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Advances in immunotherapy for MMR proficient colorectal cancer

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A B S T R A C T

Survival in mismatch-repair proficient (MMRp) metastatic colorectal cancer (mCRC) remains poor and chemotherapy is the mainstay of treatment. Immunotherapy has demonstrated durable responses and a favourable side-effect profile in various cancer types and multiple clinical trials have been conducted in MMRp mCRC. In this review we summarise emerging trial data which demonstrate promising immunotherapy combinations in MMRp mCRC. We outline barriers to success, evaluate emerging biomarkers and discuss potential strategies to increase the effectiveness of immunotherapy in MMRp mCRC.

Introduction

Survival of patients diagnosed with metastatic colorectal cancer (mCRC) remains poor, with a median overall survival (OS) of 30 months in those who start first-line chemotherapy [1,2]. Median OS in the second-line setting is 13 months with chemotherapy and anti-angiogenic agents [3,4]. Median OS in the third-line setting is 7.1 months with trifluridine-tipiracil hydrochloride chemotherapy or regorafenib [5]. There is hence a need for new therapies.

Fewer side effects and better quality of life have been reported for immunotherapy compared to chemotherapy in several cancer types [6,7]. Immunotherapy with immune checkpoint inhibitors (ICI) is transforming the management of mismatch-repair deficient (MMRd)/microsatellite unstable (MSI) mCRCs based on high response rates and in some cases durable disease control [8,9]. However, fewer than 5 % of all mCRCs are MMRd therefore the benefit is highly restricted [10].

Immunotherapy is so far largely considered ineffective in mismatch-repair proficient (MMRp)/microsatellite-stable (MSS) mCRC [8]. Yet, trials combining immune checkpoint inhibitors (ICI) with conventional cancer drugs or new immunotherapeutics have started to expose vulnerabilities in MMRp mCRCs (see Fig. 1 for an overview of the main targets that are being addressed). Lower mutation and neoantigen loads in MMRp compared to MMRd mCRCs are considered the main reason for the lack of immunotherapy responses [11]. Immune recognition in MMRp CRCs with their low antigenicity may be more easily countered by immunosuppressive mechanisms that are common in these tumours, such as transforming growth factor β (TGFβ) expression, Wnt/β-catenin pathway activation or Kirsten rat sarcoma virus (KRAS) mutations [14–18]. CRC has been categorised into four consensus molecular subtypes (CMS) [12] which display different immunological characteristics and may require different immunotherapy approaches [13]. In this review, we evaluate recent immunotherapy clinical data and MMRp mCRC subgroups that may particularly benefit from specific strategies. We summarize data that identifies likely reasons for the low success rates and how these can potentially be overcome.

Clinical trials of PD-1/PD-L1 ICI with or without CTLA-4 ICI

Single-agent immunotherapy studies failed to demonstrate significant success in mCRC patients unselected according to MMR status [14–16]. In a single-arm phase 2 study of the Programmed Cell Death-1 antibody (anti-PD-1) pembrolizumab in heavily pretreated mCRC, the MMRp cohort of 18 patients demonstrated a disappointing objective response rate (ORR) of 0 %, progression-free survival (PFS) 2.2 months and overall survival (OS) 5 months [8]. This was in stark contrast to MMRd mCRC patients in this trial, where a high ORR of 40 % (4 out of 10 patients), median PFS and OS not reached, was seen. Genomic analysis of both groups revealed a mean of 1782 somatic mutations per MMRd cancer versus 73 mutations per MMRp cancer, and 578 computationally predicted mutation-associated neoantigens in MMRd versus 21 in MMRp cancers.

Dual checkpoint inhibition of both the PD-1/L1 axis and anti-cytotoxic *T*-lymphocyte associated protein (CTLA-4) was warranted as it had improved cancer immune recognition in several other tumour types [17]. The Cancer Trials Group CO.26 randomised phase 2 trial explored the combination of durvalumab (anti-PD-L1) and

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tremelimumab (anti-CTLA-4) versus best supportive care (BSC) in mCRC patients who exhausted all standard therapies [18]. Of 179 randomised patients, 166 had MSS tumours, 2 were MSI and the remainder were unknown. The ORR with durvalumab and tremelimumab was only 0.8 % (1/118 patients). The median PFS was 1.8 months in the durvalumab and tremelimumab group and 1.9 months with BSC. However, there was a non-significant trend towards longer OS with 6.6 months in the durvalumab and tremelimumab group versus 4.1 months with BSC. MSS patients with higher plasma tumour mutational burdens (TMB) $\geq$ 28/Mb variants experienced greater OS benefit.

The TAPUR phase 2 basket study in patients with advanced cancers who had exhausted all lines of therapy reported results from mCRC patients with high TMB (defined as $\geq$ 9 mutations/megabase) treated with nivolumab (anti-PD-1) and ipilimumab (anti-CTLA-4) [19]. The ORR in this small cohort of 10 patients was 10 %, with median PFS 3.1 months, median OS 9.9 months.

An expanded phase 1 study of botensilimab (anti-4) and balstilimab (anti-PD-1) in 41 evaluable heavily pretreated patients with MMRp mCRC demonstrated an ORR of 24 % (10/41 patients) with a duration of response ranging from 0 to 17 + months [20]. ORR was higher (42 %) in patients without liver metastases or with resected or ablated liver metastases suggesting that sites of metastases may affect response to immunotherapy. Botensilimab is a next-generation anti-CTLA-4 which promotes intratumoral regulatory *T*-cell (Treg) depletion via enhanced Fc-gamma receptor signaling. Whether such regulatory *T*-cell depleting antibodies have generally higher activity in MMRp CRC needs to be assessed and could provide important insights into the immunobiology of these tumours.

ICI in combination with chemotherapy

The rationale of combining ICI with cytotoxic chemotherapy is to induce immunogenic cell death which releases tumour antigens in a way that improves CD8 *T*-cell activation [21–23]. The phase 2 BACCI trial randomised patients with advanced CRCs that had progressed on all licensed therapies to capecitabine chemotherapy and bevacizumab (monoclonal anti-vascular endothelial growth factor [VEGF]-A antibody) with or without atezolizumab (anti-PD-L1)[24]. Among 133 randomised patients, 86.7 % of cancers in the atezolizumab arm and 85.7 % in the control arm were MMRp. In MMRp mCRCs, ORR in the atezolizumab arm was 8.54 % versus 4.35 % in the control arm, which was not statistically significant ($p = 0.5$). In all randomised patients, the median PFS was 4.4 months in the atezolizumab arm and 3.3 months in the control arm ($p = 0.051$). Twelve-month OS in the atezolizumab arm was 52 % versus 43 % in the control arm which was not significant ($p = 0.4$).

The first-line phase 2 Checkmate 9x8 trial of FOLFOX (folinic acid, fluorouracil and oxaliplatin) chemotherapy with bevacizumab and nivolumab (experimental) versus FOLFOX and bevacizumab (SOC) in CRC who were unable to undergo curative resection demonstrated an ORR of 60 % in experimental arm versus 46 % in the standard of care (SOC) group [25]. Median PFS was 11.9 months in both arms ($p = 0.3$). Median OS was 29.2 months in the nivolumab arm and not reached in the SOC arm. 95 % (121/127) of patients in the nivolumab group and 90 % (61/68) patients in the SOC group were MMRp. An exploratory subgroup analysis investigating MMRp tumours only indicated that a greater proportion of patients with CMS1 and CMS3 tumours remain progression free at 12 months with nivolumab. However, numbers for

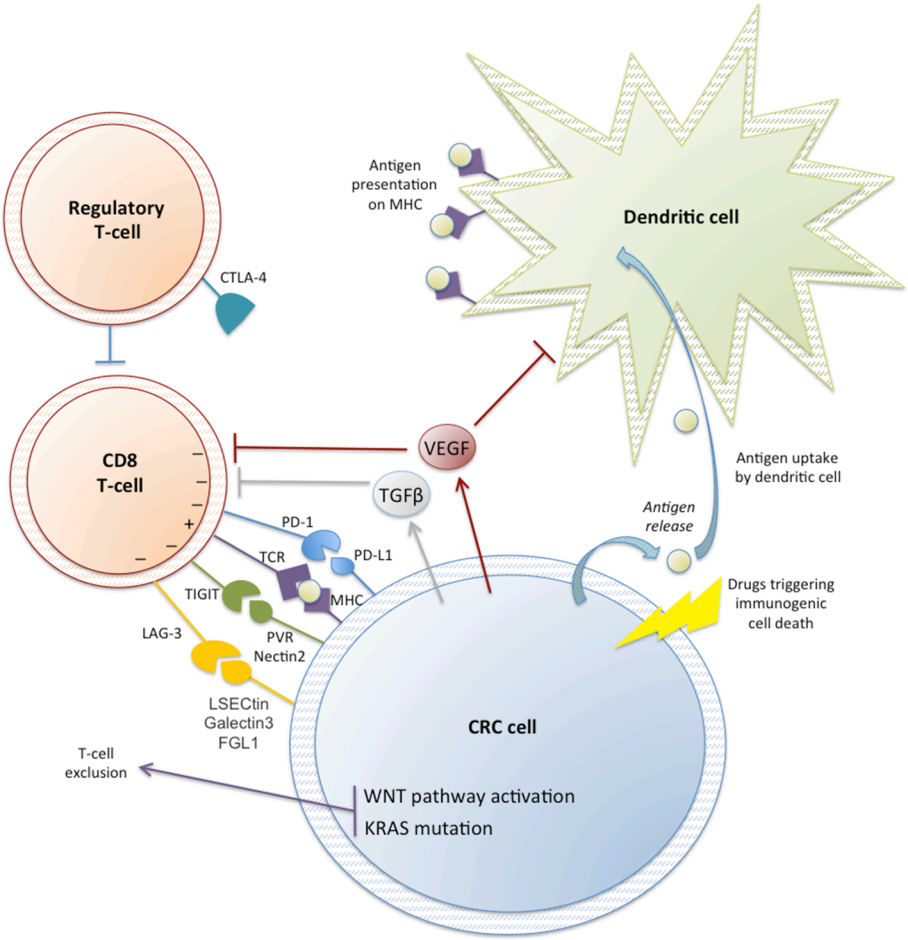

Fig. 1. Overview of the main immune-regulatory mechanisms discussed in this review. The main ligands known to date are shown for the *T*-cell co-inhibitory immune checkpoint receptors PD-1, LAG3 and TIGIT. These can be presented by cancer cells but also non-malignant cells and some are secreted rather than cell surface attached. Regulatory *T*-cells suppress the activity of effector *T*-cells and constitutively expresses CTLA-4 which makes this cell population amenable to depletion with CTLA-4 antibodies. Dendritic cells (DC) take up antigens released from dying cancer cells and present these on major histocompatibility molecules (MHC) to *T*-cells. *T*-cell receptor (TCR) recognition of a cognate antigen-MHC complex leads to *T*-cell activation. Therapies such as oxaliplatin or cetuximab have been shown to trigger immunogenic cell death which leads to neoantigen (NA) release in a way that promotes DC activation and antigen presentation on MHC. Kirsten rat sarcoma virus (KRAS) mutations and Wingless-type MMTV integration site family (WNT) pathway activation have been show to confer *T*-cell exclusion. CRC cells frequently secrete transforming growth factor β (TGFβ) and VEGF which suppress the activity of *T*-cells or dendritic cells. (+) *T*-cell stimulatory signal, (-) *T*-cell inhibitory signal.

this analysis were small and the median PFS between the nivolumab and SOC arm was equivalent (10.6 and 10.4 months).

The AtezoTRIBE randomised phase 2 trial of first-line FOLFOXIRI (fluorouracil, oxaliplatin and irinotecan) chemotherapy and bevacizumab with or without atezolizumab demonstrated a PFS benefit with the addition of atezolizumab in the whole cohort [23]. However, there was no significant increase in PFS with the addition of atezolizumab in the subgroup of MMRp patients (n = 199, PFS 12.9 months versus 11.4 months in the control group, $p = 0.071$). On multivariate analysis, high TMB and a high score in the Immunoscore Immune Checkpoint assay, which measures the densities of CD8 and PD-L1 positive cells, were independently associated with prolonged PFS in the atezolizumab group.

The MODUL study was a phase 2 multi-cohort open label randomised trial of first-line FOLFOX chemotherapy and bevacizumab in mCRC patients followed by maintenance randomisation[26]. B-Raf Proto-Oncogene, Serine/Threonine Kinase (BRAF) wild-type patients were randomised to the control group (fluoropyrimidine and bevacizumab maintenance) or atezolizumab group (atezolizumab and fluoropyrimidine and bevacizumab maintenance). The ORR was not presented and there was no difference in either PFS or OS between the two groups (PFS 7.39 months in the control group and 7.2 months in the atezolizumab group; OS 21.91 months in the control group and 22.05 in the atezolizumab group).

The single-arm phase 2 MEDITREME trial combined FOLFOX induction chemotherapy with durvalumab and tremelimumab in previously untreated RAS-mutated mCRCs. 5 % of these were MSI [27]. The study met its primary endpoint with an ORR of 61 % in 56 evaluable patients and the median PFS was 8.4 months. The interpretation of these data from a single-arm trial is difficult as much of the observed activity may be due to the chemotherapy backbone. However, these results appear favourable compared to the ORR of 36.7 % and PFS of 5.6 months reported with FOLFOX treatment in the first-line setting in RAS mutant liver-limited mCRC [28].

O^6-methylguanine DNA methyltransferase (MGMT) is silenced in 40 % of CRC and in patients with pre-treated MSS mCRC and MGMT silencing, the single-arm phase 2 MAYA trial evaluated priming with 2 cycles of temozolomide chemotherapy followed by nivolumab and ipilimumab[29]. The ORR was 39 %, median PFS was 7.1 months and OS was 18.5 months. This appears better than results from a previous phase 2 study investigating temozolomide monotherapy in mCRC with MGMT silencing, where ORR was 10 %, median PFS 1.9 months and OS 5.1 amongst 41 patients enrolled [30].

Immunotherapy in combination with anti-EGFR antibodies

Combining ICI with anti- epidermal growth factor receptor (EGFR) antibodies, which can trigger immunogenic cell death and promote *T*-cell infiltration into mCRCs, is a further rational strategy [31,32]. The AVETUX trial was a single-arm phase 2 trial of FOLFOX, cetuximab (anti-EGFR) and avelumab (anti-PD-L1) in previously untreated RAS/RAF wild-type mCRC [33]. Ninety-three percent of patients (40/43) had MSS tumours. The ORR was 79.5 % and median PFS was 11.1 months. These results are similar to the PFS for FOLFIRI cetuximab in the pooled data from the first-line randomised CRYSTAL, OPUS and TAILOR trials [34,35].

A single-arm phase 2 study of nivolumab, ipilimumab and panitumumab (anti-EGFR) in 49 patients with KRAS/NRAS/BRAF wild-type MSS mCRC who had received 1–2 prior lines of therapy (patients were excluded if they had received prior anti-EGFR therapy) met its prespecified endpoint by showing a 12-week response rate of 35 % [36]. Median PFS was 5.7 months. In comparison, panitumumab alone had response rates of 22 %, median PFS 4.1 and median OS 10.4 in chemotherapy-refractory mCRC [37].

A phase 2 single-arm study evaluating cetuximab and avelumab (anti-PD-L1) in pre-treated RAS wild-type mCRC recruited 77 patients who had a complete or partial response to first-line chemotherapy and anti-EGFR drugs, developed acquired resistance, and failed second-line therapy [38]. 92 % of patients had MSS tumours and among 65 evaluable patients the ORR was 6 % (4/65) [38], median PFS was 3.6 months and median OS was 11.6 months [39].

A phase 1 / 2 single-arm trial of nivolumab, cetuximab and encorafenib (mitogen-activated protein kinase [MAPK] pathway inhibitor) in BRAF V600E mutant, MMRp mCRC patients who had progressed through at least one prior line of chemotherapy recruited 26 patients and demonstrated an ORR of 45 %, median PFS 7.3 months and median OS 11.4 months [40]. This is a promising ORR which compares favourably to the ORR of 20–26 % in phase 3 trials of encorafenib, binimetinib and cetuximab in BRAF-mutant mCRC [41].

Immunotherapy combinations with small molecule VEGFR inhibitors

ICI in combination with small molecule VEGF receptor (VEGFR) inhibitors might enhance response through several different mechanisms including blockade of the immunosuppressive effects of VEGF (Fig. 1) and reduction of immunosuppressive cell populations in the tumour [42,43]. The REGONIVO phase Ib trial of regorafenib (a multi-kinase inhibitor VEGFR1-3 angiogenic and oncogenic kinases) and nivolumab included patients with advanced gastric cancer and mCRC who had received two or more previous lines of chemotherapy [44]. This showed an impressive 33 % ORR in 24 patients with MMRp colorectal cancer compared to the poor ORR with single-agent regorafenib (1 % in the CORRECT trial) [45] or single-agent anti-PD1 immunotherapy in MMRp mCRC (ORR 0 %)[8]. This was the first trial indicating synergy between immunotherapy and VEGFR inhibitors in MMRp mCRC. The median PFS was 7.9 months and median OS had not been reached with a follow-up duration of 15 months [44] which compares favourably to PFS and OS data for other third-line therapies [5,45]. Exploratory analyses showed strikingly higher response rates with regorafenib and nivolumab in patients with lung metastases (50 %) than in those with liver metastases (15 %). The reason for this synergy is unclear but regorafenib may reduce tumour infiltrating immune-suppressive macrophages [43] and Tregs [44]. Regorafenib potently inhibits VEGFR which may furthermore abrogate the suppressive effect of VEGF on dendritic cell maturation and on *T*-cell migration [46].

A phase 1b trial of regorafenib and nivolumab in MMRp mCRC patients in the third-line setting and beyond showed far less encouraging results in 40 evaluable patients, with ORR of 8 %, median PFS 4.3 months and median OS 11.1 months [47]. Similarly, a phase 2 single-arm trial of regorafenib and nivolumab in MMRp mCRC who had progressed on or were intolerant to standard chemotherapy showed responses in only 7.1 % (5/70) [48]. The median PFS was 1.8 months and median OS 12 months. Consistent with REGONIVO, the ORR was higher in patients without liver metastases (ORR 21.7 %) and none of the responders had liver metastases. In patients without liver metastases, median PFS was 3.5 months and median OS 12 months. In patients with liver metastases, median PFS was 1.8 months and median OS 10.9 months.

Two trials of immunotherapy in combination with regorafenib showed disappointing results. A phase 1/2 trial of pembrolizumab and regorafenib in MMRp mCRC in the third- and fourth-line setting demonstrated an ORR of 0 %, median PFS 2 months and median OS 10.9 months in 73 patients [49]. A phase 2 trial of avelumab and regorafenib in MMRp mCRC in patients who had exhausted all prior lines of therapy also demonstrated an ORR of 0 %, median PFS 3.6 months and median OS 10.8 months amongst 40 evaluable patients [50].

Several trials have since reported on the efficacy of different VEGFR inhibitors and PD-1 or PD-L1 checkpoint inhibitors in MMRp mCRC. LEAP-005 was a phase 2 study of lenvatinib (a multi-kinase inhibitor targeting VEGFR1-3 and oncogenic kinases) and pembrolizumab in previously treated advanced solid tumours [51]. ORR was 22 %, median

PFS was 2.3 months and median OS was 7.5 months amongst 32 MMRp/MSS patients.

The phase 2 CAMILLA trial combined cabozantinib (a multi-targeted inhibitor of the VEGFR2 and oncogenic kinases) with durvalumab in MMRp mCRC patients who had progressed on two or more prior lines of therapy [52]. In 29 evaluable patients, the ORR was 27.6 %, median PFS was 3.8 months and median OS 9.1 months. The response rate in the CAMILLA trial is far higher than that seen for cabozantinib monotherapy [53] suggesting synergy between cabozantinib and durvalumab.

The phase 1b COSMIC-021 trial of cabozantinib and atezolizumab in advanced pretreated solid tumours presented results from the mCRC cohort, all of whom were MMRp: amongst 31 patients, ORR was 9.7 %, median PFS was 3 months, and the OS of 14 months appeared promising [54].

VEFGFR–ICI combinations seem promising although patient selection (no liver metastases) appears key. Whether different VEGFR inhibitors are equivalent needs to be assessed. It is important to note that the doses of regorafenib[44,48] and cabozantinib[52,54] used in these immunotherapy combination trials are lower than the single-agent doses used [53] and are generally well tolerated, whereas the lenvatinib dose in LEAP-005 [51] is similar to the single-agent dose[55] and many patients required dose-reductions.

Combinations of anti-PD-1 or anti-PD-L1 and novel checkpoint inhibitors

The lymphocyte activation gene 3 (LAG3) is an inhibitory receptor on *T*-cells which is frequently overexpressed in CRC. Combining LAG3 blockade with PD-1/PD-L1 blockade aims to enhance antitumour activity[56]. The dose confirmation phase I trial of pembrolizumab with favezelimab which targets LAG3 showed an ORR of 6.3 % in 80 MMRp mCRC patients who had progressed on 2 or more prior therapies [57]. Median PFS was 2.1 months and median OS was 8.3 months. Biomarker analysis demonstrated that tumours with a PD-L1 combined positive score (CPS) ≥ 1 had a higher ORR (36 cases, ORR 11.1 %) compared to those with CPS < 1 (35 cases, ORR 2.9 %). Median PFS was only marginally longer in the CPS ≥ 1 group (2.2 months) compared to the CPS < 1 group (2.0 months) and median OS in patients with CPS ≥ 1 was 12.7 months compared to only 6.7 months in the CPS < 1 group.

Another phase 1 trial investigating dual checkpoint inhibition with BI-754091 (anti-PD-1) and BI-754111 (anti-LAG3) in patients who had previously received at least 1 previous line of systemic therapy, reported a similar ORR of 7.5 % in 40 MMRp mCRC patients [58]. Similar to the favezelimab and pembrolizumab trial, higher PD-L1 expression was associated with greater benefit.

Immunotherapy in combination with other small molecule inhibitors

KRAS activation promote *T*-cell exclusion and small molecule inhibitors of the RAS/RAF pathway have been shown to increase antigen presentation which is the rationale behind inhibiting the RAS/RAF pathway and simultaneously treating with ICI [22,59–61]. The phase 3 IMblaze 370 randomised trial evaluated atezolizumab and cobimetinib (MEK1/2 inhibitor) or atezolizumab monotherapy versus regorafenib in the third-line mCRC setting [62]. 363 patients were included and recruitment of patients with MSI tumours was capped at 5 %. In all randomized patients, the ORR was 3 % (5/183 patients) with atezolizumab and cobimetinib, 2 % (2/90) with atezolizumab, and 2 % (2/90) with regorafenib. Median PFS was 1.91 months with atezolizumab and cobimetinib, 1.94 months with atezolizumab, and 2 months in the regorafenib group. No significant OS benefit was observed: OS was 8.87 months in the atezolizumab and cobimetinib arm, 7.10 months with atezolizumab and 8.51 months with regorafenib; $p = 0.99$ for atezolizumab plus cobimetinib versus regorafenib and $p = 0.34$ for atezolizumab versus regorafenib. A phase 2 study of bevacizumab, pembrolizumab and the MEK inhibitor binimetinib (mitogen-activated protein kinase [MAPK] pathway inhibitor) in 47 MSS mCRC patients who had progressed on two prior lines of therapy showed a modest response: ORR 13 %, PFS 5.8 months [63].

DNA-hypomethylating drugs and histone-deacetylase inhibitors are thought to derepress silenced genes which may promote neoantigen expression [64,65]. A phase 2 non-randomised study of pembrolizumab and azacitidine (a DNA hypomethylating agent) found an ORR of 3 % (1/30 patients) in patients with MSS mCRC who had exhausted standard therapies[65]. Median PFS was 2.1 months and median OS 6.2 months, suggesting minimal antitumour activity. The Carosell phase 2 trial of the histone deacetylase inhibitor zabadinostat in combination with nivolumab in MMRp mCRC in the third-line setting and beyond demonstrated a median OS of 7 months amongst 46 evaluable patients [66]. In a phase 1b/2 trial with expansion cohorts in non-small cell lung cancer, melanoma and MSS/MMRp mCRC in the second-line setting and beyond, patients were treated with pembrolizumab and entinostat (a histone deacetylase inhibitor) [67]. Within the mCRC cohort, the ORR was 6 % (1/16 patients). A phase 1/2 trial of pembrolizumab and ibrutinib (Bruton's tyrosine kinase inhibitor) in MSS mCRC in the third-line setting and beyond found no objective responses in 31 patients [68]. The median PFS was 1.4 months and median OS was 6.6 months. Overall, these trials show minimal to modest benefit.

Other immunotherapy strategies

Chimeric antigen receptor (CAR)-*T*-cells target *T*-cells towards antigens expressed on cancer cells[69], and recently showed interesting results. A phase 1 trial of guanylate cyclase-C (GCC) targeting CAR-*T*-cells in MMRp mCRCs in the third–line setting and beyond reported an ORR of 28.6 % amongst 21 evaluable patients [70]. Furthermore, a recent phase 1 trial of CAR-T therapy targeting carcinoembryonic antigen (CEA) in heavily pretreated mCRC reported an ORR of 20 % (2/10 evaluable patients)[71] without showing the severe colitis seen with first-generation CEA CAR-*T*-cells [72].

A phase I trial of cibisatamab (bispecific monoclonal antibody redirecting *T*-cells to the carcinoembryonic antigen [CEA]) in patients with CEA-expressing mCRCs that failed at least two prior chemotherapy regimens showed radiological shrinkage in 11 % (4/36) and 50 % (5/10) of patients treated with monotherapy or in combination with PD-L1-inhibiting antibodies, respectively [73].

Various combinations including immunotherapy

A retrospective single-centre study evaluated clinical responses to PD-1 or PD-L1 checkpoint blockade with or without other investigational agents in 95 patients with MMRp mCRCs who had progressed after standard chemotherapy [74]. The concurrent therapy given with anti-PD-1 or anti-PD-L1 was divided into five categories: VEGFR inhibitors (n = 45), MAPK inhibitors (n = 6), CTLA-4 inhibitors (n = 9) radiotherapy (n = 9) and other agents (n = 17) [74]. The overall ORR was 8.4 % (8/95 patients). Importantly, in 41 patients without liver metastases, the ORR was 19.5 %. No patients with liver metastases achieved an objective response. Moreover, median PFS was 4 months for patients without versus 1.5 months for patients with liver metastases ($p < 0.001$) [74]. To dissect this further two groups were analysed: patients without any history of liver involvement (n = 25) and those with a history of liver resection but without active liver disease at the time of treatment (n = 16). PFS in patients without any history of liver resection was 5.5 months versus 3 months in patients with previous liver resection. This potentially indicates a difference in the biology of tumours that metastasize to the liver versus those that don't. Moreover, RAS mutations ($p = 0.02$) and right-sided tumours ($p = 0.03$) were associated with poorer PFS on multivariate analysis.

The PICASSO phase 1 trial of pembrolizumab in combination with maraviroc (C—C motif chemokine receptor 5 [CCR5] antagonist) in

treatment refractory MMRp mCRC reported an ORR of 5.3 %, median PFS 2.1 months, median OS 9.83 months in 19 evaluable patients [75]. A phase 2 single-arm study of durvalumab and tremelimumab with concurrent radiotherapy in MMRp mCRC patients who had received at least 2 prior lines of therapy demonstrated modest results with an ORR of 8.3 % (2/24 patients), median PFS 1.8 months and median OS 11.4 months [76].

Trials in localized CRC

A recent trial showed good response rates to neoadjuvant immunotherapy in 15 patients with Stage 1–3 MMRp CRCs [77]. Neoadjuvant ipilimumab and nivolumab with or without the COX2 inhibitor celecoxib led to a pathological response rate of 27 % (CI 8–55 %) in MMRp CRC that had been surgically removed after 1–4 cycles of immunotherapy. TMB did not significantly differ between responders and non-responders. Higher tumour infiltration with PD1-positive CD8 *T*-cells was identified as a candidate biomarker of response. These results indicate that localised MMRp CRCs may be more immunogenic, less immunosuppressive, or both, when compared to MMRp mCRCs.

Several phase 2 trials combined neoadjuvant ICI and chemoradiotherapy in MMRp localised rectal cancer and reported pathological complete response (pCR) rates. The phase 2 AVANA trial of chemoradiotherapy with avelumab followed by surgery reported pCR rate of 8 % in 58 MMRp patients; the phase 2 VOLTAGE-A trial of chemoradiotherapy followed by nivolumab and then surgery reported 30 % pCRs in 37 MMRp patients 2022[78]. The phase 2 trial of short-course radiotherapy followed by chemotherapy in combination with camrelizumab followed by surgery reported a 46 % pCR rate in 26 MMRp patients [78]. Some of these pCR rates appear promising and further trials are required to establish the value of this strategy.

The proposed mechanisms for higher response rates in the neoadjuvant compared to the mCRC setting is that immunoediting leads to a progressive antigen loss in the latter and chemotherapy treatment may lead to *T*-cell depletion[79].

Summary of clinical trial data to date

Table 1 summarizes the above clinical trials and Fig. 2 the ORR for these various immunotherapies in MMRp mCRC. In summary, single-agent checkpoint inhibitors did not confer clinical benefit in MMRp mCRC and none of the randomised trials including immunotherapy showed significant or clinically meaningful benefit to date. Although not significant, prolonged PFS was observed in some randomized trials, indicating that there may be some biological effect. The effect on OS is harder to judge and is not yet convincing, as several trials have not yet reported their OS outcomes. Several single-arm trials that combine immunotherapy with drugs that have high response rates, such as the encorafenib and cetuximab combination or chemotherapies, are difficult to evaluate as most of the observed activity may be due to the non-immunotherapy agents. Most trials that have been reported were in the 3rd + line setting where tumour loads are large and this may impair immunotherapy efficacy. ICI sensitivity may be higher in early stage disease based on data from a very small trial in Stage 1–3 MMRp CRC. Taken together, combinations of PD-1 checkpoint inhibition with VEGFR inhibitors, anti-LAG3 antibodies or next-generation anti-CTLA-4 antibodies are novel approaches that appeared to increase response rates modestly in at least some trials.

Barriers and biomarkers for more effective immunotherapy in MMRp mCRC

Identifying biological barriers and also biomarkers for more effective immunotherapy in MMRp mCRC needs to be a priority to improve outcomes in these tumours. The potential predictive biomarkers identified in the above trials are PD-L1 expression, the absence of liver metastases, as well as high TMB and Immunoscore [20,23,44,47,57,58,74]. The collection of pre-treatment tissues for translational analysis and ideally also of longitudinal pre-treatment and on-treatment biopsies is critically important to advance biomarker discovery and to obtain insights into the molecular and cellular dynamics that enable or preclude responses to immunotherapy.

Antigenicity

MMRp CRCs have a more than 10-fold lower mutation load than MMRd CRC [11]. A low number of mutation-encoded neoantigens (NAs) is hence considered a main reason for the poor response rate to ICIs in MMRp CRCs. Nevertheless, multiple published computational analyses predict dozens of neoantigens in most MMRp CRCs [83,84]. By applying immunopeptidomics by liquid chromatography-tandem *mass* spectrometry to five patient derived organoids (PDOs) from MMRp mCRCs, we only detected 3 neoantigens across all 5 PDO lines[85]. In contrast, computational prediction indicated a total of 196 neoantigens. Thus, neoantigen numbers are likely low and may be absent in some MMRp CRCs. In a study evaluating the immunopeptidome of localised CRC and matched non-malignant colon from 37 CRC patients who underwent curative surgical resection, results demonstrated 45 non-mutated but potentially cancer specific peptides at the cell surface in the tumour tissue among 13 CRC cases (averaging 3.5 per case) [86]. Together, high-sensitivity MS studies suggest that MMRp CRCs perhaps only present low numbers of tumour specific antigens[86,87]. Neoantigens that can be recognized by CD8 *T*-cells have been shown by the discovery of immunogenic KRAS mutations[88], serving as an example that neoantigens are clearly important in some MMRp CRCs.

Non-canonical peptides from the genome's dark matter including human endogenous retroviruses [87] or post-translationally modified peptides have been described but remain understudied in MMRp CRC. Overall, this shows a clear need for more research into tumour antigens in MMRp mCRCs and for the development of technologies that can be applied in the clinic to identify tumours that present sufficient antigens for immunotherapy to have an effect. New therapeutic approaches such as neoantigen vaccines [89] may be important to precisely direct *T*-cells specifically to the few antigens presented in these tumours whereas cancers without sufficient antigens for *T*-cell recognition may benefit from CAR-*T*-cell therapies or bispecific antibody therapies that redirect *T*-cells to cell surface antigens such as CEA or GCC. Whether the tumour mutation burden can be increased therapeutically is being tested in the Arethusa trial[90]. MMRp CRCs with O6-methylguanine-DNA-methyltransferase deficiency are being treated with mutagenic temozolamide chemotherapy and subsequently with pembrolizumab. Preliminary results showed that temozolamide led to MMR gene inactivation and increased TMB.

Immunoediting hypothesis

Recognition and killing of cancer cells by the immune system can promote a natural selection process where cancer cells that lose antigens evade surveillance and metastasise [91]. This evolution of escape variants through immunoediting may explain higher immunotherapy response rates in early stage MMRp CRCs compared to mCRCs. However, a higher degree of systemic immunosuppression may be an alternative explanation for the lack of response in patients with advanced disease and more research is clearly needed[77]. A recent murine study showed that tumour *T*-cell antigens are often downregulated in CRCs, resulting in poor expression on HLA molecules and ineffective priming of *T*-cells. Importantly it was shown in a murine transplant model that this priming defect could be overcome by treatment with a neoantigen vaccine which led to effective anti-tumour responses [92]. HLA loss of heterozygosity (LOH) and HLA downregulation are further immune escape mechanism that reduce antigen presentation and have been observed in mCRC [85]. It is important to treat with IO as early as

Table 1
Table summarizing published results from prospective clinical trials of immunotherapy in MMRp mCRC patients.

Trial name NCT/Clinical trial number	Phase and setting	N	%MMRp	Treatment arms	ORR (%)	PFS (months)	OS (months)
PD-1/PD-L1 ICI with or without CTLA-4 ICI							
Phase 2 Study of MK-3475 in Patients With Microsatellite Unstable (MSI) Tumors NCT01876511 [8]	Phase 2 Third-line and beyond	41	44 % (18 patients)	Pembrolizumab	0 % (amongst MMRp)	2.2 (amongst MMRp)	5 (amongst MMRp)
CO.26 A Phase II Randomized Study of Durvalumab and Tremelimumab and Best Supportive Care vs Best Supportive Care Alone in Patients With Advanced Colorectal Adenocarcinoma Refractory to Standard Therapies NCT02870920 [18]	Phase 2 Exhausted all prior lines	179	93 % (166 patients)	Durvalumab + Tremetlimumab + BSC versus BSC	0.8 % versus 0 %	1.8 versus 1.9	6.6 versus 4.1
Targeted Agent and Profiling Utilization Registry (TAPUR) Study NCT02693535 [19]	Phase 2 Exhausted all prior lines	12 (only 10 evaluable for efficacy)	92 % (11 patients	Nivolumab + Ipilimumab	10 %	3.1	9.9
A Phase 1 Study of AGEN1181, an Fc-Engineered anti-CTLA-4 Monoclonal Antibody as Monotherapy and in Combination With AGEN2034 (Balstilimab), an anti-PD-1 Monoclonal Antibody, in Subjects With Advanced Cancer NCT03860272 [20]	Phase 1 Exhausted all prior lines	41	100 %	Botensilimab + balstilimab	24 %	Not available	Not available
Immunotherapy in combination with chemotherapy							
BACCI: A Phase II Randomized, Double-Blind, Placebo-Controlled Study of Capecitabine Bevacizumab Plus Atezolizumab versus Capecitabine Bevacizumab Plus Placebo in Patients With Refractory Metastatic Colorectal Cancer NCT02873195 [80]	Phase 2 Exhausted all prior lines	133	86.7 % in atezolizumab arm and 85.7 % in control arm	Capecitabine + bevacizumab + atezolizumab versus Capecitabine + bevacizumab	8.54 % versus4.35 %	4.4 versus 3.3	Not available
An Open-Label Exploratory Phase 2/3 Study of Nivolumab With Standard of Care Therapy vs Standard of Care Therapy for First-Line Treatment of Metastatic Colorectal Cancer (Checkmate 9x8) NCT03414983 [25]	Phase 2 First-line	195	95 % (121/127 patients) in the nivolumab group versus 90 % (61/68 patients) in the SOC group	FOLFOX + bevacizumab + nivolumab Versus FOLFOX + bevacizumab	60 % versus 46 %	11.9 versus 11.9	29.2 versus not reached
Randomized Phase II Study of FOLFOXIRI Plus Bevacizumab Plus Atezolizumab versus FOLFOXIRI Plus Bevacizumab as First-line Treatment of Unresectable Metastatic Colorectal Cancer Patients NCT03721653 [23].	Phase 2 First line	218	91 %	FOLFOXIRI + bevacizumab + atezolizumab Versus FOLFOXIRI + bevacizumab	59 % versus 64 % (amongst whole cohort)	12.9 versus 11.4 (amongst MMRp subgroup)	Not available
A Multi-Centre Randomised Clinical Trial of Biomarker-Driven Maintenance Treatment for First-Line Metastatic Colorectal Cancer (MODUL) NCT02291289 [81]	Phase 2 First-line	445	98 %	Fluuropyrimidine + bevacizumab Versus Atezolizumab + fluoropyrimidine + bevacizumab	Not available	7.39 versus 7.2	21.91 versus 22.05
MEDITREME Phase Ib/II Trial Evaluating the Safety, Tolerability and Immunological Activity of Durvalumab (MEDI4736) (anti-PD-L1) Plus Tremelimumab (anti-CTLA-4) Combined With FOLFOX in Patients With Metastatic Colorectal Cancer NCT03202758 [27]	Phase 2 First-line	57	95 %	FOLFOX + durvalumab + tremelimumab	61 %	8.4	Not available

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Table 1 (*continued*)

Trial name NCT/Clinical trial number	Phase and setting	N	%MMRp	Treatment arms	ORR (%)	PFS (months)	OS (months)
NIVOLUMAB Plus IPILIMUMAB and TEMOZOLOMIDE in Combination in Microsatellite Stable (MSS), MGMT Silenced Metastatic Colorectal Cancer (mCRC): the MAYA Study NCT03832621 [82]	Phase 2 First-line	33	100 %	Temozolomide then nivolumab + ipilimumab	39 %	7.1	18.5
AVETUX Avelumab and Cetuximab in Combination With FOLFOX in Patients With Previously Untreated Metastatic Colorectal Cancer - The Phase II AVETUX- Colorectal Cancer (CRC) Trial NCT03174405 [33]	Phase 2 First-line	43	93 %	FOLFOX + cetuximab + avelumab	79.5 %	11.1	Not available
Immunotherapy in combination with anti-EGFR antibodies							
Phase II Multicenter Trial of Panitumumab, Nivolumab, and Ipilimumab for KRAS/NRAS/BRAF Wild-type MSS Refractory Metastatic Colorectal Adenocarcinoma NCT03442569 [36]	Phase 2 1–2 prior lines of therapy	49	100 %	Nivolumab + ipilimumab + panitumumab	35 % (12-week response rate)	5.7	10.4
CAVE (Cetuximab-AVElumab) mCRC: A Single Arm Phase II Clinical Study of the Combination of Avelumab Plus Cetuximab in Pre-treated RAS Wild Type Metastatic Colorectal Cancer Patients NCT04561336 [38,39]	Phase 2 Second- or third- line	77	92 %	Cetuximab + avelumab	6 %	3.6	11.6
Phase I/II Trial of Encorafenib, Cetuximab, and Nivolumab in Microsatellite Stable BRAFV600E Metastatic Colorectal Cancer (BMS-MDACC CA209-8P6/ARRAY IST-818-101X) NCT04017650 [40]	Phase 1 / 2 At least one prior line of chemotherapy but no more than 2 prior lines. Patients had to be EGF- and BRAF-targeted therapy naïve	26	100 %	Nivolumab + cetuximab + encorafenib	45 %	7.3	11.4
Immunotherapy combinations with small molecule VEGFR inhibitors							
Regorafenib and Nivolumab Simultaneous Combination Therapy for Advanced and Metastatic Solid Tumors: Phase I Clinical Trial (REGONIVO) NCT03406871 [44]	Phase 1 Two or more previous lines of chemotherapy	25 (in the CRC cohort; there was also a gastric cancer cohort)	96 %	Regorafenib + nivolumab	33 % (amongst the 24 patients with MMRp CRC)	7.9	Not reached
Phase I Study of Regorafenib and Nivolumab in Mismatch Repair (MMR) Proficient Advanced Refractory Colorectal Cancer NCT03712943 [47]	Phase 1 Third-line setting and beyond	40	100 %	Regorafenib + nivolumab	8 %	4.3	11.1
An Open-label, Single-arm, Phase II Study of Regorafenib and Nivolumab in Patients With Mismatch Repair-Proficient (pMMR)/Microsatellite Stable (MSS) Colorectal Cancer (CRC) NCT04126733 [48]	Phase 2 Third-line and beyond	70	100 %	Regorafenib + nivolumab	7.1 %	1.8	12
A Phase I/II Study of Regorafenib and Pembrolizumab in Metastatic Colorectal Cancer Patients in 3rd and 4th Line Setting NCT03657641 [49]	Phase 1 / 2 Third-and fourth-line setting	73	100 %	Pembrolizumab + regorafenib	0 %	2	10.9

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Table 1 (*continued*)

Trial name NCT/Clinical trial number	Phase and setting	N	%MMRp	Treatment arms	ORR (%)	PFS (months)	OS (months)
A Phase I/II Study of Regorafenib Plus Avelumab in Solid Tumors NCT03475953 [50]	Phase 2 Exhausted all prior lines	40	100 %	Avelumab + regorafenib	0 %	3.6	10.8
A Multicenter, Open-label Phase 2 Study of Lenvatinib (E7080/MK-7902) Plus Pembrolizumab (MK-3475) in Previously Treated Subjects With Selected Solid Tumors (LEAP-005) NCT03797326 [51]	Phase 2 Third-line	32	100 %	Lenvatinib + pembrolizumab	22 %	2.3	7.5
A Phase I/II Trial of Cabozantinib in Combination With Durvalumab (MEDI4736) With or Without Tremelimumab in Patients With Advanced Gastroesophageal Cancer and Other Gastrointestinal (GI) Malignancies (CAMILLA) NCT03539822 [52]	Phase 2 Third-line and beyond	29	100 %	Cabozantinib + durvalumab	27.6 %	3.8	9.1
A Phase 1b Dose-Escalation Study of Cabozantinib (XL184) Administered Alone or in Combination With Atezolizumab to Subjects With Locally Advanced or Metastatic Solid Tumors (COSMIC-021) NCT03170960 [54]	Phase 1b Third-line and beyond	31	100 %	Cabozantinib + atezolizumab	9.7 %	3	14
Combinations of anti-PD-1 or anti-PD-L1 and novel checkpoint inhibitors							
A Phase 1 Trial of MK-4280 as Monotherapy and in Combination With Pembrolizumab With or Without Chemotherapy or Lenvatinib (E7080/MK-7902) in Subjects With Advanced Solid Tumors NCT02720068 [57]	Phase 1 Third-line and beyond	80	100 %	Pembrolizumab + favezelimab	6.3 %	2.1	8.3
An Open Label, Phase I Dose-finding Study of BI 754,111 in Combination With BI 754,091 in Patients With Advanced Solid Cancers Followed by Expansion Cohorts at the Selected Dose of the Combination in Patients With Non-small Cell Lung Cancer and Other Solid Tumors NCT03156114 [58]	Phase 1 / 2 Second-line and beyond for expansion phase. anti-PD(-L)1 naïve	40	100 %	BI-754091 + BI-754111	7.5 %	Not available	Not available
Immunotherapy in combination with other small molecule inhibitors							
A Phase III, Open-Label, Multicenter, Three-Arm, Randomized Study to Investigate the Efficacy and Safety of Cobimetinib Plus Atezolizumab and Atezolizumab Monotherapy vs Regorafenib in Patients With Previously Treated Unresectable Locally Advanced or Metastatic Colorectal Adenocarcinoma (IMblaze 370) NCT02788279 [62]	Phase 3 Third-line	363	99 %	Atezolizumab + cobimetinib Versus Atezolizumab Versus Regorafenib	3 % versus 2 % versus 2 %	1.91 versus 1.94 versus 2	8.87 versus 7.1 versus 8.51
Phase II Study of Pembrolizumab in Combination With Binimetinib and Bevacizumab in Patients With Refractory	Phase 2 Third-line and beyond	47	100 %	Bevacizumab + pembrolizumab + binimetinib	13 %	5.8	Not available

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Table 1 (*continued*)

Trial name NCT/Clinical trial number	Phase and setting	N	%MMRp	Treatment arms	ORR (%)	PFS (months)	OS (months)
Colorectal Cancer NCT03475004 [63]							
A Phase 2 Study of Pembrolizumab (MK-3475) in Combination With Azacitidine in Subjects With Chemo-refractory Metastatic Colorectal Cancer NCT02260440 [65]	Phase 2 Exhausted all standard therapies	30	100 %	Pembrolizumab + azacitidine	3 %	2.1	6.2
A Phase Ib/ II Trial to Assess the Safety and Efficacy of CXD101 in Combination With the PD-1 Inhibitor Nivolumab in Patients With Metastatic, Previously-Treated, Microsatellite-Stable Colorectal Carcinoma (CAROSELL) NCT03993626 [66]	Phase 1 / 2 Third-line setting and beyond	46	100 %	Zabadinostat + nivolumab	Unknown	Unknown	7
A Phase 1b/2, Open-label, Dose Escalation Study of Entinostat in Combination With Pembrolizumab in Patients With Non-small Cell Lung Cancer, With Expansion Cohorts in Patients With Non-small Cell Lung Cancer, Melanoma, and Mismatch Repair-Proficient Colorectal Cancer NCT02437136 [67]	Phase 1b / 2 Second-line setting and beyond	16	100 %	Pembrolizumab + entinostat	6 %	Not available	Not available
A Phase I/II Study of Pembrolizumab in Combination With Ibrutinib for Advanced, Refractory Colorectal Cancers NCT03332498 [68]	Phase 1 / 2 Third-line setting and beyond	31	100 %	Pembrolizumab + ibrutinib	0 %	1.4	6.6
Other immunotherapy strategies							
A phase 1 dose-escalation study of GCC19 CART a novel coupled CAR therapy for subjects with metastatic colorectal cancer ChiCTR2100053828 [70]	Phase 1 Third-line and beyond	21	100 %	CD19-targeting CAR-*T*-cells	28.6 %	Not available	Not available
Phase I Escalating-Dose Trial of CAR-T Therapy Targeting CEA$^{\pm}$ Metastatic Colorectal Cancers NCT02349724 [71]	Phase 1 Third-line and beyond	10	Unknown	CEA-targeting CAR-*T*-cells	20 %	Not available	Not available
An Open-Label, Multicenter, Dose-Escalation Phase I Study to Evaluate the Safety, Pharmacokinetics, and Therapeutic Activity of RO6958688, A Novel *T*-cell Bispecific Antibody That Targets the Human Carcinoembryonic Antigen (CEA) on Tumor Cells and CD3 on T Cells, Administered Intravenously in Patients With Locally Advanced and/or Metastatic CEA(+) Solid Tumors NCT02324257 [73]	Phase 1 Third-line and beyond	36 mCRC patients receiving monotherapy; 10 mCRC receiving combination	Unknown	Cibisatamab monotherapy And Cibisatamab + atezolizumab	11 % (monotherapy) and 50 % (combined with atezolizumab)	Not available	Not available
Phase II Study to Assess the Efficacy of Durvalumab (MEDI4736) and Tremelimumab Plus Radiotherapy or Ablation in	Phase 2 Third-line and beyond	24	100 %	Durvalumab + tremelimumab + radiotherapy	8.3 %	1.8	11.4

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Table 1 (*continued*)

Trial name NCT/Clinical trial number	Phase and setting	N	%MMRp	Treatment arms	ORR (%)	PFS (months)	OS (months)
Patients With Metastatic Colorectal Cancer NCT03122509 [76]							
A Phase I Trial of Combined PD-1 Inhibition (Pembrolizumab) and CCR5 Inhibition (Maraviroc) for the Treatment of Refractory Microsatellite Stable (MSS) Metastatic Colorectal Cancer (mCRC) (PICASSO) NCT03274804 [75]	Phase 1 Third-line and beyond	20	100 %	Pembrolizumab + maraviroc	5.3 %	2.1	9.83

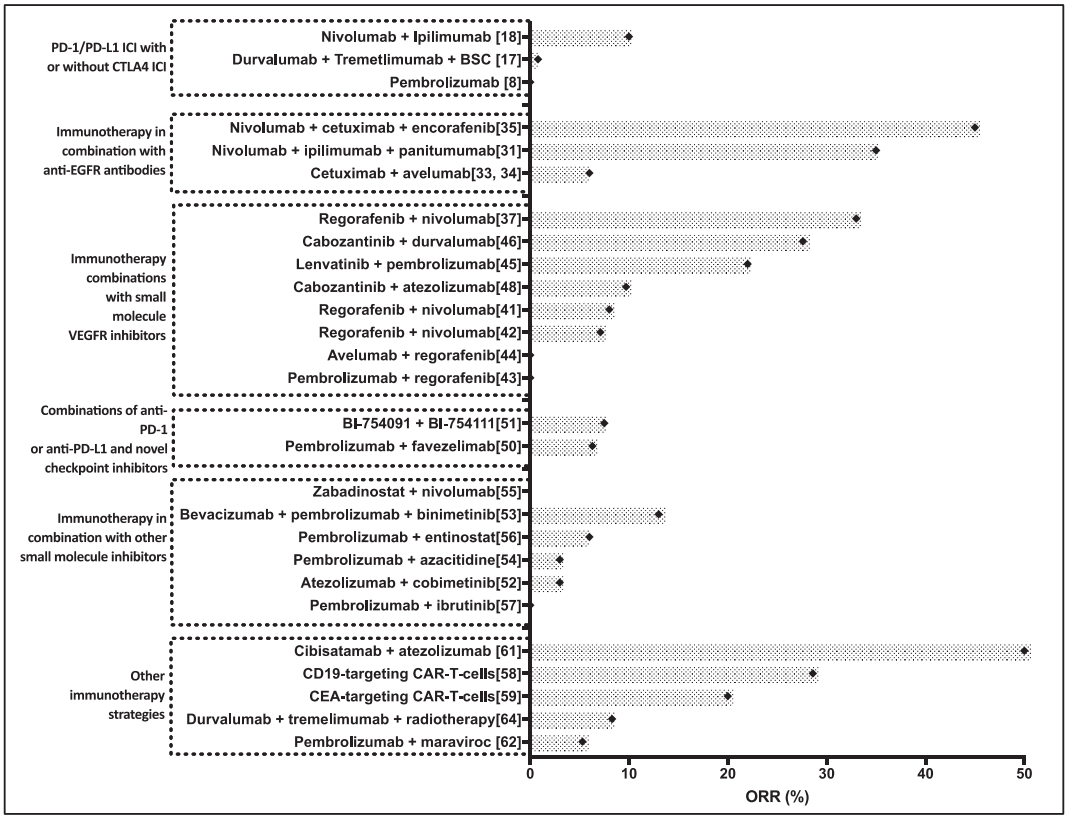

Fig. 2. Bar chart showing published ORR for clinical trials in MMRp mCRC to date. (Note trials involving combinations with chemotherapy are not included on this figure as there is a significant ORR attributable to the chemotherapy.).

possible and to develop improved techniques to identify tumours with specific immune escape mechanisms and target them with appropriate immunotherapies such as bispecifics or vaccines.

Immunosuppressive mechanisms

Forty-five percent of MMRp mCRC patients were PD-L1 CPS ≥ 1 [57]. The encouraging response rates seen in trials combining PD1 and LAG3 inhibition demonstrate that immune checkpoints beyond PD1/PD-L1 contribute to restraining immune cells. Moreover, the pro-angiogenic growth factor VEGF is frequently overexpressed in MMRp CRCs and can suppress *T*-cell priming by dendritic cells. The success of combining VEGF TKIs with checkpoint inhibitors supports the clinical relevance of this approach [44,51,52]. Yet, the current absence of data indicating similar synergies with bevacizumab, a monoclonal antibody that exclusively targets VEGFA (the ligand of VEGFR2), indicates that multiple VEGFRs need to be inhibited or that one or several of the additional tyrosine kinase receptors targeted by VEGFR TKIs are important [93].

MMRp CRC mouse models demonstrated that inhibition of the immunosuppressive cytokine TGFβ rendered tumours susceptible to ICI therapy [94]. Yet, the results of a recently terminated trial with the bispecific antibody Bintrafusp alfa (TGFβ-trap and anti-PD-L1) in MMRp CRCs are concerning in this context as this showed more aggressive tumour progression compared to historical cohorts[95].

Wnt pathway activation in cancer cells has been shown to suppress *T*-cell recruitment in melanoma and CRC patients [96–98]. The Wnt pathway is activated in over 90 % of MMRp CRCs through mutations in *adenomatous polyposis coli* (APC), β–Catenin, or through R-spondin (RSPO) gene fusions [96–99]. Activation of tumour-intrinsic Wnt/β-catenin signaling is enriched in immune excluded tumours [11].

Therapeutic phase 1–2 clinical trials of Wnt inhibition in combination with immunotherapy are underway (NCT05091346) and initial results demonstrated tolerable safety profiles[100].

A perplexing finding is that MMRp mCRCs without liver metastases respond best to checkpoint inhibitors [74]. Macrophage-mediated *T*-cell elimination in the liver microenvironment may contribute to this[101]. A further important insight came from a mouse model showing that liver metastases can increase tumour growth elsewhere in the body [102], potentially by affecting PD-1 and CTLA-4 expression This is consistent with data showing that patients with liver metastases had lower abundance of cyctotoxic *T*-cells, Tregs and macrophages in their tumours compared to those without liver metastases [48]. Thus, liver metastases may induce a systemic immunosuppressive effect [74].

KRAS mutations are associated with repression of Th1/cytotoxic immunity in CRC [103] and might impede responsiveness to immunotherapy. KRAS mutation is more commonly found in MMRp CRC than MMRd CRC [104] which may contribute to the lack of response to immunotherapy seen in MMRp CRC. Several novel KRAS inhibitors are in development (NCT05288205, NCT04699188) and these are hence rational combination partners for immunotherapy in MMRd CRCs.

In view of emerging data indicating increased benefit when PD-1/PD-L1 ICIs are combined with LAG3 antibodies or Treg-depleting CTLA-4 antibodies, it is prudent to also investigate whether additional immune checkpoints such as TIM3 or TIGIT restrain *T*-cells in CRC.

CRC microenvironment composition and subtypes

The Immunoscore is calculated based on the density of lymphocytes in the core and invasive margin of CRC primary tumours [105] and has been validated as a prognostic marker in CRC [106,107]. As mentioned above, the Atezotribe trial provided the first evidence that the Immunoscore may be a useful predictive biomarker for ICIs[23].

CRC has been categorised into four consensus molecular subtypes (CMS) [12] which differ in their immunological and microenvironmental characteristics: CMS1 overexpresses genes specific to cytotoxic lymphocytes. CMS2 and CMS3 both demonstrate a largely immune excluded phenotype with low immune and inflammatory signatures [13,108]. CMS4 displays several potentially immunosuppressive signatures such as high TGFβ signaling and high angiogenic activity [13,32]. There is strong enrichment of MMRd CRCs in the CMS1 subgroup whereas MMRp CRCs can fall into any of the four CMS subtypes [12]. A key question to address is whether CMS-specific rationally chosen therapeutics are required to overcome resistance to IO. MMRp mCRCs that responded to the EGFR antibody cetuximab showed subtype switches from CMS2 to CMS2, increase *T*-cell inflammation and upregulation of a pan-cancer immunotherapy response signature and of PD-L1 and LAG3 immune checkpoints, suggesting that specific therapies potentially overcome the immune-excluded phenotype[32]. Other epigenetic approaches are being developed although none has displayed clinical benefit yet [109].

Ongoing studies and future perspectives

A large number of immunotherapy trials in MMRp CRC are currently underway and a selection showing the range of strategies pursued are presented in Table 2. The approach of VEGFR small molecule inhibitors in combination with ICI are for example being investigated in a phase 3 trial in metastatic disease (NCT04776148) and in a phase 2 trial in early-stage tumours (NCT04715633). anti-LAG3 agents in combination with ICI have entered phase 3 clinical trials in the second-line setting and beyond in patients with PD-L1 positive tumours (NCT05328908, NCT05064059). Next-generation anti-CTLA-4 agents which promote intratumoural regulatory *T*-cell depletion via enhanced Fc-gamma receptor signaling are being explored in the neoadjuvant setting (NCT05571293). Personalized neoantigen vaccines are for example being tested in combination with chemotherapy, bevacizumab,

Table 2
Selected clinical trials of immunotherapy currently recruiting in MMRp CRC.

Study title	Phase and study number	Treatment arms	Estimated enrollment	Primary endpoint
Pembrolizumab plus lenvatinib versus standard of care for previously treated metastatic colorectal cancer (mCRC): Phase III LEAP-017 study	3 NCT04776148	Pembrolizumab Lenvatinib Regorafenib TAS-102 (trifluridine and tipiracil)	434	OS
PD-1 Inhibitors (Camrelizumab) Combined With VEGF Inhibitors (Apatinib) for Locally Advanced dMMR/MSI-H Colorectal Cancer: an Open-label, Multi-center, Phase II Clinical Trial	2 NCT04715633)	Camrelizumab Apatinib	52	Clinical complete response or pathological complete response
A Phase 3, Randomized, Open-label Study of Relatlimab-nivolumab Fixed-dose Combination versus Regorafenib or Trifluridine + Tipiracil (TAS-102) for Participants With Later-lines of Metastatic Colorectal Cancer	3 NCT05328908	Nivolumab-relatlimab fixed dose combination Regorafenib TAS-102	700	OS
A Phase 3 Study of MK-4280A (Coformulated Favezelimab [MK-4280] Plus Pembrolizumab [MK-3475]) versus Standard of Care in Previously Treated Metastatic PD-L1 Positive Colorectal Cancer	3 NCT05064059	Favezelimab/ Pembrolizumab Regorafenib TAS-102	432	OS
Novel Exploratory Study to Test Combination of Botensilimab and Balstilimab Immunotherapy in Resectable Colorectal Cancer Patients	2 NCT05571293	Botensilimab Balstilimab	12	Pathological overall response rate AE
A Phase 2/3, Randomized, Open-Label Study of Maintenance GRT-C901/GRT-R902, A Neoantigen Vaccine, in Combination With Immune Checkpoint Blockade for Patients With Metastatic Colorectal Cancer	2 / 3 NCT05141721.	GRT-C901 GRT-R902 Atezolizumab Ipilimumab Fluoropyrimidine Bevacizumab Oxaliplatin	665	PFS
Phase Ib/II Trial of SX-682 in Combination With Nivolumab for Refractory RAS Mutated (RAS) Microsatellite Stable (MSS) Metastatic Colorectal Cancer (mCRC) (STOPTRAFFIC-1)	1b/2 NCT04599140	CXCR1/2 Inhibitor SX-682 Nivolumab	53	AE

atezolizumab and ipilimumab in a randomised phase 3 trial in MMRp mCRC (NCT05141721). Other pathways that may modify the immune environment by reducing myeloid-derived suppressor cell recruitment using a small-molecule CXCR1/2 inhibitor are also being investigated (NCT04599140).

Although a large number of clinical studies are ongoing, some areas have received comparatively little attention in MMRp CRC. For example, trials of ICI specifically in patients without liver metastases should be considered. Furthermore, the gut microbiome influences immune recognition and possibly ICI efficacy in other tumour types [110,111]. Studies of microbiome modulation together with ICI therapy could assess this in MMRp CRCs. Further clinical trials are required in the neoadjuvant rather than the metastatic CRC setting, because of the higher response rate in the former.

Conclusion

Although no immunotherapy drug has been licensed in MMRd mCRC to date, several promising combinations, for example of anti-PD1 antibodies with newer anti-CTLA-4 antibodies, VEGFR inhibitors, or anti-LAG3, are emerging. Moreover, there are strong rationales for testing of novel therapeutics such as cancer vaccines, cellular and bispecific immunotherapies, and agents targeting additional immune checkpoints and immunosuppressive factors in MMRp CRC. Strong candidate biomarkers such as the presence or absence of liver metastases, and PDL1 expression have been identified for some combinations but the detailed molecular and cellular determinants of immunotherapy success are still poorly understood in these tumours. Inclusion of translational protocols into clinical trials will be crucial to accelerate the rational development of effective combinations that will eventually lead to prolonged survival.

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CRediT authorship contribution statement

Hazel Lote: Conceptualization, Writing – original draft, Writing – review & editing. **Naureen Starling:** Writing – review & editing. **Rille Pihlak:** Writing – review & editing. **Marco Gerlinger:** Conceptualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

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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