Immunotherapy in Colorectal Cancer

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Abstract

Colorectal cancer (CRC) remains one of the most common malignancies and the second leading cause of cancer-related death worldwide; treatment algorithms include surgery, chemotherapy and targeted therapies. Immunotherapy has recently emerged as an effective treatment approach in several types of cancer, including non–small cell lung cancer, melanoma and kidney cancer. In CRC, novel immune-checkpoint inhibitors such as anti-CTLA4 and PD1/PDL1 monoclonal antibodies have shown limited efficacy, although ongoing trials in mismatch repair-deficient CRC have shown significant and promising results. Here, we review the role of immune-microenvironment in colorectal cancer and current clinical data about therapeutic activity of immunotherapy in the treatment of CRC.

Keywords: colorectal cancer, immunotherapy, drug development, checkpoint inhibition, mismatch repair

1. Introduction

Colorectal cancer (CRC) is the fourth most common cancer and the second leading cause of cancer-related death worldwide. Surgery, chemotherapy, radiation therapy and targeted agents including anti-angiogenic and anti-epidermal growth factor receptor (EGFR) therapies form the backbone of treatment for CRC in various stages. Unfortunately, when diagnosed at advanced stage, CRC is still inevitably fatal. More than 50% of patients diagnosed with CRC eventually develop metastases, and almost 90% of these patients have unresectable disease [1–3]. In some patients with metastatic disease, metastectomy is still possible and can result in a cure in appropriately selected patients [2, 3]. The almost totality of metastatic CRC patients eventual-

ly develops resistance to all available standard therapies leading to cancer progression and death [4].

As we will discuss here, immunotherapy and immunomodulatory drugs may represent future therapeutic options to be included in the therapeutic armamentarium in the treatment of CRC. The importance of inflammation in CRC is partially supported by the evidence that patients with inflammatory bowel diseases, i.e., patients with ulcerative colitis and Crohn's disease are at increased risk for developing CRC [5]. It is assumed that chronic inflammation is a significant contributor to cancer development. This is supported by the fact that colon cancer risk increases with longer duration of colitis, greater anatomic extent of colitis, the concomitant presence of other inflammatory manifestations like primary sclerosing cholangitis [6] and the fact that certain drugs used to treat inflammation, such as 5-aminosalicylates and steroids, may prevent the development of CRC in this clinical setting [7]. It may be thus possible that by shaping the immune composition of the CRC microenvironment through novel immunotherapies, this may ultimately lead to a therapeutic effect in CRC.

2. The immune-cell microenvironment in colorectal cancer

An important step in tumour progression is the evasion and suppression of the host immune system [8, 9], as shown in **Figure 1**. In the normal microenvironment, the effector cells, including the natural killer (NK) cells and cytotoxic T lymphocytes (CTLs), are capable of driving potent anti-tumour suppressive activities. Tumour cells are often able to induce an
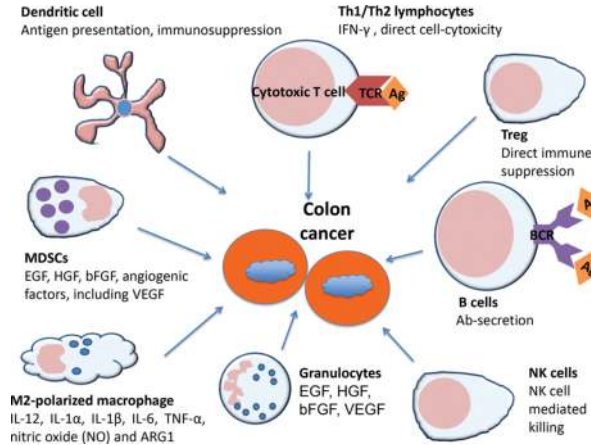
Figure 1. Immune-cell microenvironment in colorectal cancer. The evasion and suppression of the host immune system is an important step of colorectal cancer (CRC) progression. In physiologic conditions, effector cells, including the natural killer (NK) cells and cytotoxic T lymphocytes (CTLs), exert tumour surveillance and tumour suppressive activities. Tumour cells are able to induce an immunosuppressive microenvironment that protects them from the host immune system through the expansion of regulatory immune cells (i.e., myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Tregs)) and alternative activation of other immune cells, including macrophages, granulocytes and dendritic cells.

immunosuppressive microenvironment that protects them from the host immune system. Overall, tumour cells are able to shape the host microenvironment, which is rich of immune cell populations, in a suitable way for them to survive to the host immune system recognition [10, 11]. The two major immunosuppressive mechanisms in cancer are (1) expansion of regulatory immune cells (i.e., myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Tregs)) and (2) activation of the inhibitory T-cell pathways—programmed cell death-1/ programmed cell death-ligand 1 pathways (PD-1/PD-L1 pathways).

3. Myeloid-derived suppressor cells

Myeloid-derived suppressor cells are a heterogeneous and immature subset of circulating cells of myeloid derivation that can differentiate into, macrophages, granulocytes or dendritic cells (DCs) under physiologic conditions. However, under pathological conditions such as cancer or inflammation, the differentiation of these immature myeloid cells is inhibited resulting in accumulation of MDSCs in the tumour microenvironment or in the sites of inflammation [12]. For example, in cancer patients and tumour models, MDSCs accumulate in the tumour microenvironment because of the release of soluble factors by tumour cells or by other cells of the microenvironment, i.e., granulocyte macrophage colony stimulating factor (GM-CSF), interleukin-1 β and stromal-derived growth factor 1-α [13, 14]. MDSCs can then suppress T-cell proliferation through expression of several immune suppressive factors, including arginase, reactive oxygen species (ROS) and nitric oxide (NO). MDSCs can also promote the development of Treg cells *in vivo*, which are anergic and immune-suppressive [15]. Several studies have consistently shown that cancer patients with higher MDSC levels have shorter survival compared to patients with lower MDSC levels [16, 17]. Moreover, depletion of MDSCs in tumour-bearing mice using anti Gr-1 antibody [18, 19] or MDSC-targeting specific peptides have shown anti-tumour activities [20] suggesting that MDSCs can be a good target for future anti-tumour treatments. Two main subsets of MDSCs have been described, namely, granulo-cytic MDSC (G-MDSC) or polymorphonuclear (PMN)-MDSCs and monocytic MDSC (Mo-MDSC). G-MDSCs have granulocyte-like morphology characterised by increased levels of ROS and low levels of NO, whereas Mo-MDSCs have monocyte-like morphology with increased level of NO, but low levels of ROS. Human G-MDSCs and Mo-MDSCs are classically defined as $CD11b^+ CD33^+ HLA-DR^{-/low}CD14^-$ and $CD11b^+ CD33^+ HLA-DR^{-/low} CD14^+$, respectively. In tumour-bearing mice, G-MDSCs are the major MDSC subset that expands in the peripheral lymphoid organs after tumour engraftment pointing to a different biology of these cells in human and mice [21].

MDSCs promote metastasis development and primary tumour growth both in CRC patients and CRC murine models [22]. Importantly, MDSCs have also been implicated in the resistance to anti-angiogenic therapies used for the treatment CRC [23] via their ability to stimulate the expression of genes, whose products promote leukocyte recruitment, alternative angiogenic mechanisms, tumour migration, wound healing and formation of premetastatic niches in distal metastatic organs [23].

4. Regulatory T cells (Tregs)

Treg cells are a subset of CD4^{+} T lymphocytes characterised by the expression of Forkhead Box P3 (FOXP3) transcription factor [24]. Tregs are able to suppress the function of antigen presenting cells (APCs), i.e., dendritic cells, and effector T cells by direct contact or by release of anti-inflammatory cytokines (IL-10 and TGF-β). Tregs are major players in the development of tumour immunosuppressive microenvironment; these cells accumulate both in the tumour microenvironment and the peripheral blood of patients with cancer [25, 26]. The increased frequency of Tregs both in the peripheral blood and especially in the sites of tumour growth has generally been considered a marker of poor prognosis due to Treg-mediated suppression of anti-tumour immunity [27, 28]. In transgenic mouse models, it has been shown in mice that Treg depletion induces regression of solid tumours and lymphomas, following increased intratumoural accumulation of activated CD8^{+} cytotoxic T cells [29–31]. These data indicate that targeting Tregs can represent a potential anti-tumour strategy; however, the development of autoimmune diseases following administration of Treg cells has been described in these preclinical studies and may represent a limitation in the pursue of novel anti-Treg treatments in patients. In CRC, several studies have shown that Treg density in tumour specimens represents an independent negative prognostic factor [32–34]. Low-dose cyclophosphamide has been shown to reduce the numbers and function of Tregs and to induce anti-tumour, immune-mediated effects [35, 36]; this has been shown to be true in preclinical models of CRC [37], but no studies have been carried out in CRC patients so far.

5. Dendritic cells

Dendritic cells are cells of bone marrow origin defined as professional antigen presenting cells, which have the ability to present self and non-self antigens to T cells, thus promoting immunity or immune-tolerance [38]. Antigen presentation by DCs is able to induce naive T cells differentiation into effector and memory T cells; however, it can also lead to different forms of T-cell tolerance, depending on the local microenvironment stimuli and the functional status of the DCs. Myeloid-DCs (mDCs) and plasmacytoid-DCs (pDCs) are two major DC subsets that have been identified based on their origin, immune-phenotype and functional status [39]. In human, mDCs are usually defined as Lin^{-}HLADR^{+}CD11c^{+}CD123^{dim} cells, whereas pDCs are Lin^{-}CD11c^{-}CD4^{+}CD45RA^{+}CD123^{+}ILT3^{+}. Several studies have documented accumulation of DCs in tumour sites, which often correlated with poor prognosis [40–42]. The loss of tumour-derived antigen presentation ability by tumour-infiltrating DCs has been shown to be the consequence of the suppressive effects of the tumour microenvironment mediated by various cytokines [43]. For example, it has been demonstrated that tumour-infiltrating pDCs from solid tumours express high levels of inducible T-cell co-stimulator ligand (ICOS-L), which explains their ability to induce Tregs proliferation [44, 45], thus leading to local immunosuppression. Moreover, TGF-β secreted by DCs from breast cancer patients is able to induce Treg-cell proliferation and accumulation, thus leading to tumour growth [46]. The role of DCs in CRC has been controversial mostly due to the technical difficulties associated with their quantifi-

cation and identification. For these reasons, it is difficult to draw a conclusion about the role of DCs and performance of DCs as a predictor of outcome for CRC [47, 48].

6. Natural killer cells

NK cells represent a heterogeneous lymphocyte population with direct-cytotoxic anti-tumour capacity and multiple immunoregulatory properties. Natural killer group 2D (NKG2D) is one of the NK cell activating receptors that recognises various proteins expressed on the surface of target cells in response to several forms of cellular stress. One of the ligand of NKG2D is the MHC class I polypeptide-related sequence A (MICA); target tumour cells that express MICA are efficiently killed via NKG2D despite the expression of MHC class I molecules, describing a pathway of anti-tumour activity mediated by NK cells [49]. Several preclinical studies have shown the susceptibility of CRC cells to the NK cell–mediated killing [50–52], which can be enhanced by the contemporary treatment with anti-CRC drugs like anti-EGFR inhibitors [53].

Interestingly Gharagozloo et al. [54] have recently shown that metastatic CRC patients present a significant reduction in the percentage of circulating NKG2D$^+$NK cells as well as NKG2D mRNA expression in peripheral blood as compared to healthy controls, suggesting a specific defect of NK cell–mediated natural immunity in CRC patients.

7. Macrophage in colorectal cancer

Cells of the monocyte–macrophage lineage are one of the major components of the leukocyte infiltration in tumours; there is strong evidence that these cells promote inflammatory circuits that ultimately lead to tumour progression, tumour cell invasion and metastasis [55].

Macrophages recruited to the tumour-associated microenvironment may exist both in a classically activated inflammatory phenotypes (M1) with anti-tumour capacity or an alternatively activated, immunosuppressive (M2) phenotype with tumour supporting ability [56]; M1-polarised macrophage secretes a large amount of IL-12, IL-1α, IL-1β, IL-6, TNF-α, nitric oxide (NO) and ARG1, and stimulate secretion of IFN-γ by Th1 lymphocytes, thus activating Th1 immune response which in turn stimulate the tumour specific-CTL cytotoxicity. However, during tumour progression, macrophages shift towards a M2-polarised phenotype induced by the exposure of these cells to IL-4, IL-13, M-CSF/CSF-1, IL-10 and TGF-β1, among other factors present in the tumour microenvironment. In this state, macrophages are defined as tumour-associated macrophages (TAMs) and are able to support tumour growth, survival and metastasis. TAMs mostly derived by circulating monocyte which are recruited to the tumour bed by the secretion from tumour cells and the other cells of the tumour microenvironment of inflammatory cytokines such as M-CSF/CSF-1, SDF-1/CCL12 and MCP-1/CCL2. M2 macrophages then are able to secrete large amount of growth factors, such as EGF, HGF, bFGF, inflammatory factors (such as COX2) and angiogenic factors, including VEGF and angiogenic chemokines, which in turn all together promote progression of tumours (reviewed in [55, 57]).

In general, higher densities of TAMs in tumours and overexpression of key stimulators of M2 differentiation are considered markers of poor prognosis in a number of cancers [55, 57]. TAMs are associated with tumour progression and poor survival in CRC patients [58, 59], in line with *in vitro* and *in vivo* studies showing that macrophages are able to promote survival and induce proliferation of CRC cells via activation of Wnt pathway in CRC cells [60–62]. However, some other studies have shown that macrophages actually exert a tumour-suppressive activity in CRC via direct inhibition of tumour cell proliferation and via production of chemokines that attract T cells, stimulate proliferation of allogeneic T cells and activate type-1 T cells associated with anti-tumour immune responses [63]. In CRC, the role of macrophages may be ultimately context and stage dependent with implications for the design of future therapies aiming to target these cells.

8. Immune therapy in CRC

Given the complexity of the immune microenviroment and immune-cell composition of CRC, targeting this type of cancer via novel immunotherapies has been proved challenging. However, as described in the following paragraphs, recent advanced in the immunotherapy drug-development together with a better understanding of the genetic basis of immune stimulation have finally lead to the proof of concept demonstration that immunotherapy may represent an important therapeutic tool in the treatment of CRC.

9. Immune-cytokine therapy in CRC

Non-specific immunotherapy utilising cytokines such as interferon (IFN), interleukins and granulocyte macrophage colony-stimulating factor (GM-CSF) have been studied because of the potential ability to modulate and promote host immunity against tumour antigens. A Phase II trial of 29 patients with metastatic CRC using gemcitabine, oxaliplatin and 5-fluorouracil (GOLF) in combination with IL-2 and GM-CSF immune adjuvant regimen (GOLFIG) yielded promising results, with an overall response rate of 56.5%, disease control rate of 96% and median time to progression of 12.5 months [64]. A Phase III study comparing the GOLFIG regimen against the control arm of FOLFOX-4 in first line treatment of metastatic CRC was terminated early due to poor recruitment into the control arm. However, the experimental arm did show superiority in Progression Free Survival and Overall Response Rate with a trend towards improvement of overall survival; this trial does provide proof-of-concept that GOLFIG chemoimmunotherapy may represent a novel reliable option for first-line treatment of metastatic CRC [65].

10. Vaccines as therapeutic tools in CRC

Vaccine-based therapy can be delivered as whole-tumour-cell vaccines, peptide vaccines, viral vector vaccines or dendritic call vaccines, each with its inherent advantages and disadvantages

(reviewed in [66, 67]). Overall in the treatment of CRC, there have been only small Phase I and Phase II studies with suggestions that vaccines may have a role in the adjuvant setting, and limited efficacy in metastatic disease. [68].

11. Rationale of checkpoint receptor pathway as a target in colorectal cancer

Immune checkpoints refer to a very complex and articulated series of inhibitory pathways that intricate into the immune system and that are crucial for regulating self-tolerance and modulating the duration and extent of physiological immune responses in peripheral tissues in order to avoid excessive immune-activation and subsequent collateral tissue damage (**Figure 2**) [69]. It is now well established that tumour cells can co-opt certain immune-checkpoint pathways; this represents a novel and important mechanism of immune resistance, particularly against T cells that are specific for tumour antigens. Consequently, the blockade of immune checkpoints is able to unleash T-cell–mediated anti-tumour immune response in a potent and sometime curative way [69].
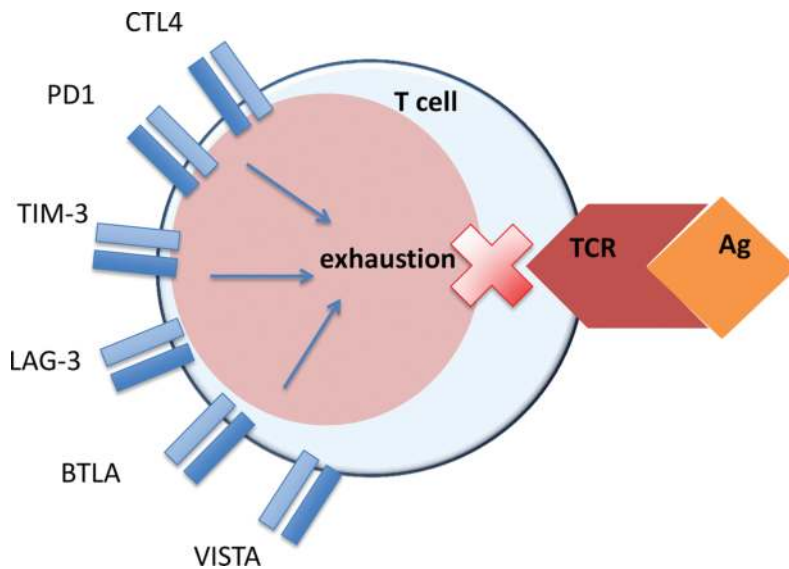
Figure 2. Immune checkpoint and immunosuppression in CRC. Immune checkpoints activate inhibitory pathways in T cell that ultimately lead to T-cell–mediated immunity suppression. Tumour cells can co-opt these immune-checkpoint pathways thus leading to T-cell exhaustion and tumour immunotolerance.CTLA4: cytotoxic T-lymphocyte-associated antigen 4; PD1: programmed cell death protein 1; TIM3: T-cell membrane protein 3; LAG3: lymphocyte activation gene 3; BTLA: B- and T-lymphocyte attenuator; VISTA: V-domain Ig suppressor of T-cell activation.

The two immune-checkpoint receptors that have been most studied in the context of clinical cancer immunotherapy, cytotoxic T-lymphocyte-associated antigen 4 (CTLA4; also known as CD152) and programmed cell death protein 1 (PD1; also known as CD279) are both inhibitory

receptors and have both shown to be appropriate targets (**Figure 2**). Importantly, several other checkpoint immune pathways have recently emerged to be additional targets for the development of new immunotherapy drugs mostly in preclinical studies (**Figure 2**); these include lymphocyte activation gene 3 (LAG3; also known as CD223), 2B4 (also known as CD244), B- and T-lymphocyte attenuator (BTLA; also known as CD272), T-cell membrane protein 3 (TIM3; also known as HAVcr2), adenosine A2a receptor (A2aR) to name a few [70].

Programmed cell death 1 is a Type I transmembrane protein, which belongs to the CD28 family [71]. PD-1 is expressed on activated and exhausted T and B cells and has two ligands PD-L1 and PD-L2. Importantly, PD-L1 is not expressed on normal epithelial tissues, but can aberrantly be expressed on a variety of solid tumours [72]. On the other hand, PD-L2 is more broadly expressed on normal healthy tissues. Binding of PD-L1 to PD-1 reduces cytokine production and activation of the target T cells, leading to an immunosuppressive microenvironment.

Clinical trials targeting PD-1/PD-L1 pathway to overcome tumour-associated immune suppression have shown promising results for a variety of solid tumours. Checkpoint inhibitor immunotherapy is currently FDA-approved for the treatment of melanoma, kidney cancer and NSCLC. However, it has been shown active in many other types of solid, including gastric, ovarian cancer, and bladder cancer, and hematologic cancers, particularly Hodgkin lymphoma [73–76]. It is currently unclear what determines response to this type of treatment and this is an area of active research giving the costs and the potential toxicity associated with these treatments.

The accumulation of somatic mutations accompanies the initiation and progression of most cancers conferring to the tumour cells unrestricted proliferative capacity [77]. The analysis of cancer genomes has revealed that tumour mutational landscapes [78] are extremely variable among patients, among different tumours from the same patient and even among the different regions of a single tumour. Two separate papers have recently shown that response to checkpoint inhibitors, i.e., anti-CTL4 and anti-PDL1 Ab, critically depend on the mutational load of the specific tumours. The first study by Snyder et al. [79] found that mutational load associates with exceptional response to the anti-CTLA-4 Ab ipilimumab in melanoma patients. Using genome-wide somatic neoepitope analysis and patient-specific HLA-typing, they identified candidate tumour neoantigens for each patient predicted to be able to activate a T-cell response in anti-CTLA-4 treated patients.

Interestingly, the probability for a tumour to carry such neoantigens was dependent on the mutational load of the specific tumour, as it was the probability to respond to anti-CTLA-4 Ab. Similar results were obtained in NSCLC patients treated with pembrolizumab, an antibody-targeting PD-1 [80]. A higher non-synonymous mutation burden in tumours was associated with improved objective response, durable clinical benefit and progression-free survival. Therapeutic benefit in these patients correlated with the molecular smoking signature, higher neoantigen burden and DNA repair pathway mutations. All these factors were associated with increased mutation burden [80].

Both studies suggest for the first time a genomic-based mechanism to the response to novel immunotherapy drugs that can potentially help with designing rational combination treatments, i.e., DNA-damaging agents plus immune-checkpoint inhibitors.

12. PD1/PDL1—immune-checkpoint inhibitors

In unselected colon cancer, the response to immune-checkpoint inhibitors has shown limited efficacy [73]. In tumours that have shown response, predictive markers to checkpoint inhibition are being evaluated—with microsatellite insufficiency (MSI) or mismatch-repair (MMR) status being the most promising thus far [81].

Pembrolizumab (MK-3475)—a highly selective humanised IgG4 monoclonal antibody that blocks the interaction of PD-1 with its ligands PD-L1 and PD-L2—has undergone extensive testing in multiple tumour types. In the KEYNOTE-028 study—a multicohort, Phase Ib trial of pembrolizumab for programmed death-ligand 1 (PD-L1) positive advanced solid tumours; there were 156 screened patients with advanced colorectal cancer, with 33 (21%) of these being PD-L1 positive and 23 went on to receive treatment. Although the safety profile was acceptable with only one patient experiencing a grade ≥3 treatment-related adverse events with elevated bilirubin; it was felt there was overall minimal anti-tumour activity. One patient who had microsatellite instability high disease experienced a partial response, with four patients (17%) having the best response of stable disease, and progressive disease in 16 patients (70%) [82].

The initial Phase I study of anti-PD-1 antibody nivolumab included 17 colorectal patients, who were heavily pre-treated; the majority of these patients had PD-L1 negative tumours and thus overall, this study showed limited clinical efficacy [83]. However, one patient with colorectal cancer treated with five doses in this study experienced a complete response at 6 months, which was ongoing after 3 years; it was noted that the patient's tumour was MSI-high, and evidence of PD-L1 expression by infiltrating macrophages and lymphocytes [84].

Based on the previous reports associating mutational load to response to checkpoint inhibitors, Le et al. hypothesised that mismatch repair–deficient tumours and mismatch repair (MMR)–deficient tumours are more responsive to PD-1 blockade than are MMR-proficient tumours. A Phase II study of 41 patients evaluating the clinical activity of pembrolizumab in metastatic carcinoma with or without MMR-deficiency showed hazard ratios for disease progression or death (0.10; 95% CI, 0.03–0.37; $P < 0.001$) and for death (0.22; 95% CI, 0.05–1.00; $P = 0.05$) that favoured patients with mismatch repair-deficient colorectal cancer [85]. Thus, ongoing studies are exploring this particular subset.

The KEYNOTE-164 study (NCT02460198) is a Phase II study currently recruiting patients with previously treated locally advanced unresectable or metastatic mismatched repair-deficient or MSI-high colorectal carcinoma to assess efficacy of pembrolizumab monotherapy [85]. In the same patient population of MSI-high colorectal cancers, the Phase III KEYNOTE-177 (NCT02563002) study will compare pembrolizumab monotherapy against standard of care chemotherapy in first line treatment of advanced CRC [86].

Regarding anti-PD-L1 compounds, atezolizumab (MPDL3280A) has shown activity in Phase I studies—with one of four patients with colorectal cancer having a durable partial response [87]. In the Phase Ib study of atezolizumab in combination with bevacizumab in refractory metastatic CRC, and that of atezolizumab and bevacizumab with FOLFOX in the oxaliplatin naïve population, this confirmed acceptable safety and clinical activity—unconfirmed ORR 8% (1/13) and 44% (8/18) in the two arms respectively [88]. However, the Phase I study of BMS936559, which included 18 colorectal patients showed no response in this tumour type [89]. There are ongoing studies with other anti-PD-L1 compounds including durvalumab/ MEDI4736 (NCT01693562) and avelumab (NCT01772004).

13. Anti-CTLA4 Therapy

Tremilimumab, a fully human immunoglobulin (Ig) G2 monoclonal antibody that blocks inhibitory signalling from CTLA4 was studied as monotherapy treatment in a Phase II single arm study, of 47 patients with refractory metastatic CRC. Tremelimumab was intended to be administered every 90 days. Clinical activity was unable to be demonstrated, with 43 of 45 evaluable patients unable to receive a second dose—with a median duration on study of 2.3 months [90]. However, a Phase I combination study of tremelimumab with durvalumab (NCT01975831) is ongoing; and ipilimumab is also being studied in combination with nivolumab (NCT02060188).

14. Other immune-checkpoint inhibitors

In MSI-high colon cancers, it has been shown that up-regulation of PD-1, PD-L1, CTLA-4, LAG-3 and IDO immune checkpoints enables evasion from Th1 response [91]. As described, PD-L1, PD-1, CTLA-4 have been and are being investigated in the treatment of CRC. Anti-LAG-3 monoclonal antibodies (BMS-986016), alone and in combination with nivolumab are also being evaluated (NCT01968109).

15. Other combined immunotherapy strategies

As there has been limited efficacy from current immunotherapy strategies, it has been proposed that combination of immunotherapy with conventional chemotherapy, radiotherapy and targeted agents should be trialled [92]. The use of DNA damaging agents may increase the mutation burden, thus increase the efficacy of checkpoint inhibition.

16. Conclusion

Advanced CRC remains inevitably lethal despite optimal management, thus novel therapeutic approaches are urgently needed. Immunotherapy, particularly novel immune-checkpoint

inhibitors, is transforming the therapeutic landscape of many types of cancer. Although in CRC the clinical data have been disappointing so far, this is probably due to the lack of knowledge of biomarkers/clinical features that can allow us the optimal selection of patients likely to respond to the specific immunotherapies. This has been proved by the identification of MMR status as a specific marker of response to anti-PD1/PDL1 treatment in CRC. In the future, a deeper understanding of immunobiology of CRC together with the development of novel immunotherapeutic agents will surely lead to new successful treatments for advanced CRC patients. This will be followed by further studies of combination of novel immunotherapies together with the present standard of care, i.e., surgery, chemotherapy and target therapies that will additionally improve the prognosis of advanced metastatic CRC.

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