune evasion mechanisms (**Figure 1**).

It was initially assumed that CRC is not an immunogenic cancer type, and therefore, immunotherapy would not be successful in this setting. Further studies identified a subset of patients harboring MSI-H/dMMR phenotype that could benefit from these therapeutic strategies [17]. Mutations in MMR genes are associated with microsatellite instability (MSI) and, therefore, a high tumor mutational burden. Consequently, these tumors contain an increased number of neoantigen, which will be recognized as foreign and will generate a robust immune response by the host. Moreover, MSI-H/dMMR tumors are characterized by the upregulation of immune checkpoints (PD-1 and PD-L1), which further enhances immune evasion [18].

2.1 Colorectal cancer molecular subtypes

Furthermore, CRC has been classified into four consensus molecular subtypes (CMS) to correlate the tumor phenotype with the clinical behavior and guide treatment. CMS1 (MSI immune subtype, 14%) tumors are frequently located in the proximal colon and are characterized by an increased immune infiltration in the tumor microenvironment (TME) (particularly CD8+, CD4+, and NK). In addition, these tumors have a high BRAFV600E mutation rate, are hypermethylated, and are associated with an impaired MMR system [19]. Owing to their particular phenotype, the immune-activated CMS1 subgroup has a clinical benefit from treatment with ICIs.

The CMS2 subtype (canonical, 37%) result from the canonical adenoma-to-carcinoma sequence. This cell phenotype is typically characterized by loss of tumor suppressor gene adenomatosis polyposis coli, followed by Kirsten rat sarcoma virus (KRAS) mutation and TP53 loss [20]. Moreover, these tumors present with low levels of hypermethylation and microsatellite stability (MSS). The CMS2 subtype is also characterized by the activation of WNT and MYC pathways, high expression of oncogenes epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), and a significant risk of distant relapse. However, CMS2 tumors have the highest 5-year overall survival (OS), at 77% among all the subtypes [21].

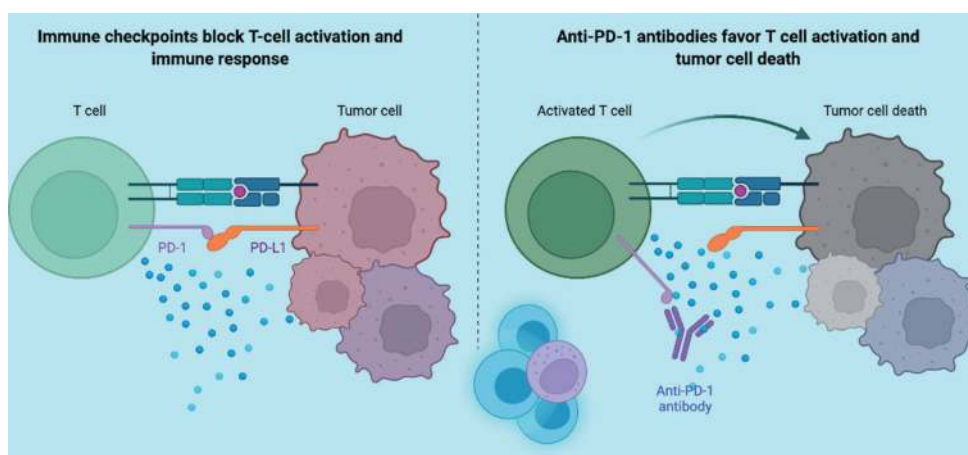


Figure 1.
Mechanism of anti-PD-1 antibodies.

CMS3 tumors (metabolic, 13%) have a chromosomal instability (CIN) genomic phenotype but with fewer copy number alterations. 30% of these tumors have microsatellite instability and an intermediate gene hypermethylation level. Moreover, CMS3 tumors are enriched with Kirsten rat sarcoma virus (KRAS) mutations [19, 20].

CMS4 (mesenchymal, 23%) has a phenotype distinguished by the activation of pathways associated with epidermal-mesenchymal transition (EMT) and by the overexpression of proteins involved in complement signaling and extracellular matrix remodeling [22]. The tumor microenvironment of CMS4 tumors is pro-inflammatory, with high levels of Treg, T helper, and myeloid derived suppressor cells. CMS4 tumors are often diagnosed in advanced stages, have a poor prognosis, and show no benefit from adjuvant chemotherapy. Regarding the metastatic setting, CMS4 tumors are resistant to anti-EGFR, independently of KRAS status [23].

In a recent translational study of over 1700 tumor samples, 55% of them had ≥ 2 CMS subgroups, suggesting that intratumoral heterogeneity is a common finding [24]. However, intratumoral heterogeneity was associated with worse OS and reduced disease-free survival (DFS) [25].

3. Clinical evidence of immune checkpoint inhibitors in colorectal cancer

Immunotherapy based on ICIs has changed the treatment paradigm in various tumor types, including lung cancer, melanoma, renal cell carcinoma, etc. These strategies showed minimal clinical activity in nonselected CRC patients [26]. The first glimpse of hope came from a phase I clinical trial investigating the efficacy of the anti-PD-1, nivolumab, in advanced solid tumors, including CRC. Of 14 CRC patients, only one with an MSI-H/dMMR phenotype had a durable complete response (CR) [27]. Further, extensive research has been developed to understand the immune contexture of MSI-H/dMMR tumors, their response to ICIs, and possible combinatorial strategies (Tables 1 and 2).

3.1 Metastatic setting

3.1.1 Pembrolizumab

Pembrolizumab is an anti-PD-1 humanized IgG4 Kappa monoclonal antibody (mAb). Its role is to target PD-1 molecules from the T cell's surface and, therefore, to prevent the interaction with its ligands, PD-L1 and PD-L2. By blocking this interaction, pembrolizumab can resuscitate the cytotoxic activity of T cells and promote the recruitment of other immune cells in the tumor microenvironment [28].

The phase Ib KEYNOTE-028 trial (NCT02054806) investigated pembrolizumab's clinical efficacy in patients with PD-L1-positive advanced solid tumors. Only one accomplished a partial response among the 23 PD-L1-positive mCRC patients. Once again, this patient reportedly had an MSI-H/dMMR phenotype, suggesting that this feature could further predict the response to ICIs [29]. Starting from the hypothesis that tumors with an increased number of somatic mutations due to dMMR might be susceptible to ICIs, the phase II KEYNOTE-016 trial investigated the clinical efficacy of pembrolizumab in MSI-H/dMMR CRC, MSS/pMMR CRC, and MSI-H/dMMR non-CRC. Among the MSI-H/dMMR CRC patients, the progression-free survival (PFS) rate and overall response rate (ORR) were 79% (seven out of nine patients) and 40% (four out of 10 patients), respectively. Similar positive results

Study name	Phase	Trial population	Treatment	ORR	PFS	OS
NCT01876511 (KEYNOTE-016)	2	Refractory dMMR mCRC refractory pMMR mCRC refractory dMMR non-CRC	Pembrolizumab	Refractory dMMR mCRC: 40% refractory pMMR mCRC: 0% refractory dMMR non-CRC: 71%	At 20 weeks refractory dMMR mCRC: 78% refractory pMMR mCRC: 11% refractory dMMR non-CRC: 67%	For dMMR mCRC and dMMR non-CRC median OS was not reached For pMMR mCRC median OS 5 months
NCT02460198 KEYNOTE-164	2	Refractory MSI-H/dMMR mCRC Cohort A ≥ 2 previous treatment lines Cohort B ≥ previous treatment lines	Pembrolizumab	Cohort A: 33% Cohort B: 33%	Estimated PFS at 12 mo Cohort A: 34% Cohort B: 41%	Estimated OS at 12 mo Cohort A: 72% Cohort B: 76%
NCT02563002 KEYNOTE-177	3	Treatment naïve MSI-H/ dMMR mCRC Arm A- pembrolizumab Arm B investigator's choice chemotherapy	Pembrolizumab vs. standard chemotherapy as 1st line treatment	Arm A: 43.8% Arm B: 33.1%	Median follow-up of 32.4 mo Arm A- 16.5 mo Arm B- 8.2 mo	Median survival not reached
NCT02060188 CheckMate 142	2	Refractory MSI-H/dMMR mCRC	Nivolumab	31%	At 12 mo: 50%	At 12 mo: 73%
	2	Refractory MSI-H/dMMR mCRC	Nivolumab + ipilimumab	55%	At 12 mo- 71% PFS rate	At 12 mo: 85% OS rate
	2	Treatment naïve MSI-H/ dMMR mCRC	Nivolumab + ipilimumab	Median follow-up of 29 mo: 69%	at 24 mo: 74% PFS rate	At 24 mo: 79% OS rate

Table 1.
 Key clinical trials of ICIs in MSI-H/dMMR mCRC.

Study name	Phase	Trial population	Treatment	Primary endpoint	Study purpose
NCT04014530 ATAPEMBRO	I/II	MSI-H/dMMR mCRC pMMR mCRC dMMR endometrial carcinoma	Ataluren (prematuration stop codon suppressor) + pembrolizumab	AEs MTD (maximum tolerated dose) of Ataluren ORR	<ul style="list-style-type: none"> To determine the safety and efficacy of the combination ataluren + pembrolizumab
NCT04008030 CheckMate 8HW	III	dMMR/MSI-H mCRC	Nivolumab Nivolumab + ipilimumab Investigator's choice chemotherapy	PFS	<ul style="list-style-type: none"> To compare the clinical benefit of nivolumab monotherapy or nivolumab + ipilimumab To compare the clinical benefit of nivolumab + ipilimumab versus investigator's choice chemotherapy
NCT03104439	II	MSI-H/dMMR mCRC MSS mCRC Pancreatic cancer	Nivolumab + ipilimumab + radiation therapy	DCR (disease control rate)	<ul style="list-style-type: none"> To establish the clinical efficacy of combining nivolumab + ipilimumab + radiation therapy
NCT02997228 COMMIT SWOG1610	III	Treatment naïve dMMR/ MSI-H mCRC	mFOLFOX6 + bevacizumab + atezolizumab mFOLFOX6 + bevacizumab + atezolizumab	PFS	<ul style="list-style-type: none"> To determine the clinical efficacy of mFOLFOX6/bevacizumab and atezolizumab compared to atezolizumab monotherapy
NCT02912559 ATOMIC Alliance A021502	III	Stage III MSI-H/dMMR CRC	mFOLFOX6 + atezolizumab mFOLFOX6	DFS	<ul style="list-style-type: none"> To compare the association of standard chemotherapy+atezolizumab versus standard chemotherapy in the adjuvant setting
NCT03186326 SAMCO	II	Previously treated MSI-H/ dMMR mCRC	Standard chemotherapy (FOLFOX/ FOLFIRI +/- targeted therapy) Avelumab	PFS	<ul style="list-style-type: none"> To compare the efficacy of avelumab versus second line standard chemotherapy
NCT03475953 REGOMUNE	I/II	Metastatic solid tumors (MSI-H/dMMR mCRC)	Avelumab + regorafenib	RP2D (recommended phase 2 dose)	<ul style="list-style-type: none"> To determine the safety and efficacy of low dose regorafenib in combination with avelumab
NCT03435107	II	Previously treated MSI-H/ dMMR mCRC and POLE mutated mCRC	Durvalumab	ORR	<ul style="list-style-type: none"> To evaluate the efficacy of durvalumab in later lines of therapy

Table 2.
Selected ongoing clinical trials of ICIs in MSI-H/dMMR CRC.

were observed in MSI-H/dMMR non-CRC cohort, with 71% ORR (five out of seven patients). Contrarily, in the MSS/pMMR cohort, the ORR was 0% and the PFS rate was 11%. In the MSI-H/dMMR CRC cohort, the median OS and PFS were not reached. Moreover, a high somatic mutational load was significantly associated with a longer PFS ($p = 0.02$) [30]. These preliminary results inspired the initiation of the KEYNOTE-164 trial. This phase II study investigated the efficacy of pembrolizumab in two cohorts of previously treated MSI-H/dMMR advanced CRC patients. Cohort A included the patients previously treated with ≥ 2 lines of standard therapy, while cohort B included the patients treated with ≥ 1 line of therapy. With a median follow-up of 31.3 months (mo) for cohort A and 24.2 mo for cohort B, the results showed an ORR of 33% (95% CI; 21–46%). The median OS was 31.4 mo (95% CI; 21.4 mo to not reached) in cohort A, and it was not reached in cohort B [31]. Furthermore, another phase II trial, KEYNOTE-158, investigated the antitumor activity of pembrolizumab in previously treated MSI-H/dMMR non-CRC patients. The ORR was 34.3%, the median PFS was 4.1 mo, and the median OS was 34.5 mo [32]. Considering these results, in May 2017, the Food and Drug Association (FDA) approved pembrolizumab to treat unresectable or metastatic MSI-H/dMMR CRC patients who progressed after conventional chemotherapy with oxaliplatin, fluorouracil, and irinotecan, and for previously treated metastatic or unresectable MSI-H/dMMR solid tumors that have no other satisfactory treatment option [33].

Based on the robust and sustained results seen in refractory mCRC, the phase III KEYNOTE-177 trial investigated the clinical efficacy of pembrolizumab as first-line treatment compared to standard chemotherapy in MSI-H/dMMR mCRC patients. At a median follow-up of 32 mo, pembrolizumab doubled the PFS compared to chemotherapy (8.2 mo versus 16.5 mo; $p = 0.0002$). The ORR was significantly higher with pembrolizumab than with standard chemotherapy (44% versus 33%). Moreover, the grade 3–5 AEs (adverse events) rate was 66% for standard chemotherapy and only 22% for pembrolizumab [34]. Even if the OS data are not mature yet, a high crossover rate to the immunotherapy arm has been reported. Based on these results, which demonstrate the superiority of pembrolizumab over standard chemotherapy, in June 2020, the FDA approved the treatment with pembrolizumab as first-line treatment in MSI-H/dMMR mCRC patients [35].

3.1.2 Nivolumab +/- Ipilimumab

Nivolumab is an anti-PD-1 humanized IgG4 mAb that, similar to pembrolizumab, disrupts the interaction between the PD-1 receptor and its ligands (PD-L1 and PDL2). The clinical benefit of nivolumab has been documented in many tumor types as melanoma, lung cancer, and renal cell carcinoma [28].

Ipilimumab is a mAb directed against the surface protein CTLA-4, expressed on activated and regulatory T cells. The CTLA-4 molecule negatively regulates T-cell function by inducing T cell anergy and tolerance [36]. Therefore, CTLA-4 blockade intends to counteract the immune tolerance to cancer cells. To support this idea, James Allison and colleagues showed that antibodies against CTLA-4 enhance the antitumor activity of immune cells in mice transplanted with fibrosarcoma and colon cancer [37].

The phase II CheckMate-142 trial was a large initiative to evaluate the clinical benefit of nivolumab alone or associated with other anticancer therapies in mCRC patients with or without MSI-H/dMMR phenotype. The study has an atypical design which initially included six cohorts: 1—nivolumab monotherapy; 2—nivolumab + ipilimumab (every 3 weeks, for four doses, followed by nivolumab monotherapy every

2 weeks); 3—nivolumab + ipilimumab (every 6 weeks, for four doses, followed by nivolumab monotherapy every 2 weeks); 4—nivolumab + ipilimumab + cobimetinib; 5—nivolumab + BMS-986016; and 6—nivolumab + daratumumab.

Out of 74 MSI-H/dMMR mCRC patients from cohort 1, 31.1% (23 out of 74) achieved an OR, while 69% (51 out of 74) had disease control for ≥ 12 weeks. Moreover, responses have been obtained in patients with or without KRAR, BRAF mutations, or a history of Lynch syndrome. Additionally, this study reported an OR of 25% in BRAF-mutated patients. These results outperform the ones obtained using standard chemotherapy (<10%) and combination strategies with EGFR, BRAF, or MEK inhibitors (10–16%) [38].

The results from cohort 3, including MSI-H/dMMR mCRC patients treated with nivolumab and ipilimumab, have also been released. At a median follow-up of 13.4 mo, the ORR was 55% (65 out of 119 patients), the median PFS was not reached, and a durable response (≥ 6 weeks) was seen in 83% of patients. Nonetheless, it is important to note that this trial was not randomized, and the direct comparison could be, at some point, misleading. The phase II CheckMate-142 trial results guided the FDA approval of nivolumab +/- ipilimumab in previously treated MSI-H/dMMR mCRC patients [39].

The CheckMate-142 trial further investigated the clinical benefit of nivolumab + ipilimumab as first-line treatment in MSI-H/dMMR mCRC patients. The trial's primary endpoint was ORR. With a median follow-up of 29 mo, the ORR was 69% and CR 13%. The median PFS and OS were not yet reached. Based on these results, nivolumab is recommended as front-line treatment in MSI-H/dMMR mCRC patients as monotherapy or with ipilimumab [40].

In recent years, other ICIs have made their way into oncological practice and are under clinical investigation, including atezolizumab (anti-PD-L1), avelumab (anti-PD-L1), and durvalumab (anti-PD-L1).

3.2 Neoadjuvant and adjuvant setting

Preclinical studies hypothesized that ICIs might be more effective in neoadjuvant and adjuvant settings. In this regard, the phase II NICHE trial included 40 stages I–III CRC cancer patients with or without MSI-H/dMMR phenotype. All the patients were treated with two doses of nivolumab and one of ipilimumab. All the patients obtained pathological responses in the MSI-H/dMMR group, suggesting that immunotherapy warrants further investigations in the neoadjuvant setting [41]. The ATOMIC study, a phase III randomized controlled trial, is currently investigating Atezolizumab + FOLFOX regimen compared to FOLFOX alone in 700 patients with MSI-H/dMMR stage III CRC. The study's primary endpoint is disease-free survival (DFS), and the results are highly expected [42]. Currently, two sizeable ongoing phase III clinical trials are investigating the addition of anti-PD-L1 avelumab (NCT03827044) or anti-PD-1 pembrolizumab (NCT02912559) to standard adjuvant chemotherapy regimens in stage III MSI-H/dMMR CRC patients.

4. Strategies beyond ICI

4.1 Adoptive cell transfer (ACT)

Another revolutionary treatment option aiming to augment the host's immune system is represented by ATCs. The approach consists of transferring the patient's

immune cells, which were previously genetically engineered, and expanded to destroy cancer cells. ACT can include tumor-infiltrating lymphocytes (TILs), natural killer (NK), chimeric antigen receptor T-cell therapy (CAR-T), or engineered T cell receptors (TCR).

ACTs have achieved impressive success in several tumor types in the last two decades, especially in hematologic malignancies, like B cell lymphoma and leukemia [43].

ACT usage for cancer treatment originates from the observation of TILs, representing a population of lymphocytes infiltrating the tumor or located at its' margins. TILs represent the host's natural antitumor immune response and can recognize tumor-specific antigens presented by MHC 1 [44].

In 2016, a group of researchers identified in the TILs from CRC metastatic lesions a polyclonal population of CD8+ cells directed against KRAS G12D. After expansion, this TILs population was further reinfused into the patient and eradicated six out of seven lung metastases. So far, harvesting TILs from colorectal tumors has faced many difficulties [45]. One of the concerning issues is the contamination with intestinal flora, which can be overcome by acquiring tumor-specific T cells from tumor-draining lymph nodes [46]. Another ideal source for aseptically harvesting TILs in CRC might be liver metastasis. However, further research is needed to overcome all the impediments to the usage of TILs in CRC and other solid tumors.

CAR-T cell therapies have been extensively studied in hematologic malignancies, with less evidence in solid tumors at the moment. This kind of personalized medicine combines genetic therapy with immunotherapy. It involves T cells harvesting from the patient, which are genetically modified to express a chimeric antigen receptor (CAR) that can recognize a tumor-associated antigen (TAA) [47, 48]. The clinical trials investigating CAR-T cells in CRC treatment targeted various TAAs, including carcinoembryonic antigen (CEA), mesothelin (MSLN), EGFR, HER2, and natural killer group 2 member D (NKG2D) [49]. A phase I clinical trial investigated CAR-T cell therapy targeting CEA in previously treated CEA-positive mCRC patients. Out of the 10 patients included in the study, seven experienced stable disease for longer than 30 months. Moreover, the study reported a sustained decline in CEA serum levels [50]. Apical surfaces of the intestinal epithelium express the membrane-bound receptor guanylyl cyclase C (GUCY2C). Magee et al. tested the efficacy of a GUCY2C-specific CAR-T cell molecule in an mCRC mice model. The result showed that GUCY2C CAR-T cells reduced the number of lung metastasis in mice, lowering morbidity and improving survival [51].

Although ACTs have shown therapeutic potential in many cancer types, there are still many obstacles to their effectiveness in solid tumors, including CRC.

4.2 Cancer vaccines

Cancer vaccines are a form of active immunotherapy thought to enhance the antitumor immune response by evoking TAA in order to be targeted by the immune system. In mCRC, several vaccine types have been studied, including peptides, dendritic cells, autologous tumor cells, and recombinant viral vectors [52]. The vaccine must supply enough tumor antigens to induce a robust immune response and, therefore, to obtain a substantial clinical benefit [53]. Unfortunately, these requests are challenging to be acquired; thus, the clinical trials investigating cancer vaccines reported mixed results.

A benefit of peptide-based vaccines is that they are affordable in terms of production and storage. A recently developed peptide vaccine, PolyPEP11018 consisting of

12 epitopes derived from seven antigens frequently expressed in mCRC, demonstrated increased CD8⁺ T cell and CD4⁺ T cell responses against three antigens after only one dose [54].

Since plasmid DNA encoding influenza nucleoprotein A was discovered to trigger a specific T cell response, DNA vaccines have received much attention. These types of vaccines consist of bacterial plasmids created to provide tumor antigens that will be further presented via MHC proteins and stimulate an immune response [55]. MYB is an oncoprotein abnormally expressed in many tumor types, including CRC. In CRC transgenic mice, MYB-based vaccines showed good therapeutic efficacy. However, several concerns about DNA vaccines include poor immunogenicity and potential interactions with the host's genome [56].

RNA-based vaccines, another widely investigated therapeutic and prophylactic form of immunotherapy, consist of a platform that encodes tumor-specific antigens. After the internalization of mRNA transcript by the target cell, the translation takes place in the cytoplasm and is followed by tumor antigen presentation via MHC proteins, triggering a robust immune response. mRNA vaccines offer several benefits, making them appealing therapeutic options. They are nonintegrating molecules, affordable, relatively fast to produce, and easy to modify [57]. A phase I/II trial is currently investigating an mRNA-based vaccine (mRNA 4650) for treating various tumor types, including gastrointestinal, melanoma, genitourinary, and CRC [58]. There are only two anticancer vaccines approved in oncological practice: Provenge (sipuleucel-T) for prostate cancer treatment and Oncophage for kidney cancer [59, 60]. At the moment, cancer vaccines are extensively studied in clinical trials and will hopefully improve treatment strategies for CRC.

5. Correct treatment sequence after the implementation of ICI in CRC armamentarium

Nowadays, most CRC patients (75%) are diagnosed with an early stage (I–III) due to performant screening programs providing a chance for cure. However, 25% of them have metastatic disease at presentation and, therefore, poor prognosis [61].

For early-stage CRC, the standard of care consists of upfront surgery of the primary tumor and regional lymph nodes, followed by adjuvant chemotherapy in selected patients [62]. Following surgical resection, the 5-year DFS is 95% for stage I, 82–88% for stage II, and 45–50% for stage III CRC [63]. The primary role of adjuvant chemotherapy is to eradicate the micrometastatic residual disease after surgery. Identifying micrometastatic residual disease is unreliable; therefore, the gold standard used to confirm the clinical benefit of adjuvant chemotherapy is the 5-year OS [64]. Since the most challenging issue of the existing treatment paradigm in early-stage CRC is the incapacity to detect micrometastatic disease, the available guidelines recommend adjuvant chemotherapy for all stage III CRC patients. For stage II CRC, the benefit of adjuvant chemotherapy is still debatable. To date, it is recommended only for patients with high-risk clinicopathologic features (positive resection margins, <12 examined lymph nodes, T4, perineural invasion, lymphovascular emboli, perforation, and obstruction). The preferred chemotherapy regimens for this setting are a combination of fluoropyrimidine and oxaliplatin (FOLFOX or CAPOX) [65]. The addition of oxaliplatin led to OS improvement, and the risk of death was further reduced by 16%, 17%, and 12% in the MOSAIC, XELODA, and NSABP C-07 trials [66–68].

In the last 20 years, the prognosis of mCRC patients has significantly improved due to remarkable progress made in precision medicine. The currently available guidelines recommend resectioning metastasis performed either upfront or after previous downsizing treatment in selected patients [69]. In a recent meta-analysis, the 5-year survival rate was approximately 38% in patients who underwent resection of the liver metastasis [70]. If, however, this goal is not realistic, systemic therapy has shown significant survival benefits for mCRC patients. The fundamental development in mCRC treatment was the addition of oxaliplatin (a platinum derivate) and irinotecan (a topoisomerase I inhibitor) to 5-FU-based chemotherapy. Therefore, FOLFOX (5-FU, folinic acid, and oxaliplatin) and FOLFIRI (5-FU, folinic acid, and irinotecan) demonstrated better response rates and DFS compared to 5-FU alone, representing the mainstay of first-line chemotherapy [71, 72].

Further, after decades of clinical and translational research, an important step toward precision medicine was discovering treatment options based on the tumor's molecular characteristics. The first biologic therapy included in the mCRC treatment strategy was bevacizumab, a mAb targeting vascular endothelial growth factor-A (VEGF-A) [73]. Bevacizumab is recommended for RAS-mutated mCRC either as first-line or second-line in combination with chemotherapy [74]. Similarly, cetuximab and panitumumab are anti-EGFR mAbs associated with chemotherapy in the first and second lines of treatment but for restricted patients harboring RAS/BRAF-WT (wild-type) tumors [75]. Moreover, aflibercept (a synthetic receptor for VEGF-B, VEGF-A, and PIGF) and ramucirumab (anti-VEGFR-2) demonstrated clinical benefit in the second-line therapy while combined with chemotherapy [76, 77]. In further line, regorafenib (a multikinase inhibitor) also showed clinical efficacy [78]. Owing to improved surgical procedures and expanded therapeutic options, most mCRC patients experience an improved survival between 24 and 36 months, allowing a continuum of care [79].

Even if MSI-H/dMMR tumors represent a small subset of mCRC (5% or all cases), the discovery and introduction of ICIs into the continuum of care has been a significant step forward in precision medicine. Based on the clinical benefit observed in clinical trials, the current guidelines recommend nivolumab ± ipilimumab and pembrolizumab as first-line and non-first-line therapy for MSI-H/dMMR mCRC patients [33, 34, 40, 80]. Surprisingly, the phase III KEYNOTE-177 trial, which compared pembrolizumab with standard first-line therapy in MSI-H/dMMR mCRC, demonstrated a doubling PFS in pembrolizumab-treated patients (16.5 months). This outcome is the longest PFS ever reported by phase III trials for any first-line therapeutic options in mCRC [34]. Additionally, pembrolizumab and nivolumab ± ipilimumab are also recommended in the neoadjuvant setting for resectable MSI-H/dMMR mCRC patients [81].

According to the CMS classification, mCRCs with MSI-H/dMMR phenotype are considered immune-activated and belong to the CMS1 subgroup. Conversely, MSS/pMMR tumors, representing 95% of all mCRCs, display a low immune infiltrate, do not respond to ICIs, and are a serious challenge for clinical management [19]. It has been revealed that radiotherapy, chemotherapy, and targeted agents can induce immunogenic cell death (ICD), releasing tumor neoantigens and increasing the immune infiltrate in the tumor microenvironment (TME). Based on this hypothesis, many clinical trials are currently investigating the combination of ICIs with other anticancer therapies in MSS/pMMR mCRC to overcome the primary resistance to immunotherapy [82, 83].

6. Biomarkers

6.1 Microsatellite instability (MSI)

“Short tandem repeats” or microsatellites are repeated noncoding DNA sequences with a length from one to six base pairs. DNA polymerases are more predisposed to make errors either by removing or by inserting additional bases in these particular regions, leading to mismatched DNA strands [84]. Therefore, the MSI molecular phenotype is a consequence of deficient MMR proteins. The most directed genes from the MMR family associated with genome instability are MLH1, MLH2, PMS2, and MSH6. It is estimated that only 15% of CRCs are microsatellite unstable (MSI-H) [85]. Germline MMR gene mutation is the hallmark of Lynch syndrome, an autosomal dominant condition associated with an increased risk of colorectal (80%), endometrial (60%), stomach, small intestine, kidney, bladder, and brain tumors [86]. However, the MSI phenotype appears due to somatic mutations in most cases, usually caused by epigenetic silencing of the MLH1 promoter. Less commonly, the inactivation of MMR proteins can occur due to somatic biallelic MMR gene mutations. It is worth mentioning that a subset of MSI-H tumors has no detected alterations in the MMR genes [87]. These tumors were shown to overexpress various micro-RNAs (miRNAs), like miRNA-21 and 122, that might silence MMR genes [88].

Considering that the human genome comprises hundreds of thousands of microsatellites, the MSI assay evaluates only five of them via polymerase chain reaction (PCR) for practical reasons. Therefore, a tumor is defined as MSI-H if at least two microsatellites have a shift in size, and a size shift in only one locus represents an MSI-L tumor. By contrast, tumors with no unstable microsatellites are defined as microsatellite stable (MSS). The immunohistochemistry (IHC) assay of key MMR proteins has a high concordance rate and similar performance characteristics to the MSI assay via PCR. Hence, loss of protein expression defines a tumor as dMMR, while the presence of all MMR proteins labels the tumors as pMMR (MMR proficient) [89]. Besides IHC and PCR, next-generation sequencing (NGS) is a novel approach for detecting MSI status with high sensitivity (95%) and specificity (98%) [90]. To further clarify the notions, MSI-H and dMMR are considered the same types of tumor, and MSS and pMMR tumors are also mostly overlapping.

Regardless of the origin (sporadic or hereditary), all the MSI-H/dMMR CRCs have some characteristic histologic features. The high mutational load resulting from the deficiency of MMR proteins leads to the accumulation of a robust number of tumor neoantigens with great immunological potential [91]. MSI-H/dMMR tumors are frequently located in the right colon, have mucinous histology, are poorly differentiated, and, more importantly, have increased TILs. Moreover, MSI-H/dMMR tumors were reported to highly express immune checkpoints (CTLA4, PD-1, and PD-L1) [92].

6.2 PD-L1 expression

The detection of PD-L1 using immunohistochemical staining is one of the most explored predictive biomarkers for the response to ICIs. Studies reported that upregulation of PD-L1 is correlated with high infiltration of effector T cells. Moreover, these tumors have a high likelihood of responding to ICI. In contrast to other tumor types

like non-small-cell lung cancer (NSCLC), melanoma, and gastric cancer, the PD-L1 expression predicted no response to ICIs in mCRC patients [93]. An update from the CheckMate-142 trial investigating nivolumab +/- ipilimumab in MSI-H/dMMR CRC demonstrated that the ORR was irrespective of PD-L1 expression [39]. Moreover, the KEYNOTE-016 trial investigating the clinical benefit of pembrolizumab in mCRC with both MSS and MSI-H phenotypes showed no statistically significant correlation between PD-L1 expression and OR or PFS [31].

The reported disparities among tumors could be explained by the dynamic nature of this surface protein, which is influenced by the TME and treatment options. Furthermore, the lack of standardization for PD-L1 expression assay limits its clinical significance [94].

6.3 POLE/POLD1

POLE (DNA polymerase epsilon) and POLD1 (DNA polymerase delta) are two enzymes responsible for the correct genome replication during the cell cycle. Somatic mutation of either POLE or POLD genes affects their proofreading function, increasing the predisposition to numerous cancer types, including CRC [95]. Similar to the MSI-H/dMMR, these tumors have an ultramutated phenotype [96]. POLE-mutated CRCs express an upregulation of immune checkpoint molecules and also have a high level of TILs. Moreover, these tumors seem to be a rare finding (1% of CRCs), appear more frequently in young male patients, and have an early stage at presentation [97].

To date, limited evidence is available regarding the clinical benefit of ICIs in POLE/POLD1-mutated tumors. An excellent response to pembrolizumab was seen in a patient suffering from endometrial cancer who had a POLE mutation seen at genomic profiling. Since MSI-H/dMMR CRCs have similar characteristics (hypermutated phenotype, upregulated immune checkpoints, and inflamed TME), it was supposed that POLE/POLD1-mutated CRCs might be better suited for ICIs [98]. Further data are, however, needed to support this hypothesis.

6.4 Immunoscore

The immunoscore represents an immunohistochemical and digital pathology-based assay derived from the immune contexture. It quantifies two lymphocyte populations, CD8+ and CD3+, both in the tumor core (TC) and invasive margins (IM). The purpose of immunoscore was to translate the immune contexture into a viable biomarker for CRC [99]. The immunoscore ranks from I0 (immunoscore 0), characterized by a low density of CD8+ and CD3+ in both TC and IM, to I4 (immunoscore 4), with a high density of both lymphocyte populations in both regions. The advantage of immunoscore appears to be dual. First, this score is reported to be a prognostic factor for DFS and OS in early CRC. Moreover, it also seems to be an important tool for novel therapeutic approaches, including immunotherapy [100].

The prognostic value of immunoscore is supported by several studies. According to the phase III NCCTG N0147 trial, a high immunoscore was statistically significantly associated with a longer 3-year DFS than a low immunoscore in stage III CRC patients [101]. An international consortium including 14 centers from 13 countries assessed the prognostic value of immunoscore in stage I–III CRC patients (samples from 2681 patients). Patients with high immunoscore had a statistically significant lower risk of recurrence at 5 years compared to low immunoscore (HR = 0.20, 95%

CI 0.10–0.38; $p < 0.0001$). In the multivariate analysis, the association between immunoscore and the time to recurrence (TTR) was independent of T stage, N stage, patient's age, sex, microsatellite instability, or other existing prognostic factors ($p < 0.0001$) [102]. Besides its prognostic value, immunoscore holds great potential as a predictive biomarker. An international study conducted by the Society for Immunotherapy of Cancer analyzed the association of immunoscore with the effect of adjuvant chemotherapy in time to recurrence (TTR) in stage III CRC patients. A high immunoscore was associated with the lowest risk of recurrence, and it showed a significant correlation with prolonged TTR, DFS, and OS in this subset of patients (all $p < 0.001$) [103]. The immune context might also predict the clinical response to ICIs. CD8+ T cells were reportedly a good predictor of response to CTLA4 blockade in melanoma patients. Moreover, CD8+ lymphocytes were associated with response to anti-PD-1 molecules [100, 104].

To date, immunoscore was introduced among the “Essential and Desirable Diagnostic Criteria” for CRC in the fifth edition of the World Health Organization (WHO) classification of digestive tumors. This detail brings us closer to the notion of TNM-I classification (“I” from “immune”) [105].

7. Conclusions and future perspectives

Immunotherapy evolved into a desirable treatment option for CRC because of the success seen in various solid tumors and the reliable side effects. However, the role of immunotherapy is still restricted to a very small subset of patients with an MSI-H/dMMR phenotype. At the moment, many clinical trials are exploring combinatorial strategies of conventional therapy and ICIs to overcome primary resistance to ICIs in CRC. To extend the clinical benefit of cancer immunotherapies, novel delivery platforms are currently under investigation, including nanoparticles, implants, biomaterials, and scaffolds. Using these delivery systems may help reduce toxicities and ensure localized and controlled drug delivery [106]. Moreover, metagenomic studies underline the critical role of microbiota in CRC pathogenesis and response to treatment, including ICIs. Nonetheless, the implementation of radiomic analyses could further identify the antitumor activity of targeted therapies or immunotherapy [107].

Future technological progress is expected to provide a more profound knowledge of the immune system and the tumor and microenvironment gene expression to ensure a continuum of care based on precision medicine.

Acknowledgements

This section of your manuscript may also include funding information.

List of abbreviations

AEs	adverse events
APC	antigen-presenting cells

CAR	chimeric antigen receptor
CAR	T-chimeric antigen receptor T-cell therapy
CEA	carcinoembryonic antigen
CMS	consensus molecular subtypes
CRC	colorectal cancer
CTLA4	cytotoxic T lymphocyte antigen 4
DCR	disease control rate
dMMR	mismatch repair deficient
EMT	epidermal-mesenchymal transition
FDA	Food and Drug Association
GUCY2C	membrane-bound receptor guanylyl cyclase C
ICIs	immune checkpoint inhibitors
IHC	immunohistochemistry
IM	invasive margins
KRAS	Kirsten rat sarcoma virus
mAb	monoclonal antibody
mCRC	metastatic colorectal cancer
MHC	major histocompatibility complex
miRNAs	micro-RNA
Mo	months
MSI-H	microsatellite instability-high
MSLN	mesothelin
MSS	microsatellite stable
NGS	next-generation sequencing
NK	natural killer
NKG2D	natural killer group 2 member D
NSCLC	non-small cell lung cancer
ORR	overall response rate
OS	overall survival
PD-1	programmed cell death protein-1
PD-L1	programmed cell death protein- ligand 1
PFS	progression-free survival
POLD1	DNA polymerase delta
POLE	DNA polymerase epsilon
PRC	polymerase chain reaction
PR2D	recommended phase 2 dose
TAA	tumor-associated antigen
TC	tumor core
TCR	engineered T cell receptors
TCR	T cell receptors
Teff	effector T cells
TILs	tumor-infiltrating lymphocytes
TME	tumor microenvironment
Treg	immunosuppressive regulatory T cells
TTR	time to recurrence
VEGF-A	vascular endothelial growth factor-A
WHO	World Health Organization

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