An update on the use of immunotherapy in patients with colorectal cancer

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An update on the use of immunotherapy in patients with colorectal cancer.

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Abstract

Introduction: Colorectal cancer (CRC) is the third most common malignancy worldwide, with recent trends demonstrating increasing incidence amongst younger patients. Despite multiple treatment options, metastatic disease remains incurable. A new therapeutic strategy to harness the host immune system, specifically with immune checkpoint inhibitors, now has reported results from a number of clinical trials.

Areas covered: This review will discuss in detail microsatellite instability (MSI) and other biomarkers for response to immunotherapy, summarize the pivotal clinical trials of immune checkpoint inhibitors in early stage and metastatic MSI colorectal cancer, explore strategies to induce treatment responses in MSS CRC and highlight the emerging treatments and novel immune based therapies under investigation.

Expert Opinion: Immunotherapy is now a standard of care for the proportion of CRC patients with MSI. While overall survival data is still awaited, the promise of profound and durable responses is highly anticipated. The lack of efficacy in MSS CRC is disappointing and strategies to convert these 'cold' tumors are needed. Further elucidation of optimal use of treatment sequencies, combinations and novel agents will improve outcomes.

Key words: Colorectal cancer, CTLA4, immune checkpoint inhibitor, immunotherapy, ipilimumab, microsatellite instability, mismatch repair, nivolumab, pembrolizumab, PDL1 rectal cancer

Article Highlights

- Colorectal cancer (CRC) is the third most common malignancy worldwide
- Standard treatment includes resection and adjuvant chemotherapy in early stage disease, and various lines of chemotherapy combined with targeted therapies in metastatic disease
- Microsatellite instability (MSI) is present in 20%, 12% and 4% of stages II, III and IV CRC respectively, is characterized by an inflamed tumor microenvironment with the presence of tumor infiltrating lymphocytes and is a biomarker for response to immune checkpoint inhibitors (ICI).
- Pembrolizumab, a PD1 inhibitor, is a standard of care for first line treatment in metastatic CRC with MSI after the Keynote-177 trial reported improvement in median progression free survival (PFS) of 16.5 months compared to 8.2 months for chemotherapy (HR 0.60 [95% CI 0.45 - 0.80]).
- Efficacy in microsatellite stable CRC is disappointing and numerous strategies are being investigated in attempts to induce responses in these 'cold' tumors.
- Ongoing research is exploring the role of ICI in early stage disease as well as developing novel immune based treatment strategies.

1. Introduction

Colorectal cancer (CRC) is the third most common malignancy diagnosed annually with over 1.8 million new cases each year. It is the second most common cause of cancer mortality worldwide [1]. The incidence is higher in men than women and there is a trend of increasing incidence rates with socioeconomic development pointing to the influence of diet, obesity and lifestyle factors [2]. Of concern, recent data shows an increase in patients diagnosed younger than 50 years [3].

The armamentarium of systemic therapies for colorectal cancer has increased, although progress in the last 15 years has been slow, apart from targeted therapies in uncommon subtypes. Standard curative therapy for colon cancer consists of surgical resection followed by adjuvant chemotherapy for stage III and high risk stage II disease, with benefit for both disease-free survival (DFS) and overall survival (OS) [4]. Recent studies are exploring the role of neoadjuvant chemotherapy in colon cancer. This approach is standard of care for locally advanced rectal cancer, using neoadjuvant long course chemoradiation or short course radiotherapy, followed by surgery and adjuvant chemotherapy, although evidence for OS benefit for the latter is weak.

In metastatic disease, various lines of chemotherapy are standard, with the addition of targeted therapy based on routine molecular testing for extended RAS and RAF mutation profiles. Standard first and second line treatments include fluoropyrimidine based chemotherapy combined with either oxaliplatin or irinotecan, with or without targeted therapies such as bevacizumab against vascular endothelial growth factor (VEGF), or cetuximab and panitumumab against epidermal growth factor receptor (EGFR) [5]. Third line options include trifluridine/tipiracil and regorafenib [6]. Many patients remain well, even after all these lines of treatment and are appropriate for clinical trials of novel agents.

There is great interest in harnessing the benefit of the new class of anti-cancer agents that target the immune system to improve the outcomes for patients with CRC. Significant progress in understanding of the complex interaction between the immune system and cancer has occurred, assisted by remarkable development in technology that have driven laboratory discovery. The fundamental principle is that the immune system recognizes cancer cells through neoantigens formed as a result of genetic mutations, chromosomal abnormalities and aberrant protein synthesis [7]. The human host immune response to a cancer is characterized by the release of pro-inflammatory cytokines and infiltration of immune cells into the tumor microenvironment [8]. In a physiological feedback mechanism designed to prevent autoimmunity, activated T cells upregulate inhibitory receptors, including CTLA4 and PD1 [9], and tumor cells, dendritic cells and macrophages upregulate counter-regulatory receptors including PDL1 and IDO [10]. Chronic T cell stimulation can result in T cell exhaustion which is associated with expression of a multitude of other checkpoint molecules including TIM-3, LAG-3, TIGIT, BTLA or VISTA [9]. Tumor cells are able to ”hijack” these immune checkpoints to suppress an anti-tumor response and thereby evade the host’s cancer immunosurveillance [11].

Understanding these mechanisms has allowed the development of therapies targeted at specific components of the cancer-immune system interaction (Figure 1). The class of Immune Checkpoint Inhibitors (ICI) have heralded a new therapeutic paradigm for oncology. Monoclonal antibodies have been developed targeting CTLA4 (ipilimumab, tremelimumab), PD1 (nivolumab, pembrolizumab) and PDL1 (atezolizumab, durvalumab, avelumab). Clinical trials have demonstrated potentially profound and durable clinical responses with these agents in a variety of cancer types and are now routinely used in clinical practice for patients with metastatic

melanoma, and lung, urothelial, renal, head and neck, triple negative breast and hepatocellular cancers [12,13].

Based on data demonstrating the efficacy of pembrolizumab in a tumor agnostic patient population with microsatellite instability high (MSI-H) or mismatch repair deficiency (dMMR) tumors, the US Food and Drug Administration (FDA) made the first ever approval in May 2017 of a cancer therapy based entirely on a biomarker [14]. This paper discusses in detail the biomarkers of response to immunotherapy (IO), in particular microsatellite instability (MSI); the clinical implications of MSI for CRC; clinical trial experience of ICI in early and metastatic CRC (mCRC), both as monotherapy and in combination with other agents; and finally emerging immune therapeutic strategies.

2. Biomarkers of response to immunotherapy

2.1 Microsatellite instability in CRC

2.1.1 Nomenclature

A microsatellite is a stretch of DNA sequence with repeated nucleotides (usually 1-6 base pairs) [15]. It is estimated that there are over 500,000 microsatellites in the human genome (almost 3%) [16]. Microsatellite instability occurs when there are a different number of repeated nucleotides (the microsatellite sequence or locus) compared to normal cells, through DNA insertion or deletion alterations. In tumors characterized by MSI, the aberrant cells have undergone clonal expansion and their abnormal number of repeated nucleotide sequences is able to be detected as a marker of the malignant phenotype [15] [17].

The mutation mismatch repair (MMR) pathway is one of the four main repair systems fundamental to maintaining the integrity of DNA, responsible for the identification and

reparation of base-base mismatches, including any sequence aberrations arising in microsatellites. The four most commonly affected human DNA MMR genes are *MLH1*, *MSH2*, *MSH6* and *PMS2* [18]. A mutation in any gene results in a faulty repair pathway, allowing accumulation of genetic damage in affected cells. Thus, MSI is a result of a deficient MMR pathway. Although frequently used interchangeably it is important to note the difference in these terms. The relevance of MSI-low (MSI-L), where <40% of microsatellites demonstrate instability, is debated. Currently, there is no meaningful clinical or pathological difference between MSI-L and microsatellite stable (MSS) tumors, and as such terminology for MSI-H may be simplified to MSI [15].

2.1.2 Mechanism and prevalence

MMR deficiency occurs through several mechanisms: an inherited germline mutation, a consequence of promoter hypermethylation of *MLH1* or rarely a biallelic somatic mutation. Lynch Syndrome is an autosomal dominant disease where a mutation is inherited most commonly in *MLH1* and *MSH2*, requiring acquisition of a somatic mutation in the corresponding wild-type allele to cause a deficiency in the MMR pathway [15]. This genotype is highly predisposed to cancer. Lynch Syndrome accounts for 2-3% of all CRC but is much commoner in young cohorts [19].

Most CRC is microsatellite stable (MSS). The likelihood of MSI is lower with more advanced stage disease, due to the favorable prognosis it infers. The incidence is approximately 20%, 12% and 4% in stages II, III and IV CRC respectively [20].

2.1.3 Testing methods

There are two widely accepted approaches: immunohistochemistry (IHC) and polymerase chain reaction (PCR) [21]. IHC is much cheaper, more accessible, has a 92% concordance to PCR and guides further genetic analysis [22].

IHC detects staining for protein expression of the four MMR genes, to act as a surrogate marker of MSI. If the tumor stains, this means the normal MMR proteins are present, indicating the genes are functional, described as normal, retained MSS or MMR proficient (pMMR). Reporting the IHC as 'positive' causes confusion and should be avoided. Absent staining of any of the proteins is abnormal i.e. MMR deficient (dMMR) or MSI.

If *MLH1* is not expressed on IHC, then further testing is indicated to distinguish between sporadic and inherited mutations. This can be done by testing either *MLH1* methylation status or *BRAF* mutation molecular analysis. Hypermethylation of *MLH1* promoter accounts for 90% of sporadic CRC and the presence of a *BRAF* mutation is almost exclusively associated with sporadic pathogenesis. While *BRAF* mutations are rarely seen with Lynch syndrome, the absence of *BRAF* mutant protein does not distinguish sporadic or inherited origin of dMMR [23].

The process of detecting MSI through a PCR-based assay, uses a standardized panel of five microsatellites defined by the modified Bethesda guidelines [21]. This is mainly used where IHC results are inconsistent or where Lynch Syndrome is suspected, to define the specific abnormality. Although Next Generation Sequencing (NGS), where a whole panel of genes are tested concurrently, has emerged as an alternative, pentaplex PCR remains the validated and accepted standard [24].

Misdiagnosis of dMMR status carries significant treatment implications, and in one retrospective analysis occurs in 10% of patients. Combining both IHC and PCR testing of dMMR reduces the rate of misdiagnosis, while NGS may also play a role in reducing this further [25].

A developing field is liquid biopsy (simply a blood test appropriately processed) to detect MSI in circulating tumor DNA (ctDNA). This alleviates need for a tissue sample and has potential for monitoring treatment response and acquisition of resistance during therapy [26]. ctDNA can be used to assess for molecular residual disease (MRD) and rates of MRD have been correlated with disease stage, radiologically measurable disease and response to therapy [27].

2.1.4 Clinicopathological correlations

Clinicopathological features of sporadic MSI/dMMR tumors include female gender, age over 70 and history of tobacco-smoking. Tumors tend to be right sided, graded as poorly differentiated and/or with mucinous differentiation and characterized by an 'inflamed' stroma with multiple tumor-infiltrating lymphocytes [28][29]. This can lead to comments that the tumor 'looks nasty' when in fact these tumors have a better prognosis stage-by-stage than MSS tumors [30]. Compared to MSS tumors, MSI in early stage CRC has an improved overall survival, with a large pooled analysis finding a hazard ratio (HR) of 0.67; (95% CI 0.58 - 0.78) [31]. In comparison, the prognostic implications of MSI in metastatic disease is controversial, though a large pooled analysis reported an adverse effect on survival [32]. Immunoescape, a process whereby tumors have developed mechanisms to evade the strong host immune infiltration characteristic of MSI, is a possible biological explanation [33]. Furthermore, the prevalence of concurrent *BRAF* mutation may be a confounding factor.

MSI also predicts for harm from single agent fluoropyrimidine adjuvant chemotherapy in early stage CRC, theorized to be due to the inhibition of the body's immune response to the tumor [34]. Non randomized data has suggested that adjuvant combination chemotherapy with oxaliplatin in stage III disease yields net benefit [18].

Approximately 10% of metastatic CRC cases have *BRAF* mutations, the vast majority being V600E. These tumors more frequently originate in the right colon, have a predilection for peritoneal metastases and is a negative prognostic marker with the risk of death in *BRAF* mutants being twice that of *BRAF* wildtype patients [35]. There is a significant intersection between *BRAF* and MSI. 35 - 52% of MSI cases have *BRAF* mutations and 21 - 55% of *BRAF* mutant cases are also MSI [32,36]. Both MSI and BRAF mutation status are prognostic. MSI is associated with decreased mortality in BRAF mutated and wildtype patients; and BRAF V600E is associated with higher mortality in both MSI and MSS patients.

2.2 Other IO biomarkers: PDL1, TMB, TILs, B2M and microbiome

2.2.1 PDL1

Programmed death-ligand 1 (PDL1) staining by IHC is frequently used in malignancies such as non-small cell lung cancer (NSCLC) to predict response to ICI [37]. This biomarker however does not appear to be robust in either MSS or MSI CRC [38,39]. In rectal cancer, low PDL1 expression has been associated with poorer prognosis [40].

2.2.2 TMB

High tumor mutational burden (TMB) has been associated with IO response in many tumor types. TMB reflects the number of somatic mutations per megabase and is detected through

NGS. It is thought that a hypermutated tumor will produce a greater number of neoantigens for presentation and immune recognition, thus facilitating an IO response. Unlike PDL1, TMB-high tumors do predict a response to IO in CRC [41]. However in CRC, this usually reflects underlying MSI. Where MSI is not found, other explanations for the accumulation of mutations should be sought, such as POLE mutations, which are rare but confer similar IO sensitivity [42]. The cut-off to obtain a response in MSI CRC was found to be between 37 and 41 mutations per megabase, while an unselected group of CRC patients had a cut-off of 52 [43]. In MSS CRC, response to IO based on TMB is limited to case reports, with the prevalence of MSS CRC with high TMB at only 3% [44,45].

2.2.3 TILs

Tumor infiltrating lymphocytes (TILs) represent the immune response in the tumor bed and reflect an active or 'inflamed' tumor microenvironment (TME) [46]. The density and location of TILs in a TME field defined on a histology slide has been quantified and validated in the Immunoscore®, a commercial test now available with predictive value in stage I-III CRC [47]. The association between high TILs or Immunoscore® and response to IO are being explored, although once again these usually coincide with MSI status.

2.2.4 B2M

Beta-2 microglobulin (B2M) mutation is known to be a marker of acquired resistance to immune checkpoint inhibitors in metastatic melanoma. B2M mutation leads to a failure of antigen presentation and thus a lack of T-cell stimulation. In a small sub-group analysis of MSI patients, five CRC patients on IO treatment were found to have acquired B2M mutation resulting in progressive, metastatic disease [48]. Separate studies with larger cohorts offer conflicting results

with B2M absence of staining being associated with a favorable prognosis in MSI CRC, with a 5-year OS of 91.7% compared to 72.1% [49,50]. This disparity might reflect that B2M mutation in metastatic deposits enable immune escape, compared with in the primary tumor. B2M mutation does not predict primary resistance in ICI naive patients. A retrospective single center study reported clinical response in 11 (85%) or 13 CRC patients with B2M mutation who received ICIs [51].

2.2.5 Microbiome

The intestinal microbiome appears to affect the efficacy of IO in many tumors, although series differ in which specific microbiota are predictive [52,53]. These studies were not specific to CRC, and understanding the role of the microbiome in CRC is particularly difficult given that many patients have had bowel surgery (often extensive) and commonly have diarrhea and constantly changing bowel function during standard chemotherapy.

3. Role of ICI in metastatic CRC

3.1 Early phase multi-tumor trials

The initial studies of immune checkpoint inhibitors were conducted prior to the significance of MSI as a predictive biomarker being established. In the first-in-human trial of nivolumab in 39 patients with various tumor types, 1 of 14 mCRC patients achieved a complete response that remained durable for over 3 years [54,55]. This patient was later shown to have an MSI tumor.

In Keynote-028, a study of pembrolizumab in 20 different tumor types, the sole MSI mCRC patient had a partial response [56]. However, the major breakthrough came with the publication of what is considered the seminal trial, Keynote-016, examining pembrolizumab 10mg/kg every

2 weeks given for various MSI and MSS tumors. Objective responses were reported in 4 of 10 patients with treatment refractory MSI mCRC [39]. Results from an expanded cohort documented objective response in 21 of 40 MSI patients, with 5 complete responses [48]. By contrast, no response was observed in MSS patients.

3.2 MSI mCRC

3.2.1 Previously treated MSI mCRC

Several trials examined single agent ICI in chemotherapy-refractory mCRC. Keynote-164 examined pembrolizumab at a fixed dose of 200mg every 3 weeks for up to 35 cycles, reporting 21 of the 63 patients treated after one line of therapy had an objective response, including 5 complete responses [57]. Of the 61 patients who had received two or more prior lines of therapy, 20 had an objective response, which included 2 patients with a complete response. Treatment related adverse events (TRAEs) in these trials of single agent pembrolizumab, was reported at 62 – 98% of patients, though most events were grade 1 - 2 or laboratory test based events. The commonest adverse events included arthralgia, diarrhea, nausea, asthenia, rash and fatigue. The incidence of grade 3-4 TRAEs was 13% and 3% of patients discontinued treatment due to pneumonitis.

Checkmate-142 was a phase II, non-randomized, open label trial of nivolumab monotherapy (3mg/kg every 2 weeks) or in combination with ipilimumab in MSI mCRC. In the monotherapy cohort of 74 previously treated patients, 2 achieved a complete response and 22 had a partial response [58]. In the combination arm (nivolumab 3mg/kg and ipilimumab 1mg/kg every 3 weeks for four doses followed by nivolumab 3mg/kg every 2 weeks), 65 of 119 patients (55%) achieved an objective response with 3% complete responses [38]. This is the largest study of

combination PD1 and CTLA4 inhibitor therapy in MSI CRC and demonstrates that this combination can achieve higher response rates.

As with combination trials of PDL-1 plus CTLA-4 inhibitors in other tumor types, the toxicity profile Is significant, with 32% of patients in Checkmate-142 experiencing a TRAE of grade 3 or higher, which included elevated liver transaminases (11%), elevated lipase (4%), anemia (3%) and colitis (3%). The most common TRAEs of any grade were diarrhea (22%), fatigue (18%) and pruritus (17%). The commonest immune related TRAEs (irAEs) of any grade were reported by organ system: skin (29%), endocrine (25%), gastrointestinal (23%), hepatic (19%) and pulmonary (5%). There were no treatment related deaths.

Trials with other ICI have been undertaken, such as the phase III trial IMblaze370 study, included a small number of patients with MSI mCRC previously treated with two prior lines of therapy [59]. 2 of 3 patients in the atezolizumab plus cobimetinib (MEK inhibitor) arm and 1 of 3 patients in the atezolizumab monotherapy arm achieved an objective response.

3.*2.2 First line MSI mCRC*

The trials described previously (Table 1) set the scene for moving ICI into earlier lines of therapy, with the hope of providing an alternative to chemotherapy, even in the first line setting. Interim results of a cohort of 45 patients with treatment naive MSI mCRC were presented recently [60]. Patients received nivolumab 3mg/kg every 2 weeks plus ipilimumab 1mg/kg every 6 weeks. Objective response rate (ORR) was 69%, with 13% achieving a complete response. The progression free survival (PFS) and OS at 24 months was 74% and 79% respectively. This regimen appeared better tolerated than trials of similar doses in later lines, with 22% TRAEs of

grade 3 or higher. The commonest any grade TRAEs were pruritus (36%), hypothyroidism (18%), arthralgia (20%), asthenia (16%) and rash (16%).

The most significant trial to date in this field is Keynote-177, a phase III, international, open label, randomized controlled study comparing pembrolizumab monotherapy (200mg every 3 weeks for up to 35 cycles) with standard doublet chemotherapy with or without anti-VEGF or anti-EGFR monoclonal antibodies in the first line setting for patients with MSI mCRC [61]. The trial enrolled 307 patients and allowed crossover on progression. The co-primary end points were PFS and OS. Interim results have recently reported median PFS of 16.5 months in the pembrolizumab arm compared to 8.2 months for chemotherapy (HR 0.60 [95% CI 0.45 - 0.80]). The PFS rate at 24 months was 48% for pembrolizumab and 19% for chemotherapy. OS data is still immature. The ORR was 43.8% for pembrolizumab and 33.1% for chemotherapy. In the pembrolizumab arm, 11.1% achieved a complete response and 32.7% achieved a partial response which was maintained in 83% of patients for at least 24 months. In the chemotherapy arm, prolonged maintenance of response was seen in only 35%.

As expected, pembrolizumab therapy was much better tolerated, with 22% grade 3 or higher adverse event compared with 66% in the chemotherapy arm. The commonest grade 3 or higher TRAEs in the pembrolizumab arm were colitis (3%), hepatitis (3%), diarrhea (2%) and fatigue (2%). Common TRAEs of any grade were diarrhea (25%), fatigue (21%), nausea (12%), decreased appetite (8%) and stomatitis (5%). The commonest irAEs of any grade were hypothyroidism (12%), colitis (7%), hyperthyroidism (4%), pneumonitis (4%), adrenal insufficiency (3%) and hepatitis (3%). There were no treatment related deaths.

3.2.3 MSI CRC with concurrent *BRAF* mutation

Clinical trial data for ICI in *BRAF* mCRC is limited. In the previously discussed Checkmate-142 trial, 12 *BRAF* mutant patients received nivolumab monotherapy. The ORR was 25%. In the nivolumab plus ipilimumab arm, there were 29 *BRAF* mutant patients and the ORR was 55% [38]. These results are similar to the whole trial population. In the recently presented Keynote-177 trial, 22% of patients were *BRAF* mutant. While outcomes have not been reported for this subgroup specifically, both *BRAF* wildtype and mutant subgroups appear to have similar PFS benefit from pembrolizumab [61]. This patient group now has multiple potential therapeutic strategies including ICI, targeted therapy combinations, chemotherapy and targeted therapy combinations [62].

3.3 MSS mCRC

3.3.1 Single agent ICI

The vast majority of patients with mCRC have MSS tumors and derive almost no benefit from single agent ICI. In a phase I trial of nivolumab in 296 patients with multiple cancer types, no response was observed in 19 patients with CRC unselected for MMR status [63]. Keynote-016 and Keynote-028 observed no responses in 18 and 22 patients respectively with MSS mCRC [39] [56].

3.3.2 ICI combinations

In an effort to induce response, combination CTLA4 and PDL1 inhibition has been studied seeking to capitalize on their synergistic actions. CCTG CO.26 is a phase II, open label, randomized trial of tremelimumab (75mg every 4 weeks for 4 cycles) plus durvalumab (1500mg every 4 weeks) compared with best supportive care (BSC) alone in 180 patients with treatment refractory mCRC, of whom 166 were MSS [64]. The median OS was 6.6 months in the treatment

group and 4.1 months in the BSC group (HR 0.72 [90% CI 0.54 - 0.97]). The HR for death in the MSS subgroup was 0.66 (90% CI 0.48 - 0.89). One patient in the treatment arm achieved a partial response. All patients in the investigational arm reported an adverse event and 62% reported a grade 3 or higher adverse event. While this trial achieved its primary endpoint, improvement in overall survival was modest. Analysis for TMB was available for 94% of patients and a cut point of 28 was prognostic and predictive. For patients with a TMB of 28 or more, there was a worse prognosis in the BSC arm (OS HR 2.59; 90% CI, 1.46-4.62) and the greatest benefit from treatment (OS HR 0.34; 90% CI, 0.18-0.63). Interpreting CCTG CO.26 is controversial. Although it is the largest study to date to report positive results in MSS CRC, the conclusion that this disease requires combination ICI therapy in order to achieve responses has not been widely adopted.

4. IO in combination with other agents

4.1 Chemotherapy

The anti-tumor activity of chemotherapeutic agents extends beyond direct cytotoxic effects. Their immunogenicity includes depleting myeloid derived suppressor cells and Treg cells, and thereby increasing antigen presentation and T cell activity [65]. Synergy between chemotherapy and ICI was studied in a phase II trial in 30 treatment naïve mCRC patients, who received FOLFOX with pembrolizumab [66]. Only three patients were MSI. ORR was 53% including one complete response. Grade 3 or 4 toxicity was seen in 37%. Similarly, Keynote-651, a phase Ib study in MSS mCRC demonstrated ORR of 60% in 15 treatment naïve patients given pembrolizumab with FOLFOX and ORR of 13% in 16 patients treated in the second line setting with pembrolizumab with FOLFIRI [67].

Temozolomide, an oral alkylating agent, has been shown to induce loss of DNA mismatch repair mechanism and hence prime tumors towards a hypermutated state [68]. This strategy of temozolomide administered prior to ICI to induce a MSI-like state (or at least high TMB) is being studied in MSS CRC using pembrolizumab (NCT03519412), nivolumab (NCT03879811), and combination nivolumab and ipilimumab (NCT03832621).

4.2 Anti-angiogenic targeted therapy

Combination with anti-angiogenic therapy has been explored based on preclinical models showing they induce immune upregulation. VEGF is extensively implicated in tumorigenesis with roles in angiogenesis, immune modulation in the tumor microenvironment and suppressing immune cell migration, activation and function [69]. Clinical benefit of this strategy has been demonstrated in other tumor types including NSCLC, renal cell carcinoma and hepatocellular carcinoma [70]. Retrospective analysis of trial data also hypothesizes benefit from antiangiogenic agents in MSI trial subpopulations [71].

Combination atezolizumab and bevacizumab was examined in 10 patients with pretreated MSI mCRC in a phase Ib trial, with ORR of 30%. This regimen was associated with 40% of patients experiencing grade 3 or 4 toxicity, most commonly proteinuria [72]. The ongoing three arm phase III COMMIT trial is comparing FOLFOX/bevacizumab in combination with atezolizumab with atezolizumab monotherapy in first line MSI mCRC (Table 2).

A similar strategy of targeting angiogenesis to convert the 'immunodesert' environment is being explored in MSS mCRC. In a phase I trial of mCRC unselected for MMR status, objective response was observed in 8% of treatment refractory patients who received atezolizumab and bevacizumab, and 36% in treatment naïve patients who received atezolizumab, bevacizumab and

FOLFOX [73]. In BACCI, a phase II, placebo controlled trial of heavily pretreated mCRC (86% MSS) the addition of atezolizumab to capecitabine and bevacizumab, resulted in statistically significant but very modest increase in PFS from 3.3 to 4.4 months [74]. However, the addition of atezolizumab to maintenance fluoropyrimidine and bevacizumab after sixteen weeks of induction FOLFOX and bevacizumab did not improve PFS or OS in the phase II MODUL trial [75].

4.3 Epidermal growth factor receptor antibody targeted therapy

EGFR is implicated in the immune response, with preclinical data demonstrating a role in opsonization and phagocytosis of colonic cancer cells, T cell activation and natural killer (NK) cell activity [76] More recently, anti-EGFR therapy has been demonstrated to downregulate DNA repair mechanisms, which is correlated with increased instability of microsatellites [77]. The combination of ICI and EGFR targeting has been evaluated in a phase Ib/II trial of cetuximab plus pembrolizumab in treatment refractory mCRC patients unselected for MSI status, demonstrating disease control for at least 16 weeks in six of nine patients and manageable toxicity [78]. The addition of chemotherapy was studied in the phase II AVETUX trial in the first line mCRC setting independent of MSI status. 43 patients received FOLFOX, cetuximab and avelumab. Interim results for the first 20 patients have been reported with ORR of 75% [79].

4.4 Tyrosine kinase inhibitors

Regorafenib, a multikinase inhibitor of angiogenic and oncogenic kinases, reduces immunosuppressive tumor associated macrophages (TAMs) and regulatory T cells (Tregs) in laboratory tumor models. REGONIVO is a Japanese phase Ib trial investigating regorafenib and nivolumab in 25 treatment refractory mCRC patients, of which 1 was MSI. A promising ORR of

36% was seen [80]. However, in the French phase II REGOMUNE trial using regorafenib and avelumab in 48 patients with treatment refractory MSS mCRC, ORR was 0% [81]. Ongoing phase III trials may provide definitive conclusions regarding this much-hoped for treatment synergy.

Preclinical studies have demonstrated that MEK inhibitors, through their blockade of the MAPK pathway, can augment T cell infiltration, major histocompatibility complex (MHC) upregulation and antigen presentation cell (APC) activation in pMMR cell lines, when combined with PD1/PDL1 inhibitors [82]. The IMblaze370 trial compared atezolizumab plus cobimetinib and regorafenib in a chemorefractory, predominantly MSS patient population following on from a phase I trial reporting an ORR of 17% [59]. However, IMblaze370 did not demonstrate any OS benefit with the combination (HR 1.00 [95% CI 0·73–1·38]) and the ORR was <3%. Other ongoing trials investigating ICI synergy with MEK inhibitors include NCT02876224 evaluating cobimetinib, atezolizumab and bevacizumab; and NCT02060188 evaluating cobimetinib, nivolumab and ipilimumab.

5. Role of ICI in early stage CRC

5.1 Adjuvant therapy in colon cancer

ICI are being investigated in the adjuvant setting in MSI in efforts to improve disease free survival and overall survival beyond standard fluoropyrimidine and oxaliplatin doublets. However, the prognosis for the patients is already superior to MSS patients, so large numbers will need to be recruited to phase III trials. The ATOMIC study (NCT02912559) is a US based trial currently recruiting and will evaluate the impact of atezolizumab concurrent with standard adjuvant FOLFOX chemotherapy and then continuing to complete a 12 month course in MSI

stage III colon cancers. The POLEM trial (NCT03827044) was planned to explore the addition of six months of avelumab after adjuvant chemotherapy but did not proceed to open after the sponsor withdrew. Nivolumab after standard adjuvant therapy in MSI CRC will be assessed in the ctDNA positive patient population in a US based phase III trial (NCT03803553).

In MSS disease, Columbia-2 is a phase II trial that will evaluate durvalumab in combination with FOLFOX adjuvant chemotherapy (NCT04145193). A current phase I trial will investigate combination ipilimumab, nivolumab and a KRAS peptide vaccine in MSS KRAS mutant CRC after standard adjuvant chemotherapy (NCT04117087).

5.2 Neoadjuvant therapy

5.2.1 Locally advanced rectal cancer

Standard treatment of locally advanced rectal adenocarcinoma involves radiotherapy, either short or long course, the latter with concurrent chemotherapy [83]. MSI/dMMR rates in early stage rectal cancer are only around 6% (and often suggest Lynch syndrome) [84,85].

In preclinical data, radiotherapy appears to be immunostimulatory, promoting PDL1 production in both the primary tumor and in the invasive front [86] [40]. Several retrospective studies support a synergistic effect of ICI and neoadjuvant chemoradiotherapy in various tumor types [87] [88] [89]. Harnessing the local and abscopal effect of radiation to boost host immune response to the tumor is a putative mechanism being explored to enhance response to ICI in MSS rectal cancers.

There are many clinical trials incorporating ICI in neoadjuvant protocols for rectal cancer, adding single agents or combinations during or after chemoradiation (CRT). These include: AveRec (NCT03299660), an Australian phase II study of 2-4 cycles of avelumab after CRT;

AVANA (NCT03854799), a phase II study of avelumab during neoadjuvant CRT; INNATE (NCT04130854), a phase II trial of an anti-CD40 agonist (APX005M) in conjunction with short-course radiotherapy; TARZAN (NCT04017455), an open-label early phase trial evaluating the addition of atezolizumab and bevacizumab after neoadjuvant radiotherapy and CHINOREC (NCT04124601), incorporating nivolumab and ipilimumab with neoadjuvant CRT.

5.2.2 Locally advanced colon cancer

Emerging evidence supports the role for neoadjuvant chemotherapy for both resectable and locally advanced colon cancer, a paradigm shift from upfront surgery that has evolved from the treatment of rectal cancer [90]. This is yet to be routinely adopted, however unexpectedly good results were seen in MSS colon cancers as well as MSI tumors in the exploratory NICHE study may promote wider use [91]. Nineteen of 20 patients with MSI tumors had a major pathological response in the subsequently resected specimen, 12 of which were complete responses. Of the 15 patients with MSS cancers, the surprise finding was 3 major pathological responses.

6. Novel immunotherapy agents

6.1 Bispecific antibodies

Bispecific antibodies are a novel therapeutic class, engineered with the ability to bind to two separate ligands. CEA-TCB (RG7802, RO6958688) binds to CEA on tumor cells and CD3 on T cells, inducing T cell migration, engagement, activation and proliferation within the tumor microenvironment. A phase I trial in treatment refractory mCRC has evaluated CEA-TCB as monotherapy with results demonstrating 45% disease control rate with 2 partial responses in 31 patients [92]. When CEA-TCB is combined with atezolizumab, 2 of 11 patients had a partial

response with a disease control rate of 82%. Common serious adverse events included diarrhea, infusion reactions and pyrexia.

6.2 Anti-tumor vaccines

Vaccines containing tumor antigens aimed at inducing an anti-tumor immune response, also known as active specific immunotherapy (ASI), continue to be investigated with varying results and no approved therapies to date. More recent strategies include use in the neoadjuvant or adjuvant setting, vaccines combined with cytotoxic agents and/or immunotherapy, and 'mixed' vaccines containing multiple tumor epitopes.

OncoVAX®, an active specific immunotherapy utilizing a Bacillus Calmette–Guérin (BCG) vaccine with autologous irradiated tumor cells, was evaluated in the adjuvant setting in a phase III trial enrolling 254 stage II or III CRC patients. A 61% reduction in recurrence rates was demonstrated in stage II disease. Further studies investigating the benefit of this vaccine in combination with adjuvant chemotherapy in stage III CRC are underway [93]. Another phase II study of the RO7198457 vaccine also in the adjuvant setting is also recruiting [NCT04486378].

In patients with MSS CRC, a pilot study has examined a multi-antigen immunotherapy agent with intradermal administration of peptides from proteins including Fascin-1, Ape-1, VCP and RCAS1, which are immunogenic proteins overexpressed in refractory cancers. Of the 15 patients studied, 86% had an objective response and 8 patients documenting an increased CD8 cell count, which is associated with delayed-type hypersensitivity assays of the involved peptides [94].

A recent pilot study of an intradermal autologous tumor vaccine studied 31 patients across a range of advanced solid tumors. There was one complete response of the four patients with

mCRC. Overall, 13% of patients achieved a complete response and 6.5% had a partial response [95].

To overcome the expense, timeliness and complexity involved in creating personalized anti-cancer vaccines, a combination vaccine containing a mixture of five epitopes was developed, and in phase I trials produced an objective response in 7 of 18 patients [96]. Another combination vaccine, PolyPEPI1018, was developed as an “off-the-shelf" vaccine containing 12 immune epitopes commonly expressed in CRC. This agent was administered to 11 patients with MSS mCRC. The vaccine was well tolerated with an objective response seen in 3 patients, including one pathological complete response after metastatectomy [97].

Current active vaccine combination therapy trials in mCRC include a pilot study using the GVAX® CRC vaccine with cyclophosphamide and SGI-110, a T-cell recruiting agent; four phase I trials of a vaccine with PD1 of PDL-1 inhibitors; and a phase I trial of a *KRAS*-mutant vaccine combined with dual checkpoint inhibition [NCT01966289, NCT02432963, NCT04046445, NCT03289962, NCT03287427, NCT04117087].

6.3 Targeting other immune pathways

The complex interaction between the immune system, the cancer cell and the tumor microenvironment is increasingly being appreciated. New targets and combinations of therapies are being tried to overcome the inherent resistance to ICI seen in MSS tumors. Results from phase I and II trials of alternative immunomodulatory therapy including neutrophil and monocyte primers, adoptive natural killer cells, and cytokine-induced killer cells have achieved only stable disease but no objective response and therefore have not progressed to later phase trials [98–

100]. A phase I/II trial of a toll-like receptor-9 agonist showed tolerability in a phase I trial and is being carried forward to a phase III trial in metastatic colorectal cancer patients [101].

For MSI tumors, there are also trials to enhance the activity of immune therapy further, in order to achieve cure as appears to be the case in other tumors such as metastatic melanoma. A phase I trial in patients with dMMR or Consensus Molecular Subtype 4 (CMS4) metastatic colorectal cancer is using an antibody targeting the Indoleamine 2,3 dehydrogenase (IDO) immune checkpoint protein, in combination with an immune-stimulatory anti-OX40 antibody and a bifunctional anti-PDL1/TGFβ fusion antibody [NCT03436563].

6.4 T cell receptor therapies

Chimeric antigen receptor T-cells (CAR-T) have not yet demonstrated significant efficacy in solid tumors, including CRC, unlike their promising use in hematological malignancies [102] [103]. New T-cell receptor (TCR) directed therapies are being developed. A novel therapy, "CoupledCAR" utilizes a viral vector encoding an anti-colorectal cancer CAR and an anti-colorectal cancer CAR-T cell, to improve in vivo expansion of CAR-T cells and therefore tumor response. When tested in a small patient group, two heavily pretreated CRC patients obtained a partial response; this early signal may lead to further clinical trials [104].

Another strategy involves direct administration of CAR-T cells instead of intravenous delivery, aiming to bypass the requirement for localization within tumor tissue. Hepatic arterial delivery of CAR-T cells targeting liver metastases induced necrosis in four of six patients in a phase I study [105], and in murine models intraperitoneal CAR-T cell delivery induced distal tumor responses and reduced peritoneal recurrence [106].

6.5 Targeting the tumor microenvironment

Novel immune therapies are also being developed with the aim of targeting components of the extracellular tumor microenvironment in order to decrease its immunosuppressive effects.

CLEVER-1 is highly expressed on tumor-associated macrophages and is associated with poor tumor response to immunotherapy. FP-1305, a humanized anti-CLEVER-1 antibody, was tested in the "MATINS" phase I/II trial of thirty patients with advanced solid tumors. Toxicities were acceptable, and circulating NK cell and B cell levels increased, regulatory T cell levels decreased and the CD8/CD4 T cell ratio was increased, suggesting anti-tumor immune activity. One pMMR colorectal cancer patient had a partial response [107].

Bintrafusp alfa, a dual PDL1 antibody/TGFβ trap, was given with radiotherapy (24 Gray in 3 fractions) to a single metastatic lesion with abscopal intent in a phase II trial involving 13 patients with CMS4 colorectal cancer. Only two patients had a best response of stable disease [108].

Aiming to improve response rates to PD1 inhibitors in dMMR colorectal cancer, DNase I was utilized to deplete Neutrophil Extracellular Traps (NETs), an immunosuppressive extracellular matrix containing proteins expulsed by neutrophils. In mouse MSI CRC models, DNase I delivered via intraperitoneal infusion followed by PD1 inhibitor also administered intraperitoneally, was effective in reducing tumor volume. The proposed immune mechanism was a decreased level of exhausted CD8+ T-cells after this combination therapy [109].

7. Conclusion

Immunotherapy for colorectal cancer has seen great success in the small subgroup of patients with dMMR/MSI tumors, but sadly not for the majority of patients. The ability of CRC tumors and their microenvironments to ‘hide and shelter’ from immune surveillance is an area of active

investigation, with studies focusing on the molecular pathways that govern these interactions. As of the present day, there is an established role for ICI in MSI mCRC, with positive results from the phase III Keynote-177 trial of pembrolizumab alone in the first line setting being superior to chemotherapy. Similarly, ICI has demonstrated efficacy in the later line setting in MSI mCRC. Ongoing trials are aimed at invoking response to immunotherapy in MSS disease, as well as defining its role in early stage CRC in the adjuvant and neoadjuvant settings.

8. Expert Opinion

The era of immunotherapy for colorectal cancer has arrived, but only for some patients. After decades of slow progress in the management of metastatic CRC, the current range of therapies with chemotherapy and targeted agents still offer only a palliative approach. The promise of immunotherapy to achieve functional cure as seen in other tumor types is much anticipated. Unique patterns of efficacy are observed with ICI, specifically profound deep responses when in contrast complete responses are rare with cytotoxic chemotherapy. Similarly, many patients have durable responses which are maintained for significant periods of time despite cessation of ICI. Finally, the toxicity profile of ICI is more favorable when compared to chemotherapy.

Supportive early phase data in MSI CRC, using pembrolizumab, nivolumab, and combination nivolumab and ipilimumab, have been confirmed in the pivotal phase III Keynote-177 trial, making the PD1 inhibitor pembrolizumab now a standard of care first line therapy in MSI disease. The OS data from this trial is eagerly awaited and long term follow up will demonstrate how durable the flattening of the “tail of the curve” will be.

Many research questions remain, including whether single agent is superior to combination IO, and whether either of these is best used with or instead of chemotherapy and/or targeted agents.

Finally, therapy sequencing remains an unanswered question, as does the choice of therapeutic target for patients with MSI *BRAF* mutant tumors.

For MSS metastatic CRC - the vast majority - the lack of efficacy of ICI remains profoundly disappointing. The CCTG CO.26 trial in refractory disease suggested modest efficacy for combined CTLA4 and PDL1 inhibition, but toxicity was significant such that, in addition to cost considerations, this has not been widely adopted. The focus remains on understanding the mechanisms for resistance and trialing strategies to improve immune responsiveness in these 'cold' tumors. Whether converting cold tumors into hot tumors is achieved through the use of combinations of ICI, chemotherapy, anti-angiogenics, tyrosine kinase inhibitors or with agents targeting other molecular pathways is to be seen. Furthermore, trials are underway investigating whether the abscopal effect can be induced with the combination of ICI and radiotherapy.

Our understanding of the complex, multifaceted interactions in the immune system and the ability to modulate these mechanisms will continue to expand. Additionally, the role of the gut microbiome in cancer immunology and the effect of microbiome disruption on the efficacy of immunotherapy is an exciting field of study. Connecting these concepts to the clinical setting must be explored. Progress is hoped to be made by expanding beyond our current therapeutic targets of CTLA4 and PD1/PDL1, and to investigate an extensive range of other immune oncology agents targeting TIM-3, LAG-3, TIGIT, BTLA, VISTA, IDO. Also, data regarding other immune based strategies such as tumor vaccines, modulating the tumor microenvironment and CAR-T will become available within the next decade.

Similar to many other tumor types, ICI in early stage disease, either neoadjuvant or adjuvant, is being explored. Some very intriguing data was recently published showing significant pathology response even in MSS tumors, although a larger cohort is needed to confirm this activity. In a

speculative view of future developments, this area may evolve into a total neoadjuvant or non-operative approach, as is developing in rectal cancer.

Biomarkers, beyond mismatch repair and tumor mutational burden, to enhance patient selection require further refinement and extensive data will be forthcoming in the next few years. The ability to predict responses and select the most effective treatment regimen for individual patients will realize the concept of personalized oncology, limit potential toxicity and reduce financial cost. Monitoring of ctDNA as a marker of tumor volume, disease activity and treatment response appears promising. The next step will be incorporating ctDNA data into therapeutic decision making and surveillance protocols. Detailed analysis of multi- omics (genes, transcripts and proteins) from serial tissue and liquid biopsies should aid the understanding of primary and secondary resistance mechanisms.

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Abbreviations

Colorectal cancer: CRC

Disease-free survival: DFS

Overall survival: OS

Vascular endothelial growth factor: VEGF

Epidermal growth factor receptor: EGFR

Immune Checkpoint Inhibitors: ICI

Microsatellite instability high: MSI-H

Mismatch repair deficiency: dMMR

metastatic CRC: mCRC

Food and Drug Administration: FDA

Immunotherapy: IO

Microsatellite instability: MSI

Mismatch repair: MMR

Microsatellite stable: MSS

Immunohistochemistry: IHC

Polymerase chain reaction: PCR

MMR proficient: pMMR

Next Generation Sequencing: NGS

Circulating tumor DNA: ctDNA

Molecular residual disease: MRD

Hazard ratio: HR

Programmed death-ligand 1: PDL1

Non small cell lung cancer: NSCLC

Tumor mutational burden: TMB

Tumor infiltrating lymphocytes: TILs

Tumor microenvironment: TME

Beta-2 microglobulin: B2M

Treatment related adverse events: TRAEs

Immune related TRAEs: irAEs

Objective response rate: ORR

Progression free survival: PFS

Best supportive care: BSC

Natural killer: NK

Tumor associated macrophages: TAMs

Regulatory T cells: Tregs

Major histocompatibility complex: MHC

Antigen presentation cell: APC

Chemoradiation: CRT

Active specific immunotherapy: ASI

Bacillus Calmette–Guérin: BCG

Consensus Molecular Subtype 4: CMS4

Indoleamine 2,3 dehydrogenase: IDO

Chimeric antigen receptor T-cell: CAR-T

T-cell receptor: TCR

Neutrophil Extracellular Traps: NETs

Table 1 – Pivotal completed trials in metastatic MSI CRC

Setting	Trial	Year	Phase	Number	Investigational arm	Primary Endpoint	Clinical impact
Treatment refractory	Keynote-016 [39]	2015	II	40	Pembrolizumab	ORR 52%	Led to MSI tumor agnostic approval by FDA in 2020
Second line Third line or greater	Keynote-164 [57]	2020	II	63 61	Pembrolizumab	ORR 33% ORR 33%	
Previously treated Treatment naive	Checkmate-142 [38,58]	2018	II	74 65 45	Nivolumab monotherapy Nivolumab + Ipilimumab Nivolumab + Ipilimumab	ORR 32% ORR 55% ORR 69%	Increase response rate with ICI combinations. First trial in first line setting.
First	Keynote	2020	III	307	Pembrolizumab	ORR 44% vs	Seminal

line	-177 [61]					33% Median PFS 16.5 vs 8.2 mo (HR 0.60 (95% CI 0.45 - 0.80)) PFS 24 months 48% vs 19%	phase III trial demonstrating benefit over chemotherapy

Abbreviations: ORR: objective response rate; MSI: microsatellite instability; FDA: U.S Food and Drug Administration; ICI: immune checkpoint inhibitor; PFS: progression free survival; mo: months; HR: hazard ratio; CI: confidence interval

Table 2 – Key ongoing trials

Source: US National Library of Medicine. ClinicalTrials.gov. https://clinicaltrials.gov/ct2/home (accessed August 2020)

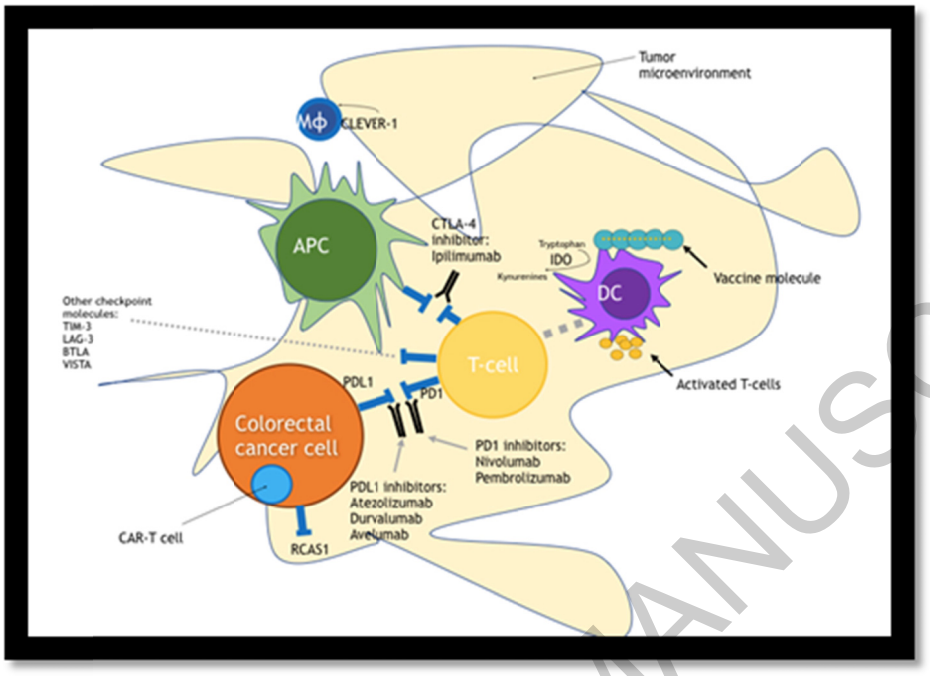
Setting	Trial	Identifier	MSI status	Phase	Investigational arm	Comparator arm	Primary Endpoint
Neoadjuvant							
Locally advanced rectal cancer	AveRec	NCT03299660	unselected	II	LCCRT followed by avelumab prior to surgery	-	Pathological response rate
Locally advanced rectal cancer	AVANA	NCT03854799	unselected	II	LCCRT + avelumab	-	pCR rate
Resectable rectal cancer	INNATE	NCT04130854	unselected	II	RT followed by atezolizumab +	-	clinical complete and near-complete

					bevacizumab		response rate
Resectable rectal cancer	CHINOREC	NCT04124601	unselected	II	LCCRT + ipiliumumab and nivolumab	LCCRT	Safety and tolerability
Adjuvant							
Stage III	ATOMIC	NCT02912559	MSI	III	FOLFOX + atezolizumab	FOLFOX	DFS
Stage III	POLEM	NCT03827044	MSI	III	Avelumab (after chemotherapy)	Observation	DFS
Metastatic							
First line	COMMIT	NCT02997228	MSI	III	FOLFOX/ bevacizumab + atezolizumab or Atezolizuma	FOLFOX / bevacizumab	PFS

					b monotherapy		
First line	Checkmate 9X8	NCT03414983	unselected	II/III	Nivolumab + FOLFOX/ bevacizumab	FOLFOX / bevacizumab	PFS
Second line	SAMCO	NCT03186326	MSI	II	Avelumab	Chemotherapy	PFS
Treatment refractory	BACCI	NCT02873195	unselected	II	Capecitabine / bevacizumab + atezolizumab	Capecitabine / bevacizumab	PFS
Regardless of previous treatment	Checkmate 8HW	NCT04008030	MSI	III	Nivolumab + ipilimumab or Nivolumab	Chemotherapy	PFS

Abbreviations: MSI: microsatellite instability; LCCRT: long course chemoradiation; pCR: pathological complete response; RT: radiotherapy; FOLFOX: 5-fluorouracil, leucovorin, oxaliplatin; DFS: disease free survival; POLE: polymerase epsilon; PFS: progression free survival

Figure 1 – Immune-tumor interaction



Abbreviations: Mϕ: macrophage; DC: dendritic cell; APC: antigen presenting cell; CTLA4: cytotoxic T lymphocyte antigen 4; PD1: programmed cell death-1 receptor; PDL1: programmed cell death ligand-1 receptor; IDO: indoleamine 2,3-dioxygenase; TIM-3: T-cell immunoglobulin and mucin domain-3; LAG-3: lymphocyte activation gene-3; BTLA: B and T lymphocyte attenuator; VISTA: V-domain immunoglobulin-containing suppressor of T-cell activation; CAR-T cell: chimeric antigen receptor T-cell; RCAS1: receptor binding cancer antigen expressed on SiSo cells; CLEVER-1: common lymphatic endothelial and vascular endothelial receptor-1