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REVIEW

Standard therapies: solutions for improving therapeutic effects of immune checkpoint inhibitors on colorectal cancer

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ABSTRACT

Immunotherapy using immune checkpoint inhibitors has opened a new era for cancer management. In colorectal cancer, patients with a phenotype of deficient mismatch repair or high microsatellite instability benefit from immunotherapy. However, the response of rest cases to immunotherapy alone is still poor. Nevertheless, preclinical data have revealed that either ionizing irradiation or chemotherapy can improve the tumoral immune milieu, because these approaches can induce immunogenic cell death among cancer cells. In this regard, combination use of standard therapy plus immunotherapy should be feasible. In this review, we will introduce the specific roles of standard therapies, including radiotherapy, chemotherapy, antiangiogenic and anti-EGFR therapy, in improving therapeutic effect of immune checkpoint inhibitors on colorectal cancer.

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1. Introduction

Colorectal cancer (CRC) is one of the top three most common cancers in the world. In China, both the incidence and mortality of CRC have been increasing in recent years. More medical sources have been paid to CRC patients, and a 5-year survival rate of 57% was reported in 2015.[1] Nowadays, immune checkpoint inhibitors (ICIs) targeting programmed cell death protein-1 (PD-1), programmed cell death-ligand 1 (PD-L1) or cytotoxic T-lymphocyte associated protein-4 (CTLA-4) have become hot topics in the field of cancer treatment. In CRC, the cases having deficient mismatch repair (dMMR) or high microsatellite instability (MSI-H) can directly benefit from ICI therapy,[2] but they only account for a small portion among all CRC cases. The rest cases with proficient MMR (pMMR), low microsatellite instability (MSI-L) or microsatellite-stable (MSS) phenotypes barely respond to ICI therapy.[3]

In addition to dMMR or MSI-H, an ideal paradigm indicates that if tumors have massive tumoricidal T cells and high PD-L1 expression, they will shrink in response to anti-PD-1 therapy.[4] However, for patients without obvious infiltration of tumoricidal T lymphocytes, the immune milieu should be improved. Can the standard therapies for CRC become candidates in this process? The current clinical practice guidelines recommend chemotherapy plus antiangiogenic therapy for treating metastatic CRC with *RAS* or $BRAF^{V600E}$ mutation.[5,6] Herein, antiangiogenic therapy enables vascular normalization to facilitate T cell infiltration into tumors, thus potentially synergizing with ICIs to control tumor progression.[7] In addition, radiotherapy, such as stereotactic body radiotherapy (SBRT), is apt to increase the production of tumor-associated antigens (TAA) and IFN-γ in tumor microenvironment (TME) along with upregulating the expression of PD-1 by T cells or major histocompatibility complex class-I (MHC-I) and PD-L1 by tumor cells.[8,9] In this regard, some of the standard therapies have exhibited their potential in improving tumoral immune milieu, thus providing a platform for combination with ICI drugs.

In this review, we will introduce the immunosuppressive profile in tumors. Then, we will discuss the impacts of standard therapies on the host immune milieu. We hope that some opinions will shed new light on the combination use of standard therapies with ICIs in CRC treatment.

2. The suppressive immune microenvironment in tumors

In fact, it is observed that cancer patients at the same TNM stage differ in their prognosis.[10] The heterogeneity of cancer indeed is widely accepted, especially as the profiles of genetic mutations have been revealed among cancers, such as CRC,[11] gastric cancer[12] or lung cancer.[13,14] Intrinsically, such mutations resist such processes as immune surveillance, recognition and clearance. In this case, although immune cells continue their migration into tumors, 'cancer immunoediting' enables tumoricidal processes to be weakened or even deprived.[4] Herein, the TME is dangerous in that it is able to thwart tumoricidal activities of tumor infiltrating lymphocytes (TIL) by inducing them to express molecules, such as PD-1 and CTLA-4.[15] Herein, interaction between PD-1 of T cells and PD-L1 of tumor cells can impair T cell survival and tumoricidal

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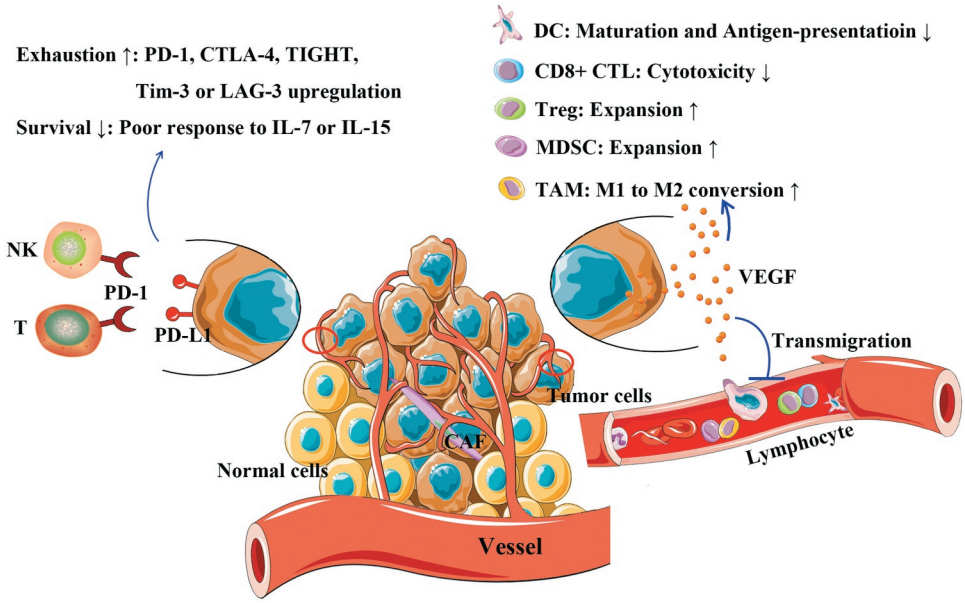

Figure 1. Construction of immunosuppressive milieu by cancer cells. PD-L1 and VEGF expressed by cancer cells are critical in constructing the immunosuppressive milieu in tumors. Herein, PD-L1 is able to elicit T cell exhaustion, thus enabling them to be with poor response to IL-7 and IL-15 stimulations along with upregulating their expressions of PD-1, CTLA-4, TIGHT, LAG4 and Tim3. VEGF is potent in increasing interstitial pressure within the tumor by promoting angiogenesis. Moreover, VEGF is able to reverse the tumoricidal functions of immune cells, such as dendritic cell (DC), cytotoxic T lymphocyte (CTL) and tumor-associated macrophage (TAM), while promotes expansion of regulatory T cell (Treg) and myeloid-derived suppressive cell (MDSC). CAF: cancer-associated fibroblast; PD-1: programmed cell death-1; PD-L1: programmed cell death-ligand 1.

Table 1. Immunosuppresive cytokines and their cellular sources.

	Treg	B	NK	DC	TAM	MDSC
TGF-β	+	-	-	+	+	+
IDO	-	-	-	+	-	+
IL-10	+	+	+	+	+	+
PD-L1	-	+	-	+	+	+

TGF-β: transforming growth factor-beta; IDO: indoleamine 2,3-dioxygenase; IL-10: interleukin- 10; PD-L1: programmed cell death-ligand 1; Treg: regulatory T cell; NK: natural killer; DC: dendritic cells; TAM: tumor-associated macrophages; MDSC: myeloid-derived suppressive cell

function.[15] Meanwhile, CTLA-4, a CD28 homologue but exhibiting higher affinities to CD80 and CD86 than CD28 molecule,[16] will antagonize the proliferation and activation of tumoricidal T cells if binding with co-stimulatory molecules,[15] a process of CTLA-4-induced dephosphorylation of T cell receptor (TCR) complex zeta chain.[17] Moreover, some cytokines contribute to immune suppression in TME as well (Figure 1). For example, vascular endothelial growth factor (VEGF) is one of the chief criminals (Figure 1). This cytokine exhibits potency in limiting the maturation and antigen-presenting functions of dendritic cells (DC) along with reducing the cytotoxic activity of $CD8^+$ T cells.[7] Meanwhile, VEGF promotes the expansion of Treg cells and myeloid-derived suppressive cells (MDSC) and the phenotypic conversion of tumor-associated macrophages (TAM) from M1 to M2.[7] In line with these findings, other factors including angiogenin-2, indoleamine 2,3-dioxygenase (IDO), transforming growth factor-beta (TGF-β), prostaglandin E2 (PGE2), IL-6 and IL-10, along with some chemokines, also facilitate immunosuppressive events[7] (Table 1). In addition, these factors enable systemic immunity to be facilitated by the tumor by increasing the frequencies of suppressor cells (e.g., MDSCs or Treg cells) while decreasing the frequencies of effector cells (e.g., cytotoxic T or Th1) in the periphery.[7] In this process, the normal function of T cells will be equally challenged. In this case, exhaustion of T cells is the most critical event. Physically, T cells will become exhausted if undergoing persistent antigen exposure. Factors such as the inhibitory ligands of antigen-presenting cells or tumor cells and priming by PGE2, IL-10 or TGF-β also account for T cell exhaustion[18] (Table 1). In fact, exhausted T cells distinguish themselves from the subsets of memory or effector T cells in several aspects, including progressive loss of effector functions, abnormal responsiveness to the homeostatic cytokines (e.g., IL-7 or IL-15), sustained inhibitory receptor expression (e.g., PD-1, CTLA-4, T cell immunoreceptor with Ig and ITIM domains [TIGIT], T cell immunoglobulin and mucin domain containing molecule-3 [Tim-3] or lymphocyte-activation gene-3 [LAG-3]), metabolic alteration in terms of glycolysis and expression of exclusive transcriptional programs directing cell differentiation[18] (Figure 1). In another route, tumor cells can secrete exosomes containing PD-L1 to manipulate distant T cell survival and function, which has been successfully tested in an experimental model of melanoma.[19] However, a recent study revealed that melanoma patients with high ratios of peripheral $PD\text{-}1^+CD8^+$ T cells to tumor burden exhibited better responses to anti-PD -1 therapy than those who did not have a high ratio.[20] Several newly published studies have identified that the cells that respond to anti-PD-1 therapy are pre-exhausted T cells in the periphery[21,22] rather than exhausted T cells pre-existing in tumors.[22,23] However, there are still several approaches allowing for tumor escape from immune cell attack. For example, tumor cells can camouflage themselves by expressing CD47 on their surfaces, thus protecting against phagocytosis by macrophages.[24] Meanwhile, tumors will attract several facilitators (e.g., MDSCs, neutrophils, Treg cells or M2-like

macrophages) and provide them with a context that induces them to reciprocally activate tumor cells. Together with this stroma maintenance, tumor cells establish a defensive network against immune cell attack (see details in *Ref.*[25]).

3. dMMR/MSI-H and ICI therapeutic responsiveness

Although various factors contribute to the immune suppression in TME, a portion of CRC cases are revealed to be inherently immune-privileged, presenting dMMR or MSI-H phenotypes in tumors.[11] As aforementioned, metastatic CRC cases with dMMR/MSI-H exhibit well responsiveness to ICI therapy.[2] As we know, microsatellites are regarded as short tandem repeats or simple sequence repeats, which consist of repeated sequences of 1 ~ 6 nucleotides in genome.[26] Normally, mismatch repair-associated proteins including MLH1, MSH2, MSH6 and PMS2 can protect against gene mutations.[27] However, deficiencies in mismatch repair-associated protein expressions can elicit MSI-H phenotypes, thus leading to excessive production of neoantigens associated with mutated genes.[27] If this is the case in CRC, tumoricidal lymphocytes will be attracted to TME after presentation of TAAs by DCs.[28] In fact, sustained exposure to foreign antigens serves as a route in eliciting T cell exhaustion.[18] In this context, ICI drugs can assist in protecting against this biological event, thus causing shrinkage of CRC tumors.[3] But from currently available data, it still can be found that not all of dMMR/MSI-H tumors can respond to ICI therapy.[3] Herein, activation of "Wingless/Integrated (Wnt)" signaling pathway has been reported to inhibit tumoricidal T cell infiltration into CRC tumors.[29] This case can be translated into dMMR/MSI-H tumors as well.[29] Meanwhile, it has been revealed that CRC cases with Wnt activation and pMMR/MSS phenotypes in tumors account for a large portion of all CRC cases,[11] but this does not mean that T infiltrates are inherently lacking in such tumors because CRC cases generally decline the amounts of cytotoxic and memory T cells in tumors as TNM stages increase.[30] Probably, this serves as a reason why metastatic CRC with pMMR/MSS phenotypes respond poorly to ICI therapy, although exact mechanisms remain elusive. In general, the amount of cytotoxic T plus memory T cells in the tumor is positively associated with CRC prognosis.[10] Moreover, such T infiltrates serve as main targets of ICI therapy.[3] In this situation, it is urgent to find a right approach to increase T infiltrates in advanced and metastatic CRC cases with pMMR/MSS phenotypes, thus enabling them to benefit from ICI therapy.

4. Standard therapy

Radiotherapy, chemotherapy or molecule-targeted therapy remain the standard of care for advanced or metastatic CRC cases. However, some cases will actually progress into refractory disease of metastases after multiple lines of therapy. At present, best supportive care can be selected as a resolution for these patients, but its low effectiveness commonly fails us. What could be upfronted after the later-line therapy? Combinational use of standard therapy plus immunotherapy appears to be feasible in this context.

4.1. Chemoradiotherapy

Radiotherapy plus concomitant 5-Fluorouracil (5-FU)/capecitabine remains the standard of care for local advanced rectal cancer (LARC) patients and serves as a neoadjuvant or adjuvant for enhancing the local-regional control rate of primary tumors. Herein, oxidative stress is one of the tumoricidal effects exerted by ionizing radiation. However, factors including nuclear factor kappa-B (NF-κB) activation,[31] ARG-1$^+$ MDSC infiltration,[32] and overexpression of the chemokine (C-X-C motif) ligand 12 (CXCL12) along with its receptors chemokine (C-X-C motif) receptor 4 (CXCR4) and chemokine (C-X-C motif) receptor 7 (CXCR7) in primary tumors have been revealed to be positively associated with poor responses to neoadjuvant chemoradiotherapy (nCRT), thus resulting in the poor clinical outcomes of patients.[33–35] As documented, 1.8 Gy ~ 2 Gy, the conventional fraction doses of radiotherapy, are sufficient for causing cell death among mature T cells in humans.[36] Providing that distant lymphocytes are recruited into tumors, most of them will undergo apoptosis during fractionated irradiation. But in fact, conventional radiotherapy alters tumoral immune profiles by inducing chemokine and cytokine upregulation, which can recruit the required immune cells into TME. For example, conventional radiotherapy is able to induce CD68$^+$ TAMs to increase the activity of thymidine phosphorylase, which upregulates monocyte chemotactic protein-1 (MCP-1) production by TAMs, thus recruiting circulating monocytes into rectal tumors.[37] Likewise, tumoral CXCL12 is able to recruit CXCR4$^+$ myeloid cells.[38] Innate immune cells attract adaptive immune cells into tumors by secreting chemoattractants. However, this is a comprehensive network that should not be limited to a certain cytokine (see details in *Ref.*[39]). In another approach, ionizing radiation is a powerful tool for inducing immunogenic cell death (ICD).[40] In this context, DCs will migrate into peripheral lymph nodes to present TAAs to T cells, while such T cells will be recruited into tumors to perform their functions.[40] In the clinic, several retrospective studies have revealed that LARC tumors with a high density of CD3$^+$ or CD8$^+$ T cells[41–46] or with a low density of Treg cells responded well to nCRT.[43,45] Moreover, nCRT has been shown to increase tumoral infiltration of CD8$^+$ T cells[42,43,45] but not Treg cells.[43,45] Based on these results, several trials were designed to test the efficacy of nCRT plus ICI drugs in LARC (Table 2). The combination is expected to increase tumor remission, thus enabling the downstaging or the pathological complete response (pCR) of the primary tumor to be more efficient. In fact, pCR surely occurs in a certain portion of LARC patients after nCRT alone.[6] Herein, a phase II trial (VOLTAGE; NCT02948348) reported that after adding five cycles of nivolumab (an anti-PD-1 mAb) before surgery, the pCR rate was 30%,[47] which is higher than that seen when using nCRT alone, which approximates the situation of nCRT followed by multiple lines of chemotherapy.[48] In line with this, another phase II study (NSABP FR-2; NCT03102047) was designed to add Durvalumab (an anti PD-L1 mAb) during the period before surgery.[47] At the very least, such strategies are encouraging for enabling a certain portion of LARC patients to avoid surgery,

Table 2. Advances in combinational use of radiotherapy plus ICI therapy in CRC.

Trial number	Phase	Enrolled patients	Radiotherapy (Dose)	ICI drugs	Treatment schedule
NCT02837263	I	CRC liver metastases	SBRT (40–60 Gy/5 fractions)	Pembrolizumab	RT+neo ICI+Surgery+ sequential ICI
NCT02437071	II	mCRC	Palliative RT dose in small-sized fractions	Pembrolizumab	RT+ sequential ICI
NCT04109755	II	MSS-LARC	SCRT (5 Gy × 5 fractions)	Pembrolizumab	Neo SCRT+ neo ICI+Surgery
NCT02586610	II	LARC	Standard CRT (50.4 Gy/28 fractions)	Pembrolizumab	Neo CRT plus concurrent ICI + Surgery
NCT02921256	II	LARC	Standard CRT (50.4 Gy/28 fractions)	Pembrolizumab	Induction CT+nCRT+ concurrent ICI + sequential ICI
NCT04030260	II	MSS/pMMR mCRC	NM	Nivolumab	RT + sequential ICI plus Regorafenib
NCT03507699	I	CRC liver metastases	SBRT (21 Gy/3 fractions)	Nivolumab + Ipilimumab	RT + sequential duplet ICI
NCT03104439	II	MSS-CRC	SBRT (8 Gy × 3 fractions)	Nivolumab + Ipilimumab	RT + sequential duplet ICI
NCT03921684	II	LARC	Standard CRT (50.4 Gy/28 fractions)	Nivolumab	Neo CRT + neo ICI plus neo CT + Surgery
NCT02948348	Ib/II	LARC	Standard CRT (50.4 Gy/28 fractions)	Nivolumab	Neo CRT + neo ICI + Surgery
NCT02888743	II	mCRC	High single dose in 3 fractions or Low single dose in hyperfractions	Durvalumab +Tremelimumab	Induction duplet ICI + RT+ sequential duplet ICI
NCT03122509	II	mCRC	NM	Durvalumab + Tremelimumab	Induction duplet ICI + RT + sequential duplet ICI
NCT03007407	II	MSS-mCRC	SBRT (Total dose in 3 fractions)	Durvalumab + Tremelimumab	RT + sequential duplet ICI
NCT03802747	I	MSS-CRC liver metastases	SBRT (/)	Durvalumab + Tremelimumab	Induction Durvalumab RT + sequential duplet ICI
NCT04083365	II	LARC	Standard CRT (50.4 Gy/28 fractions)	Durvalumab	Neo CRT + neo ICI + Surgery
NCT03102047	II	MSS-rectal cancer (Stage II–IV)	Standard CRT (50.4 Gy/28 fractions)	Durvalumab	Neo CRT + neo ICI + Surgery
NCT03101475	II	CRC liver metastases	SBRT (10 Gy × 3 fractions)	Durvalumab + Tremelimumab	Induction duplet ICI + RT + sequential duplet ICI
NCT03854799	II	LARC	Standard CRT (50.4 Gy/28 fractions)	Avelumab	Neo CRT plus concurrent ICI + neo ICI + Surgery
NCT03299660	II	LARC	Standard CRT (50.4 Gy/28 fractions)	Avelumab	Neo CRT + neo ICI + Surgery
NCT04017455	II	LARC	SCRT (5 Gy × 5 fractions)	Atezolizumab	Neo SCRT + Bevacizumab + concurrent Bevacizumab plus ICI + neo ICI + Surgery
NCT03127007	Ib/II	LARC	Standard CRT (45–50 Gy/25 fractions)	Atezolizumab	Neo CRT plus concurrent ICI + neo ICI + Surgery

ICI: immune checkpoint inhibitor; CRC: colorectal cancer; mCRC: metastatic colorectal cancer; SBRT: stereotactic body radiotherapy; SCRT: short-course radiotherapy; RT: radiotherapy; Neo: neoadjuvant; CT: chemotherapy; NM: not mentioned; MSS: microsatellite-stable; LARC: local advanced rectal cancer; CRT: chemoradiotherapy; nCRT: neoadjuvant chemoradiotherapy; pMMR: proficient mismatch repair

although evidence suggesting that patient prognosis will be improved is lacking at present. As for the rationale behind such strategies, it should first be mentioned that PD-L1 expression can indeed be shared by multiple organs, such as normal mucosa,[49] tumor cells[49] and immune cells.[50] Among a portion of LARC patients, it has been confirmed that nCRT is able to upregulate PD-L1 expression either by tumor cells or by stromal immune infiltrates.[49,50] In fact, the expression of PD-L1 is associated with the increased expression of IFN-γ after nCRT.[49] Typically, $CD8^+$ T and Th1 cells can produce IFN-γ, which serves as a strong inducer of PD-L1 expression by target cells.[19] In terms of PD-L1 expression and the density of immune infiltrates, another study revealed that a high level of PD-L1 was related to increased $CD8^+$ T cell infiltration in tumors before and after nCRT.[50] In addition, favorable clinical outcomes were achieved among LARC patients with a high tumoral density of $CD8^+$ T cells after nCRT.[46] Moreover, before nCRT, high PD-L1 expression by immune infiltrates can predict significant improvement of the disease-free survival (DFS) of LARC patients.[50] Tumors with a high density of $CD8^+$ T cells commonly have a favorable prognosis.[10] In this regard, anti-PD-(L)1 therapy should be able to compensate for nCRT to overcome $CD8^+$ T cell exhaustion.

4.2. SBRT

In contrast to conventional radiotherapy, SBRT has exhibited potential in combination with ICI therapy because a growing body of evidence suggests that SBRT has advantages over conventional radiotherapy in several aspects, including vascular normalization,[51] tumor cell lysis,[52] and sequential immune activation.[53] All of these aspects serve as the hallmark effects of SBRT. First, in contrast to the does needed for conventional radiotherapy, a single dose of 8 ~ 10 Gy can cause vascular dysfunction by increasing the activity of acid sphingomyelinase, which converts sphingomyelin into ceramide to induce endothelial apoptosis.[51] In addition, cancer stem cells (CSC) are considered critical components driving resistance to conventional radiotherapy[54] because CSCs manipulate the TME to instruct tumor responses to conform with the TME requirements in 'health and disease'.[39,55] Nevertheless, SBRT serves as a potential route to block the reciprocal interaction between CSCs and TME substrates[54] because SBRT can boost the equivalent biological dose to induce effective ICD among CSCs.[53] Due to the oncolytic effect, rapid release of TAAs potentially activates tumoricidal lymphocytes, probably leading to the regression of distant lesions, which share similar TAA profiles. Mechanistically, SBRT-induced increases in tumoral immunogenicity lead to the biological processes of $CD8^+$ T cell activation, DC maturation and antigen presentation, IFN-γ upregulation, type 1 IFN responses and MHC-I upregulation by tumor cells.[56–58] However, PD-L1 upregulation by tumor cells and PD-1 upregulation by $CD8^+$ T cells also occur.[9] To overcome PD-L1-induced T cell exhaustion, the strategy of SBRT plus ICI therapy was designed in preclinical models, and it has been translated into clinical trials for

patients with metastatic tumors to test the feasibility of this strategy.[53,58,59] In current clinical practice, SBRT has been recommended for treating CRC liver[5] and lung metastatic lesions.[60] Moreover, several trials using this combination mode for treating the metastases of refractory CRC are ongoing (Table 2). However, it is well known that if TCRs are specific for targetable cancer cell clones, such T cell subpopulations are impotent for killing other subclone cells that lack the same antigens. Therefore, a new opinion holds that SBRT should focus on all metastatic lesions to abandon its probable abscopal effect because TAA heterogenicity exists among different cancer cell subclones.[8]

4.3. Chemotherapeutic agents

Oxaliplatin serves as an ICD inducer of CRC cells.[61] After ICD, the anticancerous functions of DCs and cytotoxic T cells will be activated.[62] Mechanistically, it was found that oxaliplatin could increase the serum levels of fms-like tyrosine kinase 3 ligand (Flt3 L), which serves as an indicator of the activation of tumor antigen-presenting DCs.[63] In a preclinical model, oxaliplatin was shown to upregulate PD-L1 expression by tumoral immune cells, thus prolonging the survival of CRC-bearing mice when combined with anti-PD-L1 therapy.[64] For 5-FU, the death of MDSCs is a valid effect exerted by this agent, whereas evidence suggesting an immune-supportive role of irinotecan has seldom been reported.[62] One observation is that the numbers of $CD3^+CD4^+$ and $CD8^+CD28^+$ cells in the peripheral blood of metastatic CRC patients increase after irinotecan intervention.[65] When combining 5-FU with oxaliplatin or irinotecan, a retrospective study reported that the percent of peripheral Treg cells was decreased in the periphery.[66] Due to the immunogenic properties of CRC chemotherapy, a phase Ib/II trial has been designed to investigate the safety and efficacy of chemotherapy plus durvalumab and tremelimumab (an anti-CTLA-4 antibody) in metastatic cases with an MSS phenotype (NCT03202758)[67] (Table 3). In this study, *RAS* mutation cases were also recruited. Herein, *RAS* mutations can elicit cell autophagy, which becomes a route of chemoresistance.[68] However, a phase III trial (IMblaze370) reported that atezolizumab (an anti-PD-L1 mAb) plus a RAF-mitogen activated protein kinase (MEK) inhibitor failed to improve the clinical outcomes of *RAS*-mutated metastatic CRC patients.[69] Therefore, will *RAS* mutations influence the efficacy of chemotherapy plus ICI therapy in CRC patients with an MSS or a pMMR phenotype? This question needs answers.

4.4. Antiangiogenic therapy

Not all CRC tumors respond to chemotherapy initially. Likewise, chemoresistance will occur in a certain number of CRC patients after multiple cycles of chemotherapy. Although several factors are involved in chemoresistance, vascular abnormality accounts for this event because high interstitial pressure potentially hampers drug delivery to tumor cells.[7] For example, bevacizumab is an antiangiogenic drug that elicits vascular normalization in tumors. In regard to its synergistic effect on chemotherapy, bevacizumab significantly improves the prognosis of metastatic CRC patients compared to chemotherapy alone.[70] Despite this superiority, a retrospective study revealed that the high expression of tumoral PD-L1 negatively impacted the survival of patients with metastatic CRC, irrespective of their receipt of neoadjuvant chemotherapy plus bevacizumab.[71] In this situation, it should be asked

Table 3. Advances in combinational use of systematic therapy plus ICI therapy in CRC.

Trial number	Phase	Enrolled patients	Systematic regimen	ICI	Treatment schedule
NCT02375672	II	Advanced CRC	mFOLFOX6	Pembrolizumab	NM
NCT03626922	I	Chemo-refractory mCRC	Pemetrexed + Oxaliplatin	Pembrolizumab	Concurrent CT+ICI, followed by ICI for maximum of 35 cycles
NCT03844750	II	CRC liver metastasis	FOLFOX	Pembrolizumab	4 ~ 8 cycles of CT, then 1 dose of ICI, then liver resection
NCT03396926	II	MSS phenotype, Unresectable advanced CRC and mCRC	Capecitabine plus Bevacizumab	Pembrolizumab	Concurrent CT + Bevacizumab + ICI, then ICI-therapy for maximum of 35 cycles
NCT04072198	II	*RAS*- or *BRAF*-mutated mCRC	FOLFOXIRI plus Bevacizumab (B)	Nivolumab	8 cycles of CT + Bevacizumab + ICI, then Bevacizumab + ICI maintenance
NCT03202758	Ib/II	mCRC	FOLFOX	Druvalumab + Tremelimumab	Concurrent CT + ICI, then the Durvalumab maintenance for a maximum of 12 months
NCT04068610	Ib/II	MSS-mCRC	FOXFOX plus Bevacizumab	Durvalumab + Oleclumab	Concurrent CT + Bevacizumab + ICI until disease progression or unfordable toxicity
NCT03827044	III	MSI-H or POLE-mutated, stage III colon cancer	5-FU-based regimen	Avelumab	Surgery + adjuvant CT plus ICI for 6 months
NCT03475004	II	Refractory mCRC	Bevacizumab plus Binimetinib (MEK inhibitor)	Pembrolizumab	Bevacizumab + MEK inhibitor + ICI until disease progression or unfordable toxicity
NCT02713373	Ib/II	Recurrent CRC and mCRC	Cetuximab	Pembrolizumab	One dose of Cetuximab plus Pembrolizumab, then Pembrolizumab maintenance for a maximum of 24 weeks
NCT04017650	Ib/II	*BRAF*-mutated, MSS-mCRC or unresectable CRC	Cetuximab plus Encorafenib (Braf inhibitor)	Nivolumab	Concurrent Braf inhibitor plus Cetuximab plus Nivolumab until disease progression or unfordable toxicity
NCT03174405	II	*RAS&BRAF* wide-type mCRC	FOLFOX plus Cetuximab	Avelumab	1 cycle of FOLFOX plus Cetuximab, then combining with Avelumab, ICI-therapy for up to 12 months
NCT03608046	II	Refractory MSS-mCRC	Irinotecan plus Cetuximab	Avelumab	Irinotecan plus Cetuximab plus Avelumab for up to 19 weeks

ICI: immune checkpoint inhibitor; CRC: colorectal cancer; mCRC: metastatic colorectal cancer; CT: chemotherapy; NM: not mentioned; MSS: microsatellite-stable; MSI-H: high microsatellite instability; MEK: RAF-mitogen activated protein kinase

whether combining the current therapy with ICI therapy will potentially improve the clinical outcome of these patients. Similarly, although chemotherapy plus bevacizumab has been revealed to reduce the amount of MDSCs in the peripheral blood of metastatic CRC patients, MDSCs remain the predominant source of PD-L1, which leads to T cell exhaustion,[72] thus providing a rationale for the combination use of anti-PD-(L)1 therapy. In addition, chemotherapy plus bevacizumab appears to be insufficient for the treatment of a certain portion of CRC cases, such as those with high PD-L1 expression. Currently, many clinical trials have been designed to investigate the safety and efficacy of antiangiogenic therapy plus ICI therapy across several cancers, including CRC[7] (Table 3). Neutralization of VEGF enables bevacizumab to reduce the immunosuppressive functions of tumoral infiltrates, including MDSCs, Treg cells and M2-like TAMs.[7,62] However, a preclinical model of breast cancer has revealed that deficiency in hypoxia inducible factor-1alpha (HIF-1α) or its target VEGF in $CD8^+$ T cells can significantly limit their infiltration into tumors along with their cytotoxicity toward tumor cells.[73] For CRC, it is still unclear whether VEGF neutralization influences the tumoral amount of $CD8^+$ T cells and their functions. Nevertheless, preclinical studies in CRC have confirmed the immune-supportive effects of antiangiogenic therapy on immunotherapy.[7] Thus, it is reasonable to expect that this strategy will benefit CRC patients.

4.5. Anti-epithermal growth factor receptor (EGFR) therapy

For metastatic CRC patients with wide-type *RAS, BRAF*^{V600E}, *PIK3CA* and without *HER-2* amplification, anti-EGFR therapy is recommended in combination with chemotherapy.[5] In terms of its anti-CRC role, it has been revealed that chemotherapy plus anti-EGFR therapy can increase the number of immune infiltrates, including memory T and cytotoxic T cells, in metastatic lesions.[74] Moreover, chemotherapy plus cetuximab is able to alter the TCR repertoire diversity of $CD4^+$ T cells, while tumors bearing high TCR diversity in $CD4^+$ T cells are more apt to shrink their sizes than those with low TCR diversity.[75] In addition, an *in vitro* study has revealed that irinotecan plus 5-FU is able to induce TAA production, including EGFR, calreticulin and heat shock protein 90 (HSP90), by colon cancer cells.[76] Thus, adding cetuximab to chemotherapy further influences several biological processes performed by DCs, such as inducing DC maturation and activation, enhancing tumor phagocytosis by DCs, and generating tumor-specific cytotoxic T cells that are cross-presented by DCs.[76] In fact, cetuximab exhibits immunocompetencies inherently because the Fc portion of its IgG1 backbone is able to bind with the Fc receptor on natural killer (NK) cells, activated macrophages and DCs. Via this action, NK cell-associated antibody-dependent cell-mediated cytotoxicity (ADCC) will be activated.[62] However, NK cells always become unable to kill tumor cells during gut carcinogenesis.[77] In this context, cetuximab-primed NK-mediated tumor cell lysis is deficient. To overcome this deficiency, several strategies have been developed, such as combinations with IL-21 to reverse NK cell exhaustion,[78,79] adoptive transfer of *in vitro*-expanded tumoricidal NK cells to increase *in vivo* numbers,[80] and agonizing CD137 to induce EGFR-specific $CD8^+$ T cell generation.[81] In a similar manner, due to the upregulation of Tim-3 and PD-1 by exhausted NK cells,[77] ICI therapy has been proposed as a candidate to prevent NK cell exhaustion. Theoretically, combination with cetuximab can further enhance the tumoricidal activity of NK cells partially due to the ADCC effect. Currently, several trials investigating the therapeutic efficacy of cetuximab plus ICI therapy in CRC are ongoing (Table 3). Critically, a newly published work has identified that anti-EGFR therapy truly elicits microsatellite instability emergence in CRC cells, further ensuring the feasibility of anti-EGFR therapy in combination with ICI therapy in CRC patients.[82]

However, there remains a pitfall when combining ICI therapy with other types of therapy. For example, among CRC patients with operable metastatic lesions, it was found that anti-EGFR therapy plus chemotherapy was not able to induce the infiltration of tumoricidal T cells into all metastatic lesions at similar degrees, and a portion of metastatic lesions still lacked immune cells even after treatment.[74] This issue can also be extended to bevacizumab.[74] If so, combination uses of ICI drugs plus anti-EGFR or anti-angiogenic therapies will still not benefit all metastatic lesions in the same CRC patient. The situations in those lesions with low or no response to combination therapy are similar to the paradigms of $PD\text{-}L1^+/TIL^-$ or $PD\text{-}L1^-/TIL^-$ tumors.[4] As aforementioned, Wnt activation is associated with the low density of T cells in a CRC tumor.[29] In this context, the immune-boost therapy, such as SBRT, will be an available choice.

5. Conclusion

CRC patients with dMMR/MSI-H phenotypes in tumors can benefit from ICI therapy, but the response of tumors with pMMR/MSS phenotypes to ICI therapy is extremely poor. Moreover, a portion of CRC cases with pMMR/MSS phenotypes will become refractory after multiple lines of standard therapies. In these cases, radiotherapy, chemotherapy, antiangiogenic therapy or anti-EGFR therapy have been preclinically revealed to exhibit potencies in boosting tumoricidal immune milieus in CRC, thus providing a rational in combining with ICI drugs. On these bases, numerous trials investigating the therapeutic efficacies of standard therapies plus ICI therapy on refractory pMMR/MSS CRC are ongoing, and preliminary results from several trials indicate the combination strategies bring more benefits to CRC patients than ICI therapy alone does. Hence, standard therapies together with ICI therapy are expected to improve the prognosis of refractory cases in CRC.

Abbreviations

ADCC	antibody-dependent cell-mediated cytotoxicity
CRC	colorectal cancer
CSC	cancer stem cell
CTLA-4	cytotoxic T-lymphocyte associated protein-4
CXCL12	chemokine (C-X-C motif) ligand 12
CXCR4	chemokine (C-X-C motif) receptor 4
CXCR7	chemokine (C-X-C motif) receptor 7

DC	dendritic cell
DFS	disease-free survival
dMMR	deficient mismatch repair
EGFR	epithermal growth factor receptor
Flt3L	fms-like tyrosine kinase 3 ligand
5-FU	5-Fluorouracil
HIF-1α	hypoxia inducible factor-1alpha
HSP90	heat shock protein 90; NK, nature killer cell
ICD	immunogenic cell death
ICI	immune checkpoint inhibitor
IDO	indoleamine 2, 3-dioxygenase
LAG-3	lymphocyte-activation gene-3
LARC	local advanced rectal cancer
MCP-1	monocyte chemotactic protein-1
MDSC	myeloid-derived suppressive cell
MSI-H	high microsatellite instability
MSI-L	low microsatellite instability
MSS	microsatellite stability
MHC-I	major histocompatibility complex class-I
MEK	RAF-mitogen activated protein kinase kinase
nCRT	neoadjuvant chemoradiotherapy
NF-Kb	nuclear factor kappa-B
pCR	pathological complete response
PD-1	programmed cell death protein-1
PD-L1	programmed cell death-ligand 1
PGE2	prostaglandin E2
Pmmr	proficient mismatch repair
SBRT	stereotactic body radiotherapy
TAA	tumor-associated antigen
TAM	tumor-associated macrophage
TCR	T cell receptor
TGF-β	transforming growth factor-beta
TME	tumor microenvironment
TIGIT	T cell immunoreceptor with Ig and ITIM domains
Tim-3	T cell immunoglobulin and mucin domain containing molecule-3
VEGF	vascular endothelial growth factor
Wnt	"Wingless/Integrated"

Author's contributions

LT, TW and CP wrote this paper; MS prepared figure and tables of this review; CP conceived this topic, designed the logic flow of this review.

Conflicts of interest

The authors claim no conflicts of interest.

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