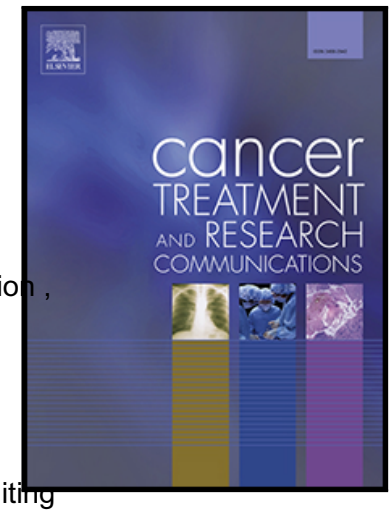

Immunotherapy in colorectal cancer: is the long-awaited revolution finally happening?

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Highlights

- Metastatic colorectal cancer remains a deadly disease with a poor prognosis
- Immune checkpoint inhibitors were found to be effective as treatment for microsatellite instability high metastatic colorectal cancer
- Combination treatments and biomarker selection are being developed to boost immunotherapy efficacy across a wider population

Immunotherapy in colorectal cancer: is the long-awaited revolution finally happening?

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Abstract
Immunotherapy has recently become a major treatment modality for several types of solid tumours, achieving remarkable and long-lasting remissions. In metastatic colorectal cancer patients (mCRC), immune checkpoint inhibitors (ICIs) were found to be effective as treatment for deficient mismatch repair (dMMR)/ microsatellite instability high (MSI-H) tumours and received regulatory approval for this indication. However, mCRC is a complex disease and dMMR/MSI-H tumours represent a minority of the cases; therefore, new strategies are needed to extend the benefits of immunotherapy to a larger population of patients. This review explores the immunological differences between dMMR/MSI-H and proficient mismatch repair (pMMR)/ microsatellite instability low (MSI-L) tumours, focuses on new proposed biomarkers to predict response to immunotherapy and illustrates results reported from the main clinical trials with immunotherapeutic agents in CRC, addressing the most promising approaches being currently developed.

1. Introduction

Colorectal cancer (CRC) is a major cause of cancer death worldwide, with 104,610 estimated new cases and 53,200 estimated deaths in the US in 2020.[1] In developed countries, early detection through screening has improved the 5-year survival of patients with CRC, but ~25% of patients still present with stage IV disease, and a further 25–50% present with early-stage disease but eventually develop metastatic

disease. The prognosis for patients with metastatic CRC (mCRC) remains poor, with an overall survival of approximately 30 months.[2–6] Thus, the development of more effective treatments for patients with this disease is an urgent unmet need.

In the past decade, immunotherapy has elicited tremendous excitement owing to its success in achieving long-term durable responses in previously difficult-to-treat solid tumours, such as melanoma and lung cancer [7]. The immune system distinguishes self from non-self through the binding of T-cell receptors (TCR) on T-cells to complexes of peptides with major histocompatibility complex (MHC) class I molecules presented on the surface of all cells, including tumour cells. Recognition of peptide–MHC class I complexes by the TCR alone is insufficient for T-cell activation; TCR–MHC signalling pathways are modulated by co-stimulatory or co-inhibitory signals, which tumour cells exploit to escape destruction [8]. Current immunotherapy strategies rely on immune checkpoint inhibitors, targeting co-inhibitory receptors, such as cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and programmed cell death protein 1 (PD-1) expressed on T-cells and other immune-cell subpopulations, or their ligands, such as programmed cell death protein 1 ligand 1 (PD-L1) expressed on tumour cells and various immune cells.

In CRC, immune checkpoint inhibitor therapy (ICI) received regulatory approval in 2017 for the treatment of tumours that are mismatch-repair-deficient (dMMR) or have high levels of microsatellite instability (MSI-H) (termed dMMR–MSI-H tumours). By contrast, current ICI therapies are ineffective in tumours that are mismatch-repair-proficient (pMMR) and are microsatellite-stable (MSS) or have low levels of microsatellite instability (MSI-L) (termed pMMR– MSI-L tumours). Following on the remarkable results of recently published clinical trials, in this review we describe the rationale for using immunotherapy in select patients with mCRC, discuss available clinical data supporting its use and highlight current clinical approaches and future directions for expanding the scope of immunotherapy in CRC.

2. dMMR–MSI-H and pMMR–MSI-L CRC

CRC can be categorized into two discrete groups on the basis of mutation patterns and ability to repair DNA microsatellite damage: tumours that have a dMMR–MSI-H signature and high overall mutation burden (>12 mutations per 106 DNA bases) and tumours that have a pMMR–MSI-L signature with a much lower mutation burden (<8.24 mutations per 106 DNA bases) [9]. MSI-H is the hallmark of tumours in patients with Lynch syndrome, but the development of this phenotype is a sporadic event in ~70–85% of all patients with dMMR–MSI-H tumours, commonly owing to somatic defects in MMR gene function such as hypermethylation of the *MLH1* promoter. MSI-induced frameshift mutations lead to the generation of a significant

number of mutations associated neoantigens (MANA), which account for the unique phenotypic characteristics of these tumours and explain the higher immunogenicity of MSI-H disease compared to MSI-L [10]. Importantly, dMMR–MSI-H tumours are heavily infiltrated by immune cells, notably CD8+ tumour-infiltrating lymphocytes (TILs), T helper 1 CD4+ TILs and macrophages and have an environment with higher levels of type I interferons compared to other types of CRC. These tumours also feature upregulation of several immune checkpoint regulators such as PD-1, PD-L1, CTLA-4, Lymphocyte activation gene 3 (LAG3) [11]. Both the high mutational burden and immune infiltration predict response to immune checkpoint blockade in several tumour types and might as well explain the different response to current immunotherapy approaches so far reported in dMMR–MSI-H and pMMR–MSI-L neoplasms [12,13].

Mismatch repair deficiency is observed in 10% to 20% of colorectal cancer patients and indicates a biologically distinct type of CRC with wide prognostic, predictive and therapeutic implications [14], because of which MMR testing is recommended by both National Health Institute for Health and Care Excellence (NICE) and European Society of Medical Oncology (ESMO) guidelines [15,16]. Current laboratory assays for MMR testing include either a multiplex polymerase chain reaction (PCR) assay [17], the "*Bethesda Panel*", or a multiplex immunohistochemistry (IHC) assay, in order to demonstrate the absence of one of four mismatch repair (MMR) enzymes (MLH1, MSH2, MSH6, and PMS2) [18]. Both assays require cost, additional tumour tissue and produce variable results, with different sensitivity and specificity reported in reference studies [19–22]. Newer approaches are being validated to address these issues, such as deep learning based identification using haematoxylin and eosin-stained slides [23].

3. Biomarkers for response to immunotherapy in CRC beyond dMMR/MSI status

The presence of dMMR/MSI-H is an established biomarker for response to immunotherapy but given the complexity of anti-tumour immune response and disease heterogeneity, this characteristic is likely not enough to predict response to ICIs [24].

3.1 PD-L1

PD-1 is expressed by activated T-cells, B-cells, and natural killer (NK) cells and can bind to its ligand, PD-L1, expressed on tumour cells [25]. However, several issues prevent PD-L1 expression from being a clinically useful biomarker, including the

lack of standardization of PD-L1 expression evaluation. In CRC, PD-L1 expression was not found to be associated with response or survival in the registration studies [26].

3.2 Tumour Mutational Burden

As stated before, mutation associated neoantigens can elicit an anti-tumour immune response [27]. In CRC a high tumour mutational burden (TMB) has been proven to predict response to immunotherapy [28,29]. High TMB is frequently observed in dMMR/MSI-H tumours but it has also been reported in neoplasms harbouring a POLE exonuclease domain mutation (1-2% of all CRC tumours). Given the similarly enhanced immunogenicity of POLE-mutated CRCs and dMMR/MSI-H CRCs, the therapeutic potential of immune checkpoint blockade in the subset of POLE- mutated CRCs is of particular interest [30]. Recent evidence suggest DNA damage response (DDR) defects, and subsequent high TMB and upregulation of PD-L1, might as well predict response to immunotherapy, even in pMMR/MSI-L tumours [31].

3.3 Immunoscore

A scoring system (Immunoscore) based on the numeration of two lymphocyte populations (CD3/CD45RO, CD3/CD8, or CD8/CD45RO), both in the core of the tumour and in the invasive margin of tumours, has been validated as a prognostic tool in early CRC [32]. It has been recently reported that higher intra-tumoral CD3+ and CD8+ T-cell densities were associated with a higher overall response rate and duration of disease control in a small sub- group of patients with dMMR/MSI-H mCRC treated with anti-PD-1 antibody pembrolizumab [33].

3.4 Gene expression signature

A comprehensive re-evaluation and comparison of CRC molecular gene-expression profiles has enabled the CRC Subtyping Consortium (CRCSC) to identify four consensus molecular subtypes (CMS) [34]. Stage-independent prognostic value and significant association with clinical, biological, and treatment features have been demonstrated and recently validated in phase III clinical studies [35–37]. CMS1-immune and CMS4-mesenchymal subtypes are both considered to be immune-reactive and highly infiltrated by immune cells as opposed to CMS2-canonical and CMS3-metabolic; therefore, both are likely to respond well to immune therapies, but they should each be treated distinctly. CMS1 tumours feature CD8+ T-cells and CD68+ macrophage infiltration as well as frequent upregulation of immune checkpoint molecules (CTLA-4, PD-1, and PD-L1) and might benefit from ICI therapy alone. CMS4 CRCs present a different pattern of immune infiltration, which is mainly suppressive; it features the infiltration of myeloid-derived suppressor cells (MDSCs),

T-regulatory cells (Tregs), monocyte-derived cells, and Th17 cells and upregulation of immunosuppressive factors such as Transforming Growth Factor beta (TGF-b), CXCL12, IL-23, IL-17. It is likely that this subtype would be best suited to strategies combining TGF-b inhibitors, Tregs/ MDSCs inhibition, and ICIs. It should be noted that CMS classification was developed using primary tumour samples and has not been validated in the metastatic setting.

3.5 Microbiome

Finally, it has been suggested that the gut microbiome also influences the outcome of cancer therapy by modulating the host inflammatory response [38]; however, its role in predicting response to immunotherapy in CRC remains largely unexplored and most of the evidence available is derived from pre-clinical models.

Administration of IL-10 CpG oligodeoxynucleotide has proven to be successful in treating early CRC in mice; the effectiveness of this combination supposedly relies on Tumour Necrosis Factor alpha (TNF-a) local release in the tumour microenvironment as induced by the *Alistipes shahii* bacterial species; interestingly, this effect is lost upon antibiotics administration [39]. Likewise, anti-CTLA-4 drug ipilimumab reduces the growth of colorectal cancer in mice with normal microflora, but has no effect germ-free specimens or when broad spectrum antibiotic therapy is applied; however it has been shown that transplant of fecal microbial composition including immunogenic Bacterioides restores efficacy of CTLA-4 inhibitors [40]. A similar effect can be obtained when fecal transplant containing Akkermansia muciniphila is combined with anti-PD-1 therapy [41].

4. Immunotherapy for dMMR–MSI-H CRC

Early studies published between 2010 and 2013 proved very limited activity for ICIs in non-selected mCRC, with anecdotal efficacy reported in MSI-H patients [42–44]. On the basis of the knowledge of the immunogenic microenvironment of MSI-H tumours and the impressive tumour response observed, several studies were launched to investigate the therapeutic potential of PD-1 inhibitors. A phase II trial (NCT01876511, KEYNOTE-016) of the anti-PD-1 antibody pembrolizumab was reported in 2015, in which three separate cohorts of patients were treated: dMMR–MSI-H CRCs, pMMR–MSI-L CRCs and dMMR–MSI-H non-CRCs [45]. In updated results presented at the 2016 American Society of Clinical Oncology (ASCO) Annual Meeting, the response rate (RR) was 50% and the disease control rate (DCR) was 89% in patients with dMMR–MSI-H tumours versus 0% and 16% for pMMR CRC, respectively. At 24 months, progression free survival (PFS) was 61%, and overall survival (OS) was 66% in MSI-H disease [46]. Interestingly, the number of somatic

mutations significantly correlated with the chance of achieving a response to therapy [47]. These results led to the regulatory approval of pembrolizumab for patients with dMMR CRC after prior treatment with fluoropyrimidine, oxaliplatin, and irinotecan. In July 2017, the FDA also approved anti-PD-1 antibody nivolumab, either alone or in combination with low dose anti CTLA-4 antibody ipilimumab, for patients with dMMR CRC based on the results of the CheckMate 142 study [48,49]. This trial enrolled in different cohorts both pre-treated and treatment-naïve dMMR mCRC patients. Previously treated patients were allocated to receive either single agent nivolumab or a combination of nivolumab plus low dose ipilimumab. Single agent nivolumab yielded an overall response rate of 31.1%, with 69% of patients showing disease control for 12 weeks or longer; twelve-month progression-free survival was 50% and 12-month overall survival was 73%. Latest available data on the combination cohort show an overall response rate (ORR) of 55% and disease control rate of 80%. Among all responders, median duration of response was not reached, with 94% of responses ongoing at data cut-off. The 9-months PFS and OS rates were 76% and 87%, respectively. Treatment with combined nivolumab and ipilimumab resulted in an increased rate of drug-related immune adverse events: 32% of patients experienced grade 3–4 treatment- related adverse events compared with 20% of patients treated with nivolumab alone [50]. CM-142 also evaluated the combination of nivolumab plus low dose ipilimumab as first line treatment in dMMR–MSI-H CRC. The most recent update reported at ASCO GI Symposium 2020 showed an ORR of 64% and a DCR of 84%; median PFS and OS were not reached. Compared to the cohort of previously treated patients, the combination showed a better safety profile with only 20% of patients experiencing treatment-related grade 3 and 4 toxicities [51]. At ASCO GI Symposium 2021 authors confirmed objective response was observed regardless of RAS/BRAF mutational status, ECOG performance status, tumour sidedness and stage at diagnosis [52]. In 2020 the investigators of the KEYNOTE-177 trial reported the results of a study comparing pembrolizumab to 5-fluorouracil–based chemotherapy with or without anti-Vascular Endothelial Growth Factor (VEGF) drug bevacizumab or anti Epidermal Growth Factor Receptor (EGFR) drug cetuximab as first line treatment in MSI-H mCRC [53]. The two primary end points were progression-free survival and overall survival. After a median follow-up of 32.4 months, pembrolizumab was found to be superior to chemotherapy with respect to progression-free survival (median, 16.5 vs. 8.2 months; hazard ratio, 0.60; P=0.0002). An overall response was observed in 43.8% of the patients in the pembrolizumab group and 33.1% in the chemotherapy group; among patients with an overall response, 83% in the pembrolizumab group had ongoing responses at 24 months. Efficacy was observed across the whole population, but early data suggest that RAS

mutant tumours might derive a smaller benefit. Updated results presented at ASCO GI 2021 show an improvement in PFS2 as well, with a median PFS2 not reached vs. 3.5 months (HR 0.63; 95% CI, 0.45-0.88) [54]. Treatment-related adverse events of grade 3 or higher occurred in 22% of the patients in the pembrolizumab group. Based on these results FDA approved pembrolizumab as first line treatment of patients with unresectable or metastatic dMMR-MSI-H colorectal cancer. Final analysis, presented at ASCO 2021 Annual Meeting, showed a median PFS of 16.5 vs 8.2 months (HR 0.59; 95% CI, 0.45-0.79 and ORR of 45.1% vs 33.1%. Hazard ratio for OS favoured pembrolizumab monotherapy, although it did not reach statistical significance; authors reported a 60% effective crossover rate in the intention-to-treat population [55]. Research in this subtype of patients is still ongoing with new results reported from a phase 2 trial investigating the activity of anti-PD-1 antibody dostarlimab in patients with dMMR or POLE mut gastro-intestinal tumours; confirmed ORR in dMMR patients was 38.7% (36% in CRC alone) with 80.9% of responses ongoing after 18 months [56]. Further strategies being investigated in this population of patients include the use of anti-PD-L1 antibody atezolizumab either alone or in combination with mFOLFOX6 plus bevacizumab compared to chemotherapy plus bevacizumab as first line treatment (NRG-GI004/SWOG-S1610, COMMIT) [57]. Preclinical evidence suggests in fact that oxaliplatin-containing chemotherapy in combination with anti-VEGF enhances the anti-tumour activity of PD-1 pathway blockade in murine CRC models [58]. While durable disease control is often observed for advanced MSI-H cancers treated with immune checkpoint inhibitors, some patients experience treatment resistance, frequently associated with increased signalling of the immunosuppressive TGF-b pathway. Bintrafusp alfa, a bifunctional fusion protein composed of the extracellular domain of the TGF-bRII receptor fused to a human IgG1 monoclonal antibody blocking PD-L1, was tested in a cohort of 15 patients (12 of which had CRC) who progressed on prior ICI therapy; unfortunately the experimental drug did not demonstrate significant anti-tumour activity [59].

5. Immunotherapy for pMMR-MSI-L CRC

Unlike in patients with dMMR–MSI-H CRC, immunotherapy alone has not demonstrated a clinical benefit in patients with pMMR–MSI-L CRC, who constitute the vast majority of patients with mCRC. For these patients, ICIs are being actively explored in combination with treatments that aim to increase the intra-tumoral immune response and render the tumour ‘immune-reactive’.

5.1 Combination immunotherapy

Dual blockade of multiple TCR co-repressors has proven effective in several solid tumours, including dMMR-MSI-H CRCs. A combination of anti PD-L1 antibody durvalumab plus anti CTLA-4 antibody tremelimumab has been compared to best supportive care alone in refractory CRC. The combination improved OS with a HR of 0.66 (p= .02) in MSS patients; the benefit was even greater in TMB-high patients (HR 0.34; p= .004) [60]. The same combination was tested in association to oxaliplatin/5-FU based chemotherapy as first line treatment in MSS RAS mutant CRC in the phase Ib/II MEDETREME trial, yielding a 6 months PFS of 62.5%; median PFS was not reached [61]. Isatuximab is a monoclonal antibody directed against CD38 receptors expressed on immunosuppressive cells in the tumour microenvironment. It was recently compared in combination with atezolizumab to tyrosine kinase inhibitor (TKI) regorafenib in the phase Ib/II MORPHEUS trial; unfortunately, superior efficacy of this combination was not shown [62]. The same platform also evaluated a combination of atezolizumab, bevacizumab and Imprime, a pathogen-associated molecular mimic which activates the innate immune response, unfortunately yielding no activity signal [63].

5.2 Radiation therapy

Preclinical and early clinical studies have suggested that radiation therapy (RT) or chemo-radiation therapy (CRT) may expose neoantigens through immunogenic cell death (ICD), thus eliciting immune-mediated antitumour responses. This immune effect is applicable not only to the irradiated tumour site, but also to distant sites through the 'abscopal effect', which theoretically could be enhanced with ICIs [64]. The data to understand the role of RT and ICIs in CRC are, however, limited. Recently, a combination of nivolumab plus ipilimumab with RT (8 Gy in three fractions to a single metastatic lesion) demonstrated promising activity in a phase II study that included 40 patients with chemo-refractory mCRC; this strategy provided a DCR of 37%, with a median DOR of over 15 months. However 33% of the patients enrolled did not receive RT due to toxicity or progressive disease [65]. In contrast with this experience, two more phase II trials showed limited activity for this approach, using both single agent and combination ICIs [66–68].

5.3 Chemotherapy and targeted therapies

Agents currently used to treat metastatic CRC can modulate anti-tumour immune response. Chemotherapy regimens can induce ICD by releasing damage-associated molecular patterns (DAMPs) [69]. Analysis of CRC liver metastases revealed that patients treated with preoperative chemotherapy had a significantly higher density of cytotoxic and memory T-cells compared with samples of untreated patients; this was

also true for patients achieving pathological or radiological responses, suggesting the development of an adaptive immune response [70,71].

VEGF is often upregulated in cancer where it contributes to tumour angiogenesis; it also plays a role in the immune microenvironment by upregulating immune checkpoint molecules (PD-1, PD-L1, CTLA-4, LAG-3) and downregulating antigen-presentation molecules. Additionally, VEGF inhibits dendritic cell (DC) maturation and increases the function of suppressor cells [72,73]. Combination of anti VEGF agents and ICIs was first tested in the MODUL trial, where maintenance treatment with combined atezolizumab/bevacizumab/fluoro- pyrimidine after first-line induction with FOLFOX/bevacizumab did not demonstrate any clinical benefit in PFS or OS compared to bevacizumab and fluoropyrimidine alone [74]; additionally a biomarker analysis was recently reported, showing that in BRAF wt population, neither PD-L1, CD8/GrB or FoxP3 appear to have predictive value [75]. Combination of anti-angiogenetic and immunotherapeutic drugs was further tested in the REGONIVO/EPOC1603 phase Ib trial, in which a combination of regorafenib plus nivolumab showed encouraging anti-tumour activity in refractory mCRC with a manageable safety profile. The combination produced a 36% RR with a 7.9 months median PFS in an Eastern population [76]. However the combination of regorafenib plus nivolumab showed a much more modest activity in a study on Western population [77], with newer reports showing a RR of 7.1% and a trend for greater benefit in patients with higher tumour immune infiltration and higher circulating angiogenesis biomarkers [78]. Furthermore, early results from a phase II trial testing a combination of capecitabine, bevacizumab and nivolumab in pre-treated MSS mCRC patients were recently published, showing a 9% RR and an expected toxicity profile [79]. Additionally, results presented at this year ASCO GI Symposium show that a combination of pembrolizumab, bevacizumab and capecitabine is safe and active in MSS CRC patients, with a DCR of 80% [80]. Results from the colorectal cancer cohort of the LEAP-005 trial were presented as well, testing a combination of the TKI lenvatinib and pembrolizumab in pMMR tumours; authors reported an ORR of 22% with median DOR not reached, a median PFS of 2.3 months and a median OS of 7.5 months [81].

Accumulating preclinical evidence suggests that anti EGFR antibody cetuximab can evoke a T-cell mediated anti-tumour immune response and stimulate NK-mediated cell-antibody-dependent cellular cytotoxicity [82,83]. Interestingly this activity is observed regardless of the RAS mutational status [84]. In the single-arm phase II AVETUX study a regimen of avelumab and cetuximab plus oxaliplatin, leucovorin, and 5-fluorouracil (mFOLFOX6) in patients with previously untreated RAS/BRAF-wild type metastatic CRC induced an 80% response rate but did not meet its primary

progression free survival end point, with a 12 months PFS rate of only 40% [85]. Interim analysis from a different proof of concept phase II trial (AVETUXIRI) was recently reported; a combination of avelumab plus cetuximab and irinotecan was tested in a population of refractory mCRC patients irrespective of RAS mutational status and obtained a similar DCR in both RAS wild type and RAS mutant cohorts (60% and 61.5%, respectively); 6 months-PFS rate was 40.0% and 38.5% in RAS wild type and mutant patients while 12 months-OS rate was reported at 53.3% and 57.7%, respectively [86]. A similar strategy was explored in the CAVE Colon phase II trial, testing a combination of avelumab plus cetuximab as a rechallenge treatment in previously treated RAS wild type metastatic CRC patients. The authors reported a 3.6 months median PFS with a 55.4% DCR; OS was reported to be 13.1 months [87]; interestingly grade 2 or 3 cutaneous toxicity seems to predict better OS [88]. Dual ICIs combination strategies are also being tested; early results from a trial evaluating a combination of nivolumab, ipilimumab and panitumumab in MSS RAS wild type mCRC show a 12-week RR of 35% and a median PFS of 5.7 months [89]. Safety profile was acceptable but there was a single grade 5 event of myocarditis.

Preclinical studies have reported that MEK inhibitors could have synergistic activity with ICIs [90]. This possibility was further explored in a phase Ib trial evaluating a combination of atezolizumab and MEK inhibitor cobimetinib, which achieved a 37% response rate [91]. However, the subsequent IMblaze370/COTEZO randomized phase III trial failed to confirm this result, showing no increase in tumour response or survival for patients treated with the combination compared to patients treated with single agent atezolizumab or regorafenib [92].

5.4 Other strategies

New approaches are currently being evaluated in clinical trials and involve modulation of the immune environment via cytokines/ chemokines, regulation of metabolic pathways, use of bi-specific fusion proteins, oncolytic viruses. One trial was recently reported, testing a combination of nivolumab plus metformin in refractory MSS colorectal cancer; preclinical data suggests in fact that metformin can improve immune exhaustion of tumour infiltrating lymphocytes and potentiate the effects of PD-1 blockade by normalizing the hypoxic tumour micro-environment. Unfortunately, while 2 of the patients treated achieved stable disease, authors reported no objective response and the trial did not proceed to the following stage of enrolment [93]. The main ongoing clinical trials are listed in Table 1.

6. Adjuvant/neo-adjuvant therapy

Efforts are being made to integrate immunotherapy strategies in the early-stage setting. The phase III randomized controlled ATOMIC trial comparing standard chemotherapy alone or in combination with atezolizumab as adjuvant therapy for patients with stage III dMMR colon cancer is currently ongoing and results are eagerly awaited [94].

Last year results from the pilot NICHE trial were published, testing a pre-operative combination of nivolumab plus ipilimumab in dMMR/pMMR colorectal cancer patients; patients received a single dose of ipilimumab and two doses of nivolumab before surgery, the pMMR group with or without celecoxib. Pathological response was observed in 20/20 dMMR tumours, with 19 major pathological responses (MPRs, ≤10% residual viable tumour) and 12 pathological complete responses. In pMMR tumours, 27% of patients showed pathological responses; notably CD8+ T-cells infiltration was predictive of response [95]. Results were recently reported from the experimental pembrolizumab arm in the platform phase II trial NRG-GI002 using total neoadjuvant therapy (TNT) in locally advanced rectal cancer. Pembrolizumab added to chemo-radiotherapy as part of TNT was safe and without unexpected short-term toxicities but failed to improve the Neoadjuvant Rectal Cancer score and the combination did not meet PFS and OS endpoints [96].

Finally, at ASCO 2021 results from the AVANA study were presented, showing a promising activity and a feasible safety profile for the combination of preoperative chemoradiotherapy plus avelumab in locally advanced rectal cancer. Authors reported a 23% pathological complete response (pCR) rate and a 61% MPR rate; interestingly, of the patients evaluable for MMR status, most were pMMR [97].

7. Conclusions

It is evident that substantial advances have been made with immunotherapy approaches in CRC. An algorithm of possible treatment sequencing based on available clinical data is shown in Figure 1. Unfortunately, most of the progress has been observed in dMMR/MSI-H CRC, which represent only a small subgroup of all colorectal tumours.

The key remaining challenge remains to identify which patients are most likely to benefit from immunotherapy, either alone or in combination with synergistic agents. It is fundamental to further develop on biomarkers to predict benefit from existing regimens and to insist on researching novel agents able to target specific features of the different subtypes of colorectal cancer, in order to expand treatment possibilities for such patients.

List of abbreviations

ASCO, American Society of Clinical Oncology
CMS, Consensus Molecular Subtype
CRC, Colorectal Cancer
CRCSC, CRC Subtyping Consortium
CRT, Chemoradiotherapy
CTLA-4, Cytotoxic T Lymphocyte Associated Antigen 4
DAMP, Damage Associated Molecular Pattern
DCR, Disease Control Rate
DDR, DNA Damage Response
dMMR, Deficient Mismatch Repair
EGFR, Epidermal Growth Factor Receptor
ESMO, European Society of Medical Oncology
FDA, Food and Drug Administration
ICI, Immune Checkpoint Inhibitor
ICD, Immunogenic Cell Death
LAG3, Lymphocyte Activation Gene 3
MANA, Mutation Associated Neoantigen
mCRC, Metastatic Colorectal Cancer
MDSC, Myeloid Derived Suppressor Cell
MHC, Major Histocompatibility Complex
MMR, Mismatch repair
MPR, Major Pathological Response
MSI-H, Microsatellite Instability High
MSI-L, Microsatellite Instability Low
MSS, Microsatellite Stable
NICE, National Institute for Health and Care Excellence
NK, Natural Killer
ORR, Overall Response Rate
OS, Overall Survival
pCR, Pathological Complete Response
PD-1, Programmed Death 1
PD-L1, Programmed Death Ligand 1
PFS, Progression Free Survival
pMMR, Proficient Mismatch Repair
RR, Response Rate
RT, Radiotherapy
TCR, T-cell receptor

TIL, Tumour Infiltrating Lymphocyte
TMB, Tumour Mutational Burden
TGF-b, Transforming Growth Factor beta
TKI, Tyrosine Kinase Inhibitor
TNFa, Tumour Necrosis Factor Alpha
Treg, T Regulatory cell
VEGF, Vascular Endothelial Growth Factor

Author's contribution

Gianluca Arrichiello: Conceptualization; Writing - Original Draft; Visualization

Luca Poliero: Conceptualization; Writing - Original Draft; Visualization

Carola Borrelli: Data Curation

Fernando Paragliola: Data Curation

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Stefania Napolitano: Supervision

Carminia Maria Della Corte: Supervision

Giulia Martini: Supervision; Writing - Review & Editing

Erika Martinelli: Project administration; Supervision; Writing - Review & Editing

Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Currently active immunotherapy studies in CRC (Table 1)

Metastatic Colorectal Cancer					
Sub-population	**LoT**	**Combination Strategy**	**Interventions**	**Trial Type**	**Trial Identifier**
dMMR/MSI-H	1L	Immune regulation	Pembro	Phase 3	NCT02563002
None	>1L	Immune regulation	Pembro	Phase 2	NCT02460198
None	N/A	Immune regulation	Durva + Treme	Phase 2	NCT02870920
dMMR/MSI-H	N/A	Immune regulation	Nivo + Ipi	Phase 3	NCT04008030
dMMR/MSI-H	2L	Immune regulation	Avelumab	Phase 2	NCT03186326
pMMR/MSI-L	N/A	Immune regulation	Nivolumab	Phase 2	NCT03981148
None	N/A	Immune regulation	BO-112 + Pembro	Phase 2	NCT045078140
pMMR/MSI-L	N/A	Immune regulation	Nivo + Relatlimab	Phase 2	NCT03642067
pMMR/MSI-L	N/A	VEGF	Nivo + Regorafenib	Phase 2	NCT04126733
None	N/A	VEGF	Atezo + Bev	Phase 2	NCT02982694
None	N/A	VEGF	Ave + Regorafenib	Phase 1/2	NCT03475953
None	N/A	VEGF	Atezo + Cabozantinib	Phase 1/2	NCT03170960
None	N/A	VEGF	Pembro + Regorafenib	Phase 1/2	NCT03657641
None	N/A	VEGF	Pembro + Lenvatinib	Phase 2	NCT03797326
None	>2L	EGFR	Ave + Cetuximab	Phase 2	NCT04551336
pMMR/RASwt	N/A	EGFR	Nivo + Ipi + Panitumumab	Phase 2	NCT03442569
None	N/A	EGFR	Pembro + Cetuximab	Phase 2	NCT02713373
KRAS G12C mut	N/A	EGFR	Pembro + MRTX849 + Cetuximab	Phase 1/2	NCT03785249
None	N/A	MEK	Nivo / Ipi / Cobimetinib / Daratumumab / anti LAG 3	Phase 2	NCT02060188
None	N/A	MEK	Pembro + Bev + Binimetinib	Phase 2	NCT03475004

None	N/A	MEK	Nivo + Ipi + Trametinib	Phase 1/2	NCT03377361
pMMR/MSI-L	N/A	MEK	Durva + Trametinib	Phase 2	NCT03428126
pMMR/RASmut	N/A	MEK	Nivo + Ipi + Binimetinib	Phase 2	NCT03271047
pMMR/BRAFmut	N/A	BRAF/MEK	Nivo + Encorafenib + Binimetinib	Phase 1/2	NCT04017650
None	1L	Chemotherapy/VEGF	Atezo + Bev + FOLFOXIRI	Phase 2	NCT03721653
pMMR/MSI-L	N/A	Chemotherapy/VEGF	Pembro + Bev + CAPOX	Phase 2	NCT04262687
pMMR/MSI-L	N/A	Chemotherapy/VEGF	Pembro + Bev + Capecitabine	Phase 2	NCT03396926
RAS/BRAFmut	>1L	Chemotherapy/VEGF	Nivo + Bev + FOLFOXIRI	Phase 2	NCT04072198
None	>1L	Chemotherapy/VEGF	Atezo + Bev + Capecitabine	Phase 2	NCT02873195
None	1L	Chemotherapy/VEGF	Nivo + FOLFOX	Phase 2/3	NCT03414983
dMMR/MSI-H	N/A	Chemotherapy/VEGF	Atezo + Bev + FOLFOX	Phase 3	NCT02997228
pMMR/MSI-L	>1L	Chemotherapy/EGFR	Ave + Cetuximab +Irinotecan	Phase 2	NCT03608046
None	N/A	Chemotherapy/EGFR	Ave + Cetuximab +FOLFOXIRI	Phase 2	NCT04513951
None	1L	Chemotherapy/EGFR	Ave + Cetuximab +FOLFOX	Phase 2	NCT03174405
None	N/A	Chemotherapy/EGFR	Pembro +FOLFOX	Phase 2	NCT02375672
pMMR/MSI-L	N/A	Chemotherapy	Pembro + Temozolomide	Phase 2	NCT03519412
pMMR/MSI-L	N/A	Chemotherapy	Nivo + Ipi + Temozolomide	Phase 2	NCT03832621
None	N/A	Chemotherapy	Nivo + CDDP + Temozolomide	Phase 2	NCT04457264
None	N/A	Chemotherapy	Durva + Treme + FOLFOX	Phase 1/2	NCT0320758
None	N/A	Radiation Therapy	Atezo + RT	Phase 2	NCT02992912
None	N/A	Radiation Therapy	Pembro + RT	Phase 2	NCT02437071
None	N/A	Radiation Therapy	Durva + Treme + RT	Phase 2	NCT03101475

None	N/A	Radiation Therapy	Durva + Treme + RT	Phase 2	NCT02888743
None	N/A	Radiation Therapy	Nivo + Ipi + RT	Phase 2	NCT03104439
None	N/A	Multiple	Rego / Atezo / Imprime PGG / Bev / Selicrelumab/ Isatuximab / Idasanutlin / AB928	Phase 1/2	NCT03555149
dMMR/MSI-H	N/A	TGF-beta	M7824	Phase 1/2	NCT03436563
None	N/A	Cytokines	ICI + N-803	Phase 2	NCT03228667
None	N/A	Cytokines	Pembro + poly-ICLC	Phase 1/2	NCT02834052
pMMR/MSI-L	N/A	PI3K	Nivo + Copanlisib	Phase 1/2	NCT03711058
pMMR/MSI-L	N/A	Other	Nivo + metformin	Phase 2	NCT03800602
Early Colorectal Cancer					
Tumour Type	**LoT**	**Combination Strategy**	**Interventions**	**Trial Type**	**Trial Identifier**
Lynch Syndrome	Adj	Immune regulation	Nivo	Phase 2	NCT03631641
None	Adj	Personalized treatment	Nivo / FOLFIRI / Enco + Bini + Cetuximab	Phase 3	NCT03803553
dMMR/MSI-H	Adj	Chemotherapy	Atezo + FOLFOX	Phase 3	NCT02912559
dMMR/POLEmut	Adj	Chemotherapy	Ave + FOLFOX	Phase 3	NCT03827044
Rectal Cancer	NeoAdj	Radiation Therapy	Ave + Capecitabine + RT	Phase 2	NCT03854799
Rectal Cancer	NeoAdj	Radiation Therapy	Pembro + RT	Phase 2	NCT04109755
Rectal Cancer	NeoAdj	Radiation Therapy	Atezo + CRT	Phase 1/2	NCT03127007
Rectal Cancer	NeoAdj	Radiation Therapy	Nivo + Ipi + CRT	Phase 2	NCT04124601
Rectal Cancer	NeoAdj	Radiation Therapy	Durva+ CRT	Phase 2	NCT04293419
pMMR RC	NeoAdj	Radiation Therapy	Durva+ CRT	Phase 2	NCT03102047

Future possible treatment options in metastatic CRC (Figure 1)