Prediction of immunotherapy efficacy and immunomodulatory role of hypoxia in colorectal cancer

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Abstract: Immunotherapy has been used in the clinical treatment of colorectal cancer (CRC); however, most patients fail to achieve satisfactory survival benefits. Biomarkers with high specificity and sensitivity are being increasingly developed to predict the efficacy of CRC immunotherapy. In addition to DNA alteration markers, such as microsatellite instability/mismatch repair and tumor mutational burden, immune cell infiltration and immune checkpoints (ICIs), epigenetic changes and no-coding RNA, and gut microbiomes all show potential predictive ability. Recently, the hypoxic tumor microenvironment (TME) has been identified as a key factor mediating CRC immune evasion and resistance to treatment. Hypoxia-inducible factor-1α is the central transcription factor in the hypoxia response that drives the expression of a vast number of survival genes by binding to the hypoxia response element in cancer and immune cells in the TME. Hypoxia regulates angiogenesis, immune cell infiltration and activation, expression of ICs, and secretion of various immune molecules in the TME and is closely associated with the immunotherapeutic efficacy of CRC. Currently, various agents targeting hypoxia have been found to improve the TME and enhance the efficacy of immunotherapy. We reviewed current markers commonly used in CRC to predict therapeutic efficacy and the mechanisms underlying hypoxia-induced angiogenesis and tumor immune evasion. Exploring the mechanisms by which hypoxia affects the TME will assist the discovery of new immunotherapeutic predictive biomarkers and development of more effective combinations of agents targeting hypoxia and immunotherapy.

Keywords: colorectal cancer, hypoxia, immune checkpoint inhibitors, immunotherapy, tumor microenvironment

Introduction

Colorectal cancer (CRC) is the third most common cancer and has a high mortality due to poor treatment options. Over the past decade, immunotherapy has achieved satisfactory efficacy in a variety of highly mutated solid tumors. Immune checkpoint inhibitors (ICIs) primarily combine with immuno-suppressive molecules, such as programmed cell death 1 (PD-1), programmed death ligand 1 (PD-L1), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), indoleamine 2, 3-dioxygenase, and lymphocyte-activation gene 3 (LAG-3), on the surface of immune cells and tumor cells to block cancer immune escape. Three ICIs (pembrolizumab, nivolumab, and ipilimumab) have been approved by the US Food and Drug Administration (FDA) for CRC containing mismatch repair defects (dMMR) or with high levels of microsatellite instability (MSI-H). Pembrolizumab and nivolumab combined with PD-1 to block the binding of PD-1 and PD-L1/PD-L2-mediated immune evasion, while ipilimumab mainly blocked the binding of CTLA-4 to cluster of differentiation 80/86 (CD80/CD86) on the surface of antigen-presenting cells (APCs) (Figure 1). However, the use of ICIs has been greatly limited by the lack of sensitive predictive biomarkers and emergence of immunotherapy resistance. The complex immunotherapy resistance mechanisms suggest that we need to develop more effective combination therapy regimens and predictive
biomarkers to provide improved survival benefits to patients with CRC.

Hypoxia is a common feature of the tumor microenvironment (TME) and an independent factor for poor prognosis in tumors.4 Tumor hypoxia is mainly regulated by hypoxia-inducible factor (HIF), a heterodimer composed of an O2-sensitive α-subunit (HIF-1α, HIF-2α, or HIF-3α) and a constitutively expressed β-subunit (HIF-1β), in which HIF-1α expression and stability play key roles in tumors adapting to hypoxia.5 Hypoxia-induced HIF-1α expression promotes the formation of disordered blood vessels, which does not improve hypoxia in the TME, and increases the risk of metastasis of CRC.6,7 Hypoxia also regulates the immune response of CRC patients by impairing antitumor innate and adaptive immunity through multiple mechanisms.8,9 A hypoxic environment induces the expression of immune checkpoints (ICs) such as PD-L1 through HIF-1α, promotes immunosuppressive cells such as myeloid-derived suppressor cells (MDSCs), regulatory T cells (Treg cells), and tumor-associated macrophages (TAMs), and inhibits tumor-infiltrating lymphocyte (TILs) infiltration and activation.10,11 Recently, therapies targeting HIF-1α and its downstream molecules have demonstrated promising antitumor effects.12,13

In this review, we focus on the predictors of immunotherapy in CRC and the impact of hypoxia on the TME and explore the complex relationship between HIF-1α and antitumor immunotherapy, which will provide new ideas for predicting and improving the efficacy of immunotherapy.

Predictors of immunotherapy for CRC

Although several ICIs have been used to treat CRC, their efficacy remains poor. The difference in therapeutic efficacy among different CRC patients is attributed to tumor heterogeneity, including DNA alterations, immune cell infiltration, ICs expression, epigenetic changes, noncoding RNA, and the gut microbiome. Biomarkers predicting the efficacy of immunotherapy are important for screening potential beneficiaries. Here, we summarize some predictive markers for CRC immunotherapy (Table 1), and explain their relationship to hypoxia.
Table 1. Predictive markers of immunotherapy for CRC.

| Indicators                              | Markers       | Features                                                                 | Reference                                                                 |
|-----------------------------------------|---------------|---------------------------------------------------------------------------|---------------------------------------------------------------------------|
| DNA alterations                          | MSI-H/dMMR    | High TMB, enriched immune infiltration, high mutational rates, increased TAAs, increased density of TILs and memory T cells | Lizardo et al.,\textsuperscript{14} Le et al.,\textsuperscript{15}          |
| TMB-H                                   |               | Increased neoantigen load, mutations accumulation, MMR system defects     | Schrock et al.,\textsuperscript{16} Fabrizio et al.\textsuperscript{17}   |
| POLE/POLD                               |               | PD-1, PD-L1, CTLA-4 and FoxP3 upregulation; enriched immune infiltration, pMMR hypermutated CRC | Bourdais et al.,\textsuperscript{18} Domingo et al.,\textsuperscript{19} Hwang et al.,\textsuperscript{20} |
| CMS                                     |               | CMS1: MSI-H, BRAF mutations; immune infiltration; CTLA-4, PD-1, PD-L1 upregulation CMS2: the activation of Wnt and Myc pathways CMS3: KRAS mutations and deregulation of metabolic pathways CMS4: TGF-β pathway activation; angiogenesis; stromal activation and Treg, MDSCs, TAMs, and Th17 cells infiltration | Guinney et al.,\textsuperscript{21} Picard et al.,\textsuperscript{22} Chida et al.,\textsuperscript{23} Becht et al.,\textsuperscript{24} Dienstmann et al.,\textsuperscript{25} |
| Immune cells infiltration and ICs       | Immune cells  | CD4$^+$ T cells, CD8$^+$ T cells, NK cells, DCs, MDSCs, Treg cells, TAMs | Zhong et al.,\textsuperscript{26} Craig et al.,\textsuperscript{27} Saito et al.,\textsuperscript{28} Oshi et al.,\textsuperscript{29} |
| ICs                                     |               | PD-1, PD-L1, CTLA-4, TIGIT, LAG-3, HLA, and TIM-3                         | Yin et al.,\textsuperscript{30} Chen et al.,\textsuperscript{31} Makaremi et al.,\textsuperscript{32} Saleh et al.,\textsuperscript{33} |
| Immunoscore                             |               | CD3$^+$ CT, CD3$^+$ IM, CD8$^+$ CT, CD8$^+$ IM High density of each region was 1 score, resulting in the Immunoscore ranging from I0 to I4 | Pagès et al.,\textsuperscript{34} Mlecnik et al.,\textsuperscript{35} |
| Epigenetic change and noncoding RNA     |               | DNA methylation: DNA methylation signature for CD8$^+$ TILs mRNA methylation: m$^6$A; m$^1$A; the RNA modification ‘writer’ Score [WM_Score] | Zou et al.,\textsuperscript{36} Wang et al.,\textsuperscript{37} Gao et al.,\textsuperscript{38} Chen et al.,\textsuperscript{39} |
| Noncoding RNA                           | ImmuMiRNA: identifying miRNA modulators of immune-associated pathways | Liu et al.,\textsuperscript{40} | |
| Gut microbiome                          | Bacteroidetes | Chemokine secretion [CXCR5, CCL22, and CCLX12], T-cell infiltration, dMMR, CTLA-4 blockade | Cremonesi et al.,\textsuperscript{41} Hale et al.,\textsuperscript{42} Vétizou et al.,\textsuperscript{43} |
|                                          | Fusobacteria  | Lower-level T-cell infiltration; NK cells and TILs inhibition; secretion of IL-1β, IL-6, and IL-8; CMS1 phenotype; TIGIT binding; MSI | Gur et al.,\textsuperscript{44} Prospera et al.,\textsuperscript{45} Nosho et al.,\textsuperscript{46} Hamada et al.,\textsuperscript{47} |
|                                          | Proteobacteria| Chemokine secretion [CXCL9]; T-cell infiltration | Cremonesi et al.,\textsuperscript{41} Temraz et al.,\textsuperscript{48} |
|                                          | Firmicutes    | Chemokine secretion [CCR5, CXCXR3]; T-cell infiltration | Cremonesi et al.,\textsuperscript{41} Temraz et al.,\textsuperscript{48} |

BRAF, B-type Raf kinase; CCL, CC chemokine ligand; CMS, consensus molecular subtype; CRC, colorectal cancer; CT, core tumor; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; CXCL, C-X-C motif chemokine; CXCR, C-X-C receptor; DCs, dendritic cells; FoxP3, forkhead box P3; HLA, human leukocyte antigen; ICs, immune checkpoints; IL, interleukin; IM, invasive margin; KRAS, Kirsten rat sarcoma; LAG-3, lymphocyte-activation gene 3; m$^6$A, N$^6$-methyladenosine; m$^1$A, N$^1$-methyladenosine; MDSCs, myeloid-derived suppressor cells; MMR, mismatch repair; MSI, microsatellite instability; NK cells, natural killer cells; PD-1, programmed cell death 1; PD-L1, programmed death ligand 1; POLD, polymerase δ; POLE, polymerase ε; TAA, tumor-associated antigens; TAMs, tumor-associated macrophages; TGF-β, transforming growth factor β; Th17 cells, T-helper 17 cells; TIGIT, T-cell immunoreceptor with Ig and ITIM domains; TILs, tumor-infiltrating lymphocytes; TIM-3, T-cell immunoglobulin-3; TMB, tumor mutational burden; Treg cells, regulatory T cells.
**DNA alterations**

MSI and MMR systems are considered important markers for predicting the therapeutic effects of ICIs for CRC.\(^\text{15,49}\) MSI is divided into MSI-H, MSI-low (MSI-L), and microsatellite stability (MSS).\(^\text{50}\) The MMR system can detect DNA replication errors in the S phase, while MSI may be derived from defects in the MMR system. CRC with dMMR/MSI-H has a higher density of TILs than MSS and sustained ICIs response.\(^\text{14}\) Generally, MSI status testing involves polymerase chain reaction, next-generation sequencing to identify short repeating DNA segments and alterations in MMR genes, and immunohistochemistry analysis for the MMR proteins.\(^\text{51,52}\) A new method [single-molecule molecular inversion probes (smMIPs)] targets high-throughput sequencing of numerous microsatellite loci for MSI using smMIPs with 100% diagnostic sensitivity and specificity in CRC.\(^\text{53}\) In addition, artificial intelligence has been used to predict MSI/dMMR directly.\(^\text{54}\)

Tumor mutational burden (TMB) is caused by DNA deletion, insertion, and frameshift mutations in the replication process of cancer cells and considered a useful biomarker for identifying patients for whom immunotherapy might be advantageous.\(^\text{16,55}\) CRC with a high TMB generates more neoantigens than that with lower TMB, which increases the immunogenicity of tumors.\(^\text{56}\) The FDA-approved pembrolizumab for the treatment of unresectable or metastatic solid tumors with TMB ≥10 mutations per megabase (mut/Mb).\(^\text{57}\) Furthermore, TMB ≥12 mut/Mb has also been used as a threshold for predicting immunotherapy efficacy in CRC populations.\(^\text{17}\) TMB in MSI-H mCRC is generally elevated but still quite variable.\(^\text{17}\) High TMB can still be detected in CRC patients with MSS and benefits from immunotherapy.\(^\text{58}\) In addition, polymerase ε and polymerase δ mutations occur in a subset (less than 3%) of patients with MSS/MMR proficiency (pMMR), which is associated with a higher number of TILs and leads to the upregulation of genes encoding PD-1, PDL-1, and CTLA-4, suggesting a favorable prognosis with immunotherapy.\(^\text{18-20}\) In addition, according to the CRC molecular gene expression profile, a consensus molecular subtype (CMS) classification is developed based on both tumor and infiltrating stroma gene expression.\(^\text{22}\) CMS includes four 'consensus' molecular subtypes: CMS1–CMS4.\(^\text{21}\) CMS1 and CMS4 show greater infiltration of immune cells, whereas CMS2 and CMS3 lack immunoactivating components. In CRC with MSI-H/dMMR, CMS1 is mainly accompanied by infiltration of CD8+ TILs, and CD68+ macrophages and upregulated expression of ICs, which also predicts the efficacy of ICIs.\(^\text{23,24}\) CMS4 was infiltrated by Treg cells, MDSCs, monocyte-derived cells, and T-helper 17 cells (Th17 cells). These immunosuppressive cells should be monitored when targeting ICs.\(^\text{28,59}\) Other DNA alterations, such as BRAF mutations, need to be investigated further. Hypoxia is also one of the main features of solid tumors. HIF-1α expression may represent a novel marker to separate the MSI-L group from the MSS and MSI-H groups. A study shows downregulation of HIF-1α in the MSI-L group compared with MSS and MSI-H groups.\(^\text{50}\) Moreover, suppression of the MMR pathway by hypoxia has been previously documented with specific downregulation of the MMR proteins MLH1, MSH2, and MSH6, leading to genomic instability.\(^\text{61,62}\) Therefore, additional studies are necessary to shed lighter on potential biomarkers and to explore the molecular mechanism of hypoxia regulating DNA alterations.

**Immune cells infiltration and ICs**

Although MSS/pMMR and MSI/dMMR are important predictors of CRC, abundant TILs more directly indicate antigenicity of tumor cells.\(^\text{63}\) Immune cells in TME include CD4+ T cells, CD8+ T cells, natural killer (NK) cells, dendritic cells (DCs), MDSCs, Treg cells, and TAMs. As the main immune cells that kill tumors, the number of CD8+ T cells and NK cells infiltrating the TME reflects the antitumor immune response. DCs mainly recognize tumor antigens through the combination of major histocompatibility complex (MHC) molecules and T-cell receptor, present them to CD8+ T cells and other lymphatic T cells, and participate in the elimination of tumor cells through DC-NK crosstalk. Immune status is prognostic for survival in CRC and is associated with hypoxia and angiogenesis.\(^\text{23,27}\) Fewer CD8- and CD3-positive immune cells were found in hypoxic tumor centers than in tumor margins, suggesting an insensitivity to immunotherapy drugs. Apart from the CD8 and CD3 phenotypes, the number of immune cells with a CD4 phenotype has also been identified as a biomarker of the immune status and prognosis of CRC.\(^\text{27}\) Treg cells are a subset of CD4+ T
lymphocytes that predict a worse outcome in CRC with a higher Treg proportion.\textsuperscript{28,29,64} Imnoscore is based on the quantification of cytotoxic and memory T cells in the central and invasive margins (IMs) of the tumor.\textsuperscript{65,66} Imnoscore is based on the numeration of two lymphocyte populations (CD3/CD45RO, CD3/CD8, or CD8/CD45RO) in the core of the tumor (CT) and in the IM of tumors.\textsuperscript{34}

The expression of various ICs, including PD-1, PD-L1, CTLA-4, T-cell immunoreceptor with Ig and ITIM domains (TIGIT), LAG-3, human leukocyte antigen (HLA), and T-cell immunoglobulin-3 (TIM-3), suggests the potential efficacy of ICIs.\textsuperscript{30,32} The high expression of ICs provides targets for immunotherapy and predicts its efficacy.\textsuperscript{67} PD-1 is mainly expressed on the surface of CD8\textsuperscript{+} T cells and inhibits the immune-killing effect by binding to PD-L1 or PD-L2 on the surface of tumor cells. CTLA-4 on CD4\textsuperscript{+} and CD8\textsuperscript{+} T cells participate in immune tolerance and immune suppression by competing with CD28 to bind to CD80/CD86 on the surface of APCs.\textsuperscript{68} MSS CRC constitutes the majority of cases and responds poorly to immunotherapy targeting PD-1, PD-L1, and CTLA-4. Nevertheless, targeting TIM-3 or TIGIT in conjunction with blocking PD-1/PD-L1 or other immunotherapies can restore the function of CD8\textsuperscript{+} T cells and enhance immunotherapy efficacy.\textsuperscript{69} Hypoxia is a key factor affecting immune cell infiltration and ICs expression in TME, which predict the effect of immunotherapy. The specific molecular mechanisms by which hypoxia regulates immune cell infiltration and ICs expression will be detailed in the following sections.

**Epigenetic change and noncoding RNA**

Gene epigenetic modifications and noncoding RNA can also predict the efficacy of immunotherapy in CRC. CD8\textsuperscript{+} T-cell distribution in the TME of CRC was associated with the methylation of CD8\textsuperscript{+} T cells. A DNA methylation signature for the CD8\textsuperscript{+} TILs characteristic score system was constructed based on CD8\textsuperscript{+} T-cell differential methylation sites, with lower scores, indicating increasing CD8\textsuperscript{+} T-cell infiltration.\textsuperscript{36} N6-methyladenosine (m\textsuperscript{6}A), a critical regulator of transcript expression, is the most frequent internal modification of mRNA in the human body. The m\textsuperscript{6}A modification decreases the mutation burden and immune activation and is related to reduced neoantigen load and poor response to immunotherapy.\textsuperscript{37} The m\textsuperscript{6}A methylation-mediated intercellular communication between TME and tumor cells contributes to CRC progression.\textsuperscript{38} The epigenetic inheritance of CRC is also regulated by hypoxia, and it has been reported that fat mass and obesity-associated protein (FTO), an m\textsuperscript{6}A demethylase, inhibited CRC metastasis and progression.\textsuperscript{70} Hypoxia restrained FTO protein expression, mainly due to an increase in ubiquitin-mediated protein degradation. Except for m\textsuperscript{6}A, epigenetic modification of m\textsuperscript{1}A, alternative polyadenylation, and adenosine-to-inosine RNA editing are also predictors of CRC immunotherapy.\textsuperscript{39} Long noncoding RNAs (lncRNAs) and microRNAs (miRNAs) have been screened as prognostic biomarkers for cancers and used to construct predictive models to predict immunotherapy efficacy.\textsuperscript{71,72} LncRNAs can be epigenetically regulated via m\textsuperscript{6}A and are involved in predicting the tumor-immune-stromal microenvironment and immunotherapy efficacy.\textsuperscript{73} Liu et al.\textsuperscript{40} proposed an integrated algorithm, immuMiRNA, to identify miRNA modulators of immune-associated pathways and established an immune-associated miRNA prognostic signature consisting of three miRNAs (miR-194-3P, miR-216a-5p, and miR-3677-3p) to predict immunotherapy efficacy. In addition, small nucleolar RNA host gene 11 (SNHG11) is upregulated by promotor hypomethylation in CRC and can promote CRC cell migration and metastasis under hypoxia.\textsuperscript{74} Mechanistically, SNHG11 binds to the von Hippel-Lindau tumor suppressor product (pVHL) recognition sites on HIF-1\textalpha, thus blocking the interaction of pVHL with HIF-1\textalpha and preventing its ubiquitination and degradation. Interestingly, HIF-1\textalpha was also able to regulate CRC proliferation by inducing noncoding RNA such as LINC00511.\textsuperscript{75} More studies are still needed to elucidate the mechanism of action of hypoxia and noncoding RNA.

**Gut microbiome**

Hypoxia is an important environmental factor affecting the abundance of intestinal flora.\textsuperscript{76} Studies have shown that changes in oxygen content caused by external and internal factors have regulatory effects on a variety of intestinal
bacteria, including *Enterococaceae*, *Prevotella*, *Enterococci*, *Actococcus*, *Fusobacteria*, *Pseudomonas*, and *Escherichia–Shigella*. Dysregulation of gut microbiota is not only an important inducement to CRC, but also modulate the immune system and affect immunotherapy efficacy. *Bacteroidales* improved the efficacy of CTLA-4 blockade, whereas *Bifidobacterium* was associated with the clinical benefits of PD-L1 blockade.43,79 *Escherichia coli*, *Bacteroides fragilis* and *Firmicutes* stimulate T-cell trafficking into CRC tumor tissues by increasing the expression of T cell-recruiting chemokines.41 However, colibactin-producing *Escherichia coli* impairs CD3+ and CD8+ T-cell infiltration in CRC and leads to tumor resistance to immunotherapy.80 Interestingly, *Fusobacteria* correlates with tumor evasion by inhibiting NK cells and TILs and increasing the expression of inflammatory mediators.44,45 *Fusobacterium nucleatum* induces PD-L1 expression by activating stimulator of interferon genes signaling and increasing the accumulation of interferon-gamma (IFN-γ)+ CD8+ TILs, and enhances the antitumor response to PD-1/PD-L1 blockade during treatment.81 *Fusobacterium* and *Bacteroidetes* in CRC were shown to be associated with lower-level T-cell infiltrates and MSI, whereas enrichment of *Bacteroides fragilis* and *Fusobacterium nucleatum* was shown in dMMR CRC.42,46

**Hypoxia regulated the TME of CRC**

Hypoxia affects tumor immune cell infiltration, immune cell activity, ICs, and cytokine secretion and plays a predictive role in immunotherapy.82 HIF-1α is the primary molecule responsible for hypoxia. The stability and activity of HIF-1α are regulated by post-translational modifications, hydroxylation, acetylation, and phosphorylation. Under normoxia, HIF-1α is rapidly degraded via pVHL-mediated ubiquitin-proteasome pathway, which is triggered by the hydroxylation of prolines and acetylation of lysine within a polypeptide segment known as the oxygen-dependent degradation domain of HIF-1α. Under hypoxic conditions, the HIF-1α subunit becomes stable and interacts with coactivators (such as the response element-binding protein), regulating the expression of target genes, including angiogenesis, epithelial mesenchymal transition, maintenance of cancer stem cells, metabolic reprogramming, tumor cell survival and proliferation, drug resistance, and tumor immune regulation.83 In the following sections, we review the effect of hypoxia and its marker HIF-1 on the antitumor immunity of CRC.

**Effects of hypoxia on angiogenesis in CRC**

Tumor angiogenesis is critical to the TME. However, the distribution of the tumor vasculature network is irregular and does not improve hypoxia in the tumor center. Hypoxia results in angiogenesis via binding to the hypoxia response element (HRE) and inducing more vascular endothelial growth factor (VEGF), specifically VEGF-A.84 VEGF-A promotes the differentiation of CD8+ T cells to exhausted subsets with high expression of PD-1 and TIM-3.85,86 Interestingly, VEGF-A is partly regulated by HIF-1α, and VEGF-A expression in CD8+ T cells contributes to T-cell infiltration in breast cancer.87 The binding of VEGF to the VEGF receptor (VEGFR) 2 on the DCs membrane upregulates the expression of cofilin 1, which impairs the motility and differentiation of DCs.88 HIF-1α is induced by leucine-rich-alpha-2-glycoprotein 1 in a concentration- and time-dependent manner, leading to VEGF-A expression.89 HIF-1α binds to the HRE element in the promoter region of cyclooxygenase-2 (COX-2). The expression of COX-2 leads to elevated levels of prostaglandin E2 (PGE2), which induces PGE2-mediated angiogenesis.90 PGE2 can also promote escape from immune surveillance by activating PD-L1 expression and suppressing DCs maturation.91,92 Furthermore, noncoding RNA also regulates HIF-α-mediated angiogenesis. Circ-Erbin, a circular RNA highly expressed in CRC cells, induces HIF-1α expression by activating the miR-125a-5p-5p/miR-138-5p/4E binding protein 1 axis to promote angiogenesis.93 HIF-1α can transactivate myocyte enhancer factor 2D (MEF2D) expression by binding to the MEF2D promoter, inducing the expression of proangiogenic cytokines.94 Dyskeratosis congenita 1 promotes HIF-1α expression by modulating its promoter activity, which promotes CRC angiogenesis and metastasis by increasing VEGF expression.6 Hypoxic CRC promotes tumor growth and angiogenesis by delivering exosomes. Hypoxia is dependent on HIF-1α to promote exosomal Wnt4 upregulation, followed by β-catenin activation in endothelial cells to promote endothelial cell proliferation and migration.95 A possible interaction between HIF-1α and tumor necrosis factor receptor-associated factor 6 (TRAF6) has been identified.96 TRAF6, an
upstream effector of the nuclear factor-kappa B (NF-κB) pathway, increases perfused vasculature and vessel permeability in hypoxic CRC. In conclusion, in CRC, multiple mechanisms, including hypoxia, induce HIF-1α to promote disordered angiogenesis in the TME and mediate immune evasion (Figure 2).

**Effects of hypoxia on cancer stem cells**

Cancer stem cells are tightly linked with PD-1/PD-L1 regulation and hypoxia. Hypoxic TME through promoting core-to-edge transition of tumor cells increases their stemness and resistance, and predisposes cancer into metastasis. By regulating pH in tumor cells and by regulating the function of cell surface molecules such as extracellular carbonic anhydrase, cancer stem cell function is increased and cell invasion is enhanced in hypoxic TME, while also contributing to immune evasion. Enriched PD-L1 expression in cancer stem-like cells (CSCs) contributes to CSCs immune evasion. PD-L1 is closely associated with cancer stem cell immune escape and has been reported to regulate self-renewal of cancer stem cells, which is dependent on hypoxic HIF-1α and HIF-2α activation. The interaction of CSCs with the immune system is an important mechanism of tumor immune escape. Some studies have shown that CSC can interact with TAM to promote M2 polarization by secreting immunosuppressive molecules, such as transforming growth factor β (TGF-β) and interleukin (IL)-4. Meanwhile,
hypoxia-induced CSCs can secrete CC chemokine ligand 20 to recruit TAMs to inhibit T-cell responses and promote immune tolerance. In turn, TAM-derived IL-6 can promote the expansion and phenotypic expression of CSCs through signal transducer and activator of transcription (STAT) 3 signaling pathway.

**Effects of hypoxia on immunosuppressive cells in CRC**

Hypoxia contributes to the infiltration and activation of immunosuppressive cells, mediates immune evasion by secreting immunosuppressive molecules, or directly inhibits the function of immune cells. Here, we examined the effects of hypoxia and HIF-1 on the infiltration, differentiation, and function of immunosuppressive cells, such as Treg cells, MDSCs, and TAMs (Figure 3).

Tregs identified by the expression of forkhead box P3 (FoxP3) are a subset of CD4+ T lymphocytes that dampen the immune response against cancer cells. High FoxP3 expression may be an independent prognostic factor for patients with...
MMR-proficient CRC. High Treg infiltration in CRC is associated with increased expression of ICs, implying susceptibility to immunotherapy. The role of HIF-1α in Treg differentiation is complex. HIF-1α can identify the HRE site in the FoxP3 promoter to promote its expression and directly bind to FoxP3 protein to promote its ubiquitination and degradation, thereby inhibiting Treg cell differentiation. Th17 and Treg cells are derived from a common precursor and can be induced by different cytokines to perform opposing functions. HIF-1α can directly induce the transcription of receptor-related orphan receptor γt to promote Th17 cell differentiation and drive the transcription of IL-17 genes, with the assistance of IL-6. Under hypoxic conditions, HIF-1α also promotes peripheral Treg cell activity, inducing carcinogenesis and the progression of CRC cells. Hypoxia also promotes CD68+ macrophages in CRC to express C-X-C motif chemokine (CXCL) 11 and attract FoxP3+ Treg cell infiltration. In response to hypoxia, FoxP3+ Treg cells express IL-17 and become FoxP3+IL-17+ Treg cells, which can suppress tumor-specific CD8+ T cells.

TAMs are critical components of TME. TAMs mainly have two phenotypes: an M1-like phenotype (antitumor) and an M2-like phenotype (pro-tumor). The number of M2 macrophages increases in the hypoxic TME of CRC. HIF-1α induced metabolic reprogramming of macrophages in the TME, including upregulation of amino acid metabolism and oxidative stress pathways, induces the transformation of macrophages into TAM with a tumor-promoting effect. HIF-1α-induced lncRNA pituitary tumor-transforming 3, pseudogene (PPTG3P) contributes to CRC glycolysis and the M2 phenotype of macrophages under hypoxic conditions. M2 macrophages were induced by hypoxia-triggered forkhead box O1 (FoxO1) deficiency, which could negatively regulate MHC-II genes. Hypoxia augments macrophage-mediated T-cell suppression in a manner dependent on the expression of HIF-1α and targeting HIF-1α in macrophages can promote the proliferation and activation of CD8+ T cells. TAMs produce TGF-β to support HIF-1α expression in CRC cells, thereby upregulating tribles pseudokinase 3 (TRIB3), which results in activation of the β-catenin/Wnt signaling pathway. TRIB3 can also reduce tumor-infiltrating T cells by inhibiting STAT 1-mediated CXCL10 transcription by enhancing the epidermal growth factor receptor signaling pathway in CRC. HIF1-α is significantly associated with the expression of VEGFR2 in M2-TAM, and TGF-β1 is produced through the VEGF/VEGFR2 signaling pathway. Hypoxia also activates RAS signaling in CRC, which drives the production of colony-stimulating factor 2 (CSF2) and lactate. CSF2 synergizes with lactate to elicit functional reprogramming of TAMs and exerts a tumor-supportive capacity. Succinate exerts its effects via succinate receptor 1, which mediates signaling through the phosphatidylinositol 3-kinase (PI3K)/HIF-1α pathway. Multiple cancer cells, including CRC cells, secrete succinate into the TME, facilitating macrophage migration and mediating TAMs polarization. However, some studies have reported different results, that upregulation of HIF-1α in macrophages contributes to the overexpression of M1 markers, whereas HIF-2α promotes M2 polarization.

MDSCs, with the ability to suppress immune cell responses, are a heterogeneous group of cells derived from the bone marrow and are considered precursors of DCs and macrophages. MDSCs, considered a predictive marker of immunotherapy, limit the therapeutic efficacy of ICIs by leading to T-cell dysfunction and promoting angiogenesis. MDSCs comprise two main populations: monocytic MDSCs (M-MDSCs) and granulocytic MDSCs (G-MDSCs). HIF-1α redirects differentiation toward the classically activated (M1) phenotype and alters the function of MDSCs. MDSCs from tumors primarily use arginase (Arg) and nitric oxide (NO) to suppress T-cell function, and hypoxia causes the upregulation of Arg1 and inducible nitric oxide synthase (iNOS) in MDSC. HIF-1α directly binds to a transcriptionally active HRE in the miR-210 proximal promoter. MiR-210 modulated MDSCs function by increasing Arg1, NO, CXCL12, and IL-16 production. HIF-1α also upregulates lncRNA PVT1 expression in G-MDSCs under hypoxia, which can increase the levels of Arg1 and reactive oxygen species in G-MDSCs and regulate their immunosuppressive ability. S100A9, a G-MDSC exosome, is mediated by hypoxia-induced Rab27a (a GTPase required for exosome secretion) and enhances the stemness of CRC. Overexpression of the V-domain Ig suppressor of T-cell activation (VISTA) is associated with
MDSC function. In CRC, hypoxia upregulates VISTA expression by HIF-1α binding to the VISTA promoter. VISTA, in turn, contributes to MDSC-mediated T-cell suppression under hypoxia. Increased HIF-1α upregulates CXCL1, CXCL3, and CXCL8 expression in MDSCs, contributing to enhanced recruitment of MDSCs to tumors.

**Effects of hypoxia on immunoeffector cells in CRC**

Hypoxia promotes the infiltration and activation of immunosuppressive cells and inhibits cells that mediate antitumor immunity. In this section, we discuss the mechanism by which hypoxia and its marker, HIF-1, inhibit DCs, NK cells, and CD8+ T cells (Figure 4).

As the most important APC, DCs can specifically recognize, process, and present diverse cancer antigens and mediate tumor immunity via the activation of CD8+ and CD4+ T cells. The effects of hypoxia on the differentiation and maturation of DCs have been well studied. Hypoxia keeps DCs in the TME in an immature state and contributes to the inhibition of T-cell response, which results in decreased motility, phagocytosis, and CD83 expression. The hypoxic TME also promotes apoptosis of DCs by inhibiting the PI3K/Akt pathway. Plasmacytoid DCs (pDCs) play immunosuppressive roles in the TME. Hypoxia-induced extracellular adenosine (ADO) significantly enhances pDCs recruitment into tumors. ADO-stimulated pDCs promote Treg cell induction and suppress proliferation and cytotoxicity of CD8+ T cells. In addition, hypoxia-induced VEGF expression and downregulation of IFN hamper DCs maturation. Hypoxic DCs have been shown to alter chemokine expression and promote the upregulation of proinflammatory cytokines such as IL-1β and tumor necrosis factor (TNF)-α. HIF-1α upregulates CC chemokine receptor (CCR) 5 and impairs the upregulation of

**Figure 4.** Effects of hypoxia on immunoeffectors cells in CRC. Hypoxia leads to immune evasion of CRC by inhibiting infiltration, activation, maturation, and function of immune effector cells and antigen-presenting cells through multiple pathways.

ADO, adenosine; CCR, CC chemokine receptor; CXCL, C-X-C motif chemokine; HIF-1α, hypoxia-inducible factor 1α; IFN, interferon; IL, interleukin; MICA, MHC class I chain-related protein A; NF-κB, nuclear factor-kappa B; NKG2D, natural killer cell group 2D; NKP44, natural killer cell p44-related protein; NKP30, natural killer cell p30-related protein; OPN, osteopontin; PTTG3P, pituitary tumor-transforming 3, pseudogene; TGF, transforming growth factor; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.
CCR7, a necessary molecule for the homing of mature DCs, is also present in the TME and may contribute to the infiltration of MDSCs and Tregs. 

**Effects of hypoxia on the alteration of ICs in CRC**

As inhibitory regulatory molecules, ICs may be regulated by hypoxia. We discussed several ICs and how they caused immune evasion in the hypoxic TME. Elevated PD-L1 expression causes escape from adaptive immunity via the promotion of apoptosis of T cells. On the surface of MDSCs, TAMs, DCs, and tumor cells in hypoxic TME, PD-L1 expression was rapidly, significantly and selectively upregulated, which was dependent on HIF-1α but not HIF-2α. HIF-1α can upregulate PD-L1 expression by binding to transcriptionally active HRE in the PD-L1 proximal promoter. Pyruvate kinase isoform M2 (PKM2) assists HIF-1α in binding to HRE. PKM2 regulates PD-L1 expression not only in CRC tumor cells but also in TAMs, DCs, and T cells. Hypoxia can induce the secretion of circEIF3K exosomes from cancer-associated fibroblasts (CAFs), which increases the stabilization of PD-L1 by reducing the production of miR-214 in CRC. In addition to PD-L1, hypoxia also causes upregulation of CTLA-4 on MDSCs, TAMs, DCs, and tumor cells through HIF-1α. CTLA-4 expression appears to be associated with hypoxic-induced VEGF and angiogenesis, and targeting both the hypoxic pathway and CTLA-4 has shown promising efficacy. Hypoxic tumor regions in the TME are associated with increased VISTA expression in tumor infiltrating MDSCs. Targeting VISTA under hypoxia relieved MDSC-mediated T-cell suppression. Further studies showed that HIF-1α binding to a conserved HRE in the VISTA promoter upregulated...
VISTA in myeloid cells. HLA-G is a non-classical MHC-I molecule that was considered to be involved in immune evasion and prognosis of CRC.\textsuperscript{31} HLA-G suppresses IFN-\(\gamma\) and TNF-\(\alpha\), leading to a reduction in the antitumor effects of NK and T cells.\textsuperscript{157} HLA-G has been shown to be induced in melanoma and glioma by HIF-1\(\alpha\) binding to HRE.\textsuperscript{158,159} However, hypoxia may decrease HLA-G expression in constitutively expressed HLA-G cell lines. Hypoxia maintenance may result in channeling cell energy into productive gene expression at the cost of HLA-G.\textsuperscript{158} Similarly, the overexpression of CD47, CD137, and other ICs may be associated with hypoxia.\textsuperscript{160} Further investigations are needed to elucidate the relationship between hypoxia and ICs in CRC.

**Targeting hypoxia to enhance the efficacy of immunotherapy for CRC**

Currently, improvements in the hypoxic TME are being explored to enhance the efficacy of immunotherapy in CRC. Some treatments have been found to improve immune status and immunotherapy efficacy by improving hypoxia in CRC. Approaches to improve hypoxia mainly include targeting HIF-1\(\alpha\), antiangiogenesis, promoting vascular normalization, and increasing tumor oxygenation (Table 2).
| Target | Agents | Effect | Reference |
|--------|--------|--------|-----------|
| Targeting HIF-1α | Decursin | Promoting HIF-1α proteasomal degradation and improved T-cell activation in TME | Ge et al.161 |
| | YYFZBJS | Inhibiting HIF-1α-mediated Treg cells activation | Zhang et al.109 |
| | Panaxadiol | Inhibiting PD-L1 expression by blocking the interaction between HIF-1α and STAT3 | Wang et al.162 |
| | Echinomycin | Suppressing PD-L1 expression on tumor cells and tumor-infiltrating myeloid cells but induced PD-L1 in normal tissues | Bailey et al.163 |
| Antiangiogenesis and promoting vascular normalization | Apatinib + anti-PD-1 | Increasing CD8+ TILs, restored CD8+ T-cell exhaustion and downregulated co-expression of PD-1, TIM-3, LAG-3 and TIGIT, and reduced accumulation of Treg cells, M2-like TAMs and MDSCs | Wang et al.164 |
| | CU06-1004 | Resulting in more tumor-infiltrating NK and T cells | Park et al.165 |
| | Catalpol | Inhibiting expressions of angiogenic markers VEGF, VEGFR2, HIF-1α to reduce the expressions of IL-1β, IL-6, IL-8, COX-2, and iNOS | Zhu et al.166 |
| | Fruquintinib + anti-PD-1 | Enhancing chemotactic factor release, increasing CD8+ T-cell infiltration and activation, decreasing ration of Treg cells, and promoting M1/M2 ratio of macrophage | Wang et al.167 |
| | Cu²⁺-ion-based intracellular bio-nanoreator | Delivery of small interfering RNA targeting VEGF | Chen et al.168 |
| | Ziv-aflibercept | Antiangiogenesis | Rahma et al.169 |
| Increasing oxygenation | Hyperbaric oxygen + melatonin | Resulting in decreasing HIF-1α expression and diminishing immune evasion | Li et al.170 |
| | Metformin | Inhibiting oxygen consumption and increasing TILs and enhance T-cell cytotoxicity activity | Ali171 |

COX-2, cyclooxygenase-2; HIF-1α, hypoxia-inducible factor 1α; IL, interleukin; iNOS, inducible nitric oxide synthase; LAG-3, lymphocyte-activation gene 3; PD-1, programmed cell death 1; PD-L1, programmed cell death ligand 1; MDSCs, myeloid-derived suppressor cells; mTOR, mechanistic target of rapamycin; ROS, reactive oxygen species; STAT3, signal transducer and activator of transcription 3; TAMs, tumor-associated macrophages; TIGIT, T-cell immunoreceptor with Ig and ITIM domains; TILs, tumor-infiltrating lymphocytes; TIM-3, T-cell immunoglobulin-3; TLR4, Toll-like receptor 4; Treg cells, regulatory T cells; VEGF, vascular endothelial growth factor; YYFZBJS, Yi-Yi-Fu-Zi-Bai-Jiang-San.

Decursin is a novel HIF-1α inhibitor that promotes proteasomal degradation and improves T-cell activation in the TME. Decursin reduces the hypoxic area; PD-L1 expression; and accumulated CD3+ T, CD4+ T, and CD8+ T-cell infiltration, but FoxP3 Treg cells and MDSC-mediated Arg1 are attenuated.161 Yi-Yi-Fu-Zi-Bai-Jiang-San, a traditional Chinese medicine, improves the TME of CRC by inhibiting HIF-1α-mediated Treg cell activation.109 Panaxadiol enhances the activity of CTLs and restores their capacity to kill tumor cells.162 The HIF-1α inhibitor echinomycin potentiates the cancer immunotherapeutic effects of anti-CTLA-4 therapy and prevent the high incidence of immune-related adverse events induced by the combination of anti-CTLA-4 and anti-PD-1/PD-L1.163 The combination of targeting TIGIT and HIF-1α is a novel strategy against CRC.172

Antiangiogenesis therapy improves the efficiency of ICIs in VEGFA-overexpressing CRC.
Apatinib, a VEGFR2 tyrosine kinase inhibitor, has the potential to reverse immunosuppression in hypoxic CRC. Apatinib plus anti-PD-1 increases CD8+ TILs, restores CD8+ T-cell exhaustion, and reduces the accumulation of Treg cells, M2-like TAMs and MDSCs. CU06-1004, an endothelial dysfunctional blocker, causes increased levels of TILs and tumor apoptosis within the TME. Catalpol inhibits the expression of IL-1β, IL-6, IL-8, COX-2, and iNOS by inhibiting the expression of angiogenic markers. The combination of fruquintinib and anti-PD-1 decreases angiogenesis, enhances vascular normalization, and reprograms the immune microenvironment. A Cu2+ ion-based intracellular bio-nanoreactor stimulates robust tumoricidal immunity by delivering small interfering RNA targeting VEGF. A phase IB clinical study (NCT02298959) showed that ziv-aflibercept, an antiangiogenic agent, plus pembrolizumab in patients with advanced solid tumors, including CRC, demonstrates an acceptable safety profile with antitumor activity. Oxygen supplementation is the simplest way to improve hypoxia and improve immunotherapy. Hyperbaric oxygen therapy and melatonin can alter the hypoxic microenvironment, resulting in decreased HIF-1α expression and diminished immune evasion in CRC. Employing metformin as a method to improve hypoxic TME could increase TILs and enhance T-cell cytotoxicity activity, thus improving sensitivity to anti-PD-1 immunotherapy.

**Conclusion**

The efficacy of immunotherapy is not as expected in most CRC patients. More sensitive and extensive biomarkers are needed to predict the efficacy of immunotherapy for CRC. We summarized some common markers and systems for efficacy prediction. CRC can create a hypoxic TME that impairs the efficacy of immunotherapy and antitumor immunity. Markers of hypoxia, especially HIF-1, showed strong correlations and predictors of immunotherapy outcomes, and some strategies for improving hypoxia have shown synergy with immunotherapy. In the future, more effective strategies and immunotherapy combinations are needed to improve hypoxia, and further searches should be made for the most appropriate combination approaches to provide a broader space for the treatment of CRC patients.

**Declarations**

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

All authors have read and agreed to the published version of the manuscript.

**Author contribution(s)**

Zhuangzhuang Zheng: Methodology, Writing – original draft.
Chenbin Bian: Formal analysis, Writing – original draft.
Huanhuan Wang: Investigation.
Jing Su: Software.
Lingbin Meng: Visualization, Writing – review & editing.
Ying Xin: Conceptualization, Funding acquisition, Validation, Writing – review & editing.
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**Competing interests**

The authors declare that there is no conflict of interest.

**Availability of data and materials**

All data generated or analyzed during this study are included in this published article.
References

1. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 Countries. CA Cancer J Clin 2021; 71: 209–249.

2. Chen YP, Zhang Y, Lv JW, et al. Genomic analysis of tumor microenvironment immune types across 14 solid cancer types: immunotherapeutic implications. Theranostics 2017; 7: 3585–3594.

3. Frank S, Skelton WP, Starr JS, et al. Immunotherapy for colorectal cancer: a review of current and novel therapeutic approaches. J Natl Cancer Inst 2019; 111: 1131–1141.

4. Wang B, Zhao Q, Zhang Y, et al. Targeting hypoxia in the tumor microenvironment: a potential strategy to improve cancer immunotherapy. J Exp Clin Cancer Res 2021; 40: 24.

5. Jiang BH, Rue E, Wang GL, et al. Dimerization, DNA binding, and transactivation properties of hypoxia-inducible factor 1. J Biol Chem 1996; 271: 17771–17778.

6. Hou P, Shi P, Jiang T, et al. DKC1 enhances angiogenesis by promoting HIF-1α transcription and facilitates metastasis in colorectal cancer. Br J Cancer 2020; 122: 668–679.

7. Catalano V, Turdo A, Di Franco S, et al. Tumor and its microenvironment: a synergistic interplay. Semin Cancer Biol 2013; 23: 522–532.

8. Zhang L, Wang S, Wang Y, et al. Effects of hypoxia in intestinal tumors on immune cell behavior in the tumor microenvironment. Front Immunol 2021; 12: 645320.

9. Carmona-Rodriguez L, Martinez-Rey D, Fernández-Aceñero MJ, et al. SOD3 induces a HIF-2α-dependent program in endothelial cells that provides a selective signal for tumor infiltration by T cells. J Immunother Cancer 2020; 8: e000432.

10. Barsoum IB, Smallwood CA, Siemens DR, et al. A mechanism of hypoxia-mediated escape from adaptive immunity in cancer cells. Cancer Res 2014; 74: 665–674.

11. Noman MZ, Desantis G, Janji B, et al. PD-L1 is a novel direct target of HIF-1α, and its blockade under hypoxia enhanced MDSC-mediated T cell activation. J Exp Med 2014; 211: 781–790.

12. Wei TT, Lin YT, Tang SP, et al. Metabolic targeting of HIF-1α potentiates the therapeutic efficacy of oxaliplatin in colorectal cancer. Oncogene 2020; 39: 414–427.

13. Su J, Zhao Q, Zheng Z, et al. Prospective application of ferroptosis in hypoxic cells for tumor radiotherapy. Antioxidants 2022; 11: 921.

14. Lizardo DY, Kuang C, Hao S, et al. Immunotherapy efficacy on mismatch repair-deficient colorectal cancer: from bench to bedside. Biochim Biophys Acta Rev Cancer 2020; 1874: 188447.

15. Le DT, Durham JN, Smith KN, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. Science 2017; 357: 409–413.

16. Schrock AB, Ouyang C, Sandhu J, et al. Tumor mutational burden is predictive of response to immune checkpoint inhibitors in MSI-high metastatic colorectal cancer. Ann Oncol 2019; 30: 1096–1103.

17. Fabrizio DA, George TJ Jr., Dunne RF, et al. Beyond microsatellite testing: assessment of tumor mutational burden identifies subsets of colorectal cancer who may respond to immune checkpoint inhibition. J Gastrointest Oncol 2018; 9: 610–617.

18. Bourdais R, Rousseau B, Pujals A, et al. Polymerase proofreading domain mutations: new opportunities for immunotherapy in hypermutated colorectal cancer beyond MMR deficiency. Crit Rev Oncol Hematol 2017; 113: 242–248.

19. Domingo E, Freeman-Mills L, Rayner E, et al. Somatic POLE proofreading domain mutation, immune response, and prognosis in colorectal cancer: a retrospective, pooled biomarker study. Lancet Gastroenterol Hepatol 2016; 1: 207–216.

20. Hwang HS, Kim D and Choi J. Distinct mutational profile and immune microenvironment in microsatellite-unstable and POLE-mutated tumors. J Immunother Cancer 2020; 9: e002797.

21. Guinney J, Dienstmann R, Wang X, et al. The consensus molecular subtypes of colorectal cancer: a retrospective, pooled biomarker study. J Immunother Cancer 2016; 22: 4057–4066.

22. Carmona-Rodriguez L, Martinez-Rey D, Fernández-Aceñero MJ, et al. SOD3 induces a HIF-2α-dependent program in endothelial cells that provides a selective signal for tumor infiltration by T cells. J Immunother Cancer 2020; 8: e000432.

23. Chida K, Kawazoe A, Suzuki T, et al. Transcriptomic profiling of MSI-H/dMMR gastrointestinal tumors to identify determinants of responsiveness to anti-PD-1 therapy. Clin Cancer Res 2022; 28: 2110–2117.

24. Becht E, de Reyniès A, Giraldo NA, et al. Immune and stromal classification of colorectal cancer is associated with molecular subtypes and relevant for precision immunotherapy. Clin Cancer Res 2016; 22: 4057–4066.

25. Dienstmann R, Vermeulen L, Guinney J, et al. Consensus molecular subtypes and the evolution
of precision medicine in colorectal cancer. Nat Rev Cancer 2017; 17: 79–92.

26. Zhong F, Lin Y, Jing X, et al. Innate tumor killers in colorectal cancer. Cancer Lett 2022; 527: 115–126.

27. Craig SG, Humphries MP, Alderdice M, et al. Immune status is prognostic for poor survival in colorectal cancer patients and is associated with tumour hypoxia. Br J Cancer 2020; 123: 1280–1288.

28. Saito T, Nishikawa H, Wada H, et al. Two FOXP3(+)CD4(+) T cell subpopulations distinctly control the prognosis of colorectal cancers. Nat Med 2016; 22: 679–684.

29. Oshi M, Sarkar J, Wu R, et al. Intratumoral density of regulatory T cells is a predictor of host immune response and chemotherapy response in colorectal cancer. Am J Cancer Res 2022; 12: 490–503.

30. Yin J, Wang H, Hong Y, et al. Identification of an at-risk subpopulation with high immune infiltration based on the peroxisome pathway and TIM3 in colorectal cancer. BMC Cancer 2022; 22: 44.

31. Chen QY, Chen YX, Han QY, et al. Prognostic significance of immune checkpoints HLA-G/ILT-2/4 and PD-L1 in colorectal cancer. Front Immunol 2021; 12: 679090.

32. Makarem S, Asadzadeh Z, Hemmat N, et al. Immune checkpoint inhibitors in colorectal cancer: challenges and future prospects. Biomedicines 2021; 9: 1075.

33. Saleh R, Taha RZ, Toor SM, et al. Expression of immune checkpoints and T cell exhaustion markers in early and advanced stages of colorectal cancer. Cancer Immunol Immunother 2020; 69: 1989–1999.

34. Pagès F, Kirilovsky A, Mlecnik B, et al. In situ cytotoxic and memory T cells predict outcome in patients with early-stage colorectal cancer. J Clin Oncol 2009; 27: 5944–5951.

35. Mlecnik B, Bindea G, Angell HK, et al. Integrative analyses of colorectal cancer show immunoscore is a stronger predictor of patient survival than microsatellite instability. Immunity 2016; 44: 698–711.

36. Zou Q, Wang X, Ren D, et al. DNA methylation-based signature of CD8+ tumor-infiltrating lymphocytes enables evaluation of immune response and prognosis in colorectal cancer. J Immunother Cancer 2021; 9: e002671.

37. Wang L, Hui H, Agrawal K, et al. m(6) A RNA methyltransferases METTL3/14 regulate immune responses to anti-PD-1 therapy. Embo J 2020; 39: e104514.

38. Gao Y, Wang H, Chen S, et al. Single-cell N(6)-methyladenosine regulator patterns guide intercellular communication of tumor microenvironment that contribute to colorectal cancer progression and immunotherapy. J Transl Med 2022; 20: 197.

39. Chen H, Yao J, Bao R, et al. Cross-talk of four types of RNA modification writers defines tumor microenvironment and pharmacogenomic landscape in colorectal cancer. Mol Cancer 2021; 20: 29.

40. Liu Z, Lu T, Wang Y, et al. Establishment and experimental validation of an immune miRNA signature for assessing prognosis and immune landscape of patients with colorectal cancer. J Cell Mol Med 2021; 25: 6874–6886.

41. Cremonesi E, Governa V, Garzon JFG, et al. Gut microbiota modulate T cell trafficking into human colorectal cancer. Gut 2018; 67: 1984–1994.

42. Hale VL, Jeraldo P, Chen J, et al. Distinct microbes, metabolites, and ecologies define the microbiome in deficient and proficient mismatch repair colorectal cancers. Genome Med 2018; 10: 78.

43. Vétizou M, Pitt JM, Daillère R, et al. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. Science 2015; 350: 1079–1084.

44. Gur C, Ibrahim Y, Isaacson B, et al. Binding of the Pap2 protein of Fusobacterium nucleatum to human inhibitory receptor TIGIT protects tumors from immune cell attack. Immunity 2015; 42: 344–355.

45. Proença MA, Biselli JM, Succi M, et al. Relationship between Fusobacterium nucleatum, inflammatory mediators and microRNAs in colorectal carcinogenesis. World J Gastroenterol 2018; 24: 5351–5365.

46. Nosho K, Sukawa Y, Adachi Y, et al. Association of Fusobacterium nucleatum with immunity and molecular alterations in colorectal cancer. World J Gastroenterol 2016; 22: 557–566.

47. Hamada T, Zhang X, Mima K, et al. Fusobacterium nucleatum in colorectal cancer relates to immune response differentially by tumor microsatellite instability status. Cancer Immunol Res 2018; 6: 1327–1336.

48. Temraz S, Nassar F, Nasr R, et al. Gut microbiome: a promising biomarker for immunotherapy in colorectal cancer. Int J Mol Sci 2019; 20: 4155.
51. Mihaylova VT, Bindra RS, Yuan J, et al. Decreased expression of the DNA mismatch repair gene Mlh1 under hypoxic stress in mammalian cells. *Mol Cell Biol* 2003; 23: 3265–3273.

52. Chan N and Bristow RG. “Contextual” synthetic lethality and/or loss of heterozygosity: tumor hypoxia and modification of DNA repair. *Clin Cancer Res* 2010; 16: 4553–4560.

53. Bortolomeazzi M, Keddar MR, Montorsi L, et al. Immunogenomics of colorectal cancer response to checkpoint blockade: analysis of the KEYNOTE 177 trial and validation cohorts. *Gastroenterology* 2021; 161: 1179–1193.

54. Masuda K, Kornberg A, Miller J, et al. Multiplexed single-cell analysis reveals prognostic and nonprognostic T cell types in human colorectal cancer. *JCI Insight* 2022; 7: e154646.

55. Bruni D, Angell HK and Galon J. The immune contexture and Immunoscore in cancer prognosis and therapeutic efficacy. *Nat Rev Cancer* 2020; 20: 662–680.

56. Pagès F, Mlecnik B, Marliot F, et al. International validation of the consensus Immunoscore for the classification of colon cancer: a prognostic and accuracy study. *Lancet* 2018; 391: 2128–2139.

57. Ganesh K, Stanislav ZK, Cercek A, et al. Immunotherapy in colorectal cancer: rationale, challenges and potential. *Nat Rev Gastroenterol Hepatol* 2019; 16: 361–375.

58. Seidel JA, Otsuka A and Kabashima K. Anti-PD-1 and Anti-CTLA-4 therapies in cancer: mechanisms of action, efficacy, and limitations. *Front Oncol* 2018; 8: 86.

59. Thibaudin M, Limagne E, Hampe L, et al. Targeting PD-L1 and TIGIT could restore intratumoral CD8 T cell function in human colorectal cancer. *Cancer Immunol Immunother* 2022; 71: 2549–2563.

60. Ruan DY, Li T, Wang YN, et al. FTO downregulation mediated by hypoxia facilitates colorectal cancer metastasis. *Oncogene* 2021; 40: 5168–5181.

61. Ma C, Zhang X, Zhao X, et al. Predicting the survival and immune landscape of colorectal cancer patients using an immune-related lncRNA pair model. *Front Genet* 2021; 12: 690530.

62. Huang J, Liu H, Zhao Y, et al. MicroRNAs expression patterns predict tumor mutational burden in colorectal cancer. *Front Oncol* 2020; 10: 550986.

63. Song W, Ren J, Xiang R, et al. Cross-talk between m(6)A- and m(5)C-related lncRNAs...
to construct a novel signature and predict the immune landscape of colorectal cancer patients. *Front Immunol* 2022; 13: 740960.

74. Xu L, Huan L, Guo T, et al. LncRNA SNHG11 facilitates tumor metastasis by interacting with and stabilizing HIF-1α. *Oncogene* 2020; 39: 7005–7018.

75. Sun S, Xia C and Xu Y. HIF-1α induced lncRNA LINC00511 accelerates the colorectal cancer proliferation through positive feedback loop. *Biomed Pharmacother* 2020; 125: 110014.

76. Han N, Pan Z, Liu G, et al. Hypoxia: the “Invisible Pusher” of gut microbiota. *Front Microbiol* 2021; 12: 690600.

77. Gao Z, Guo B, Gao R, et al. Microbiota disbiosis is associated with colorectal cancer. *Front Microbiol* 2015; 6: 20.

78. Li K, Dan Z, Gesang L, et al. Comparative analysis of gut microbiota of native Tibetan and Han populations living at different altitudes. *PLoS One* 2016; 11: e0155863.

79. Sivan A, Corrales L, Hubert N, et al. Commensal Bifidobacterium promotes antitumor immunity and facilitates anti-PD-L1 efficacy. *Science* 2015; 350: 1084–1089.

80. Lopès A, Billard E, Casse AH, et al. Colibactin-positive Escherichia coli induce a procarcinogenic immune environment leading to immunotherapy resistance in colorectal cancer. *Int J Cancer* 2020; 146: 3147–3159.

81. Gao Y, Bi D, Xie R, et al. Fusobacterium nucleatum enhances the efficacy of PD-L1 blockade in colorectal cancer. *Signal Transduct Target Ther* 2021; 6: 398.

82. McGettrick AF and O’Neill LAJ. The role of HIF in immunity and inflammation. *Cell Metab* 2020; 32: 524–536.

83. Lee JW, Bae SH, Jeong JW, et al. Hypoxia-inducible factor (HIF-1α): its protein stability and biological functions. *Exp Mol Med* 2004; 36: 1–12.

84. Rasheed S, McDonald PJ, Northover JM, et al. Angiogenesis and hypoxic factors in colorectal cancer. *Pathol Res Pract* 2008; 204: 501–510.

85. Bannoud N, Dalotto-Moreno T, Kindgard L, et al. Hypoxia supports differentiation of terminally exhausted CD8 T cells. *Front Immunol* 2021; 12: 660944.

86. Liu Z, Zhao Q, Zheng Z, et al. Vascular normalization in immunotherapy: a promising mechanisms combined with radiotherapy. *Biomedicine & pharmacotherapy* 2021; 139: 111607.

87. Palazon A, Tyrikas PA, Macias D, et al. An HIF-1α/VEGF-A axis in cytotoxic T cells regulates tumor progression. *Cancer Cell* 2017; 32: 669. e5–683.e5.

88. Long J, Hu Z, Xue H, et al. Vascular endothelial growth factor (VEGF) impairs the motility and immune function of human mature dendritic cells through the VEGF receptor 2-RhoA-cofilin1 pathway. *Cancer Sci* 2019; 110: 2357–2367.

89. Zhang J, Zhu L, Fang J, et al. LRG1 modulates epithelial-mesenchymal transition and angiogenesis in colorectal cancer via HIF-1α activation. *J Exp Clin Cancer Res* 2016; 35: 29.

90. Greenhough A, Smartt HJ, Moore AE, et al. The COX-2/PGE2 pathway: key roles in the hallmark of cancer and adaptation to the tumour microenvironment. *Carcinogenesis* 2009; 30: 377–386.

91. Prima V, Kaliberova LN, Kaliberov S, et al. COX2/mPGES1/PGE2 pathway regulates PD-L1 expression in tumor-associated macrophages and myeloid-derived suppressor cells. *Proc Natl Acad Sci U S A* 2017; 114: 1117–1122.

92. Garufi A, Pistritto G, Ceci C, et al. Targeting COX-2/PGE(2) pathway in HIPK2 knockdown cancer cells: impact on dendritic cell maturation. *PLoS One* 2012; 7: e48342.

93. Chen LY, Wang L, Ren YX, et al. The circular RNA circ-ERBIN promotes growth and metastasis of colorectal cancer by miR-125a-5p and miR-138-5p/4EBP-1 mediated cap-independent HIF-1α translation. *Mol Cancer* 2020; 19: 164.

94. Xiang J, Sun H, Su L, et al. Myocyte enhancer factor 2D promotes colorectal cancer angiogenesis downstream of hypoxia-inducible factor 1α. *Cancer Lett* 2017; 400: 117–126.

95. Huang Z and Feng Y. Exosomes derived from hypoxic colorectal cancer cells promote angiogenesis through Wnt4-induced β-catenin signaling in endothelial cells. *Oncol Res* 2017; 25: 651–661.

96. Glaus Garzon JF, Pastrello C, Jurisica I, et al. Tumor cell endogenous HIF-1α activity induces aberrant angiogenesis and interacts with TRAF6 pathway required for colorectal cancer development. *Neoplasia* 2020; 22: 745–758.

97. Mortezaee K. Hypoxia induces core-to-edge transition of progressive tumoral cells: a critical review on differential yet corroborative roles for HIF-1α and HIF-2α. *Life Sci* 2020; 242: 117145.

98. McDonald PC, Chafe SC and Dedhar S. Overcoming hypoxia-mediated tumor progression: combinatorial approaches targeting PH regulation, angiogenesis and immune dysfunction. *Front Cell Dev Biol* 2016; 4: 27.
99. Hsu JM, Xia W, Hsu YH, et al. STT3-dependent PD-L1 accumulation on cancer stem cells promotes immune evasion. Nat Commun 2018; 9: 1908.

100. Yin S, Guo Y, Wen X, et al. Increased expression of PD-L1 in endometrial cancer stem-like cells is regulated by hypoxia. Front Biosci 2022; 27: 23.

101. Dai X, Guo Y, Hu Y, et al. Immunotherapy for targeting cancer stem cells in hepatocellular carcinoma. Theranostics 2021; 11: 3489–3501.

102. Muppala S. Significance of the tumor microenvironment in liver cancer progression. Crit Rev Oncog 2020; 25: 1–9.

103. Nappo G, Handle F, Santer FR, et al. The immunosuppressive cytokine interleukin-4 increases the clonogenic potential of prostate stem-like cells by activation of STAT6 signalling. Oncogenesis 2017; 6: e342.

104. Ye LY, Chen W, Bai XL, et al. Hypoxia-induced epithelial-to-mesenchymal transition in hepatocellular carcinoma induces an immunosuppressive tumor microenvironment to promote metastasis. Cancer Res 2016; 76: 818–830.

105. Wan S, Zhao E, Kryczek I, et al. Tumor-associated macrophages produce interleukin 6 and signal via STAT3 to promote expansion of human hepatocellular carcinoma stem cells. Gastroenterology 2014; 147: 1393–1404.

106. Katz SC, Bamboat ZM, Maker AV, et al. Regulatory T cell infiltration predicts outcome following resection of colorectal cancer liver metastases. Ann Surg Oncol 2013; 20: 946–955.

107. Clambey ET, McNamee EN, Westrich JA, et al. Hypoxia-inducible factor-1 alpha-dependent induction of FoxP3 drives regulatory T-cell abundance and function during inflammatory hypoxia of the mucosa. Proc Natl Acad Sci U S A 2012; 109: E2784–E2793.

108. Dang EV, Barbi J, Yang HY, et al. Control of T(H)17/T(reg) balance by hypoxia-inducible factor 1. Cell 2011; 146: 772–784.

109. Zhang Y, Chai N, Wei Z, et al. YYFZBJS inhibits colorectal tumorigenesis by enhancing Tregs-induced immunosuppression through HIF-1α mediated hypoxia in vivo and in vitro. Phytomedicine 2022; 98: 153917.

110. Yang S, Wang B, Guan C, et al. Foxp3+IL-17+ T cells promote development of cancer-initiating cells in colorectal cancer. J Leukoc Biol 2011; 89: 85–91.

111. Ma C and Dong X. Colorectal cancer-derived Foxp3(+) IL-17(+) T cells suppress tumour-specific CD8(+) T cells. Scand J Immunol 2011; 74: 47–51.

112. Qi L, Chen J, Yang Y, et al. Hypoxia correlates with poor survival and M2 macrophage infiltration in colorectal cancer. Front Oncol 2020; 10: 566430.

113. Alexander RK, Liou YH, Knudsen NH, et al. Bmal1 integrates mitochondrial metabolism and macrophage activation. Elife 2020; 9: e54090.

114. Wang Y, Yu G, Liu Y, et al. Hypoxia-induced PTTG3P contributes to colorectal cancer glycolysis and M2 phenotype of macrophage. Bioscience reports 2021; 41: BSR20210764.

115. Yang JB, Zhao ZB, Liu QZ, et al. FoxO1 is a regulator of MHC-II expression and anti-tumor effect of tumor-associated macrophages. Oncogene 2018; 37: 1192–1204.

116. Gojkovic M, Cunha PP, Darmasaputra GS, et al. Oxygen-mediated suppression of CD8(+) T cell proliferation by macrophages: role of pharmacological inhibitors of HIF degradation. Front Immunol 2021; 12: 633586.

117. Liu C, Zhang W, Wang J, et al. Tumor-associated macrophage-derived transforming growth factor-β promotes colorectal cancer progression through HIF1-TRIB3 signaling. Cancer Sci 2021; 112: 4198–4207.

118. Shang S, Yang YW, Chen F, et al. TRIB3 reduces CD8(+) T cell infiltration and induces immune evasion by repressing the STAT1-CXCL10 axis in colorectal cancer. Sci Transl Med 2022; 14: eabf0992.

119. Min AKT, Mimura K, Nakajima S, et al. Therapeutic potential of anti-VEGF receptor 2 therapy targeting for M2-tumor-associated macrophages in colorectal cancer. Cancer Immunol Immunother 2021; 70: 289–298.

120. Liu H, Liang Z, Zhou C, et al. Mutant KRAS triggers functional reprogramming of tumor-associated macrophages in colorectal cancer. Signal Transduct Target Ther 2021; 6: 144.

121. Wu JY, Huang TW, Hsieh YT, et al. Cancer-derived succinate promotes macrophage polarization and cancer metastasis via succinate receptor. Mol Cell 2020; 77: 213.e5–227.e5.

122. Wang T, Liu H, Lian G, et al. HIF1α-induced glycolysis metabolism is essential to the activation of inflammatory macrophages. Mediators Inflamm 2017; 2017: 9029327.

123. Takeda N, O’Dea EL, Doedens A, et al. Differential activation and antagonistic function...
of HIF-{alpha} isoforms in macrophages are essential for NO homeostasis. *Genes Dev* 2010; 24: 491–501.

124. Hou A, Hou K, Huang Q, et al. Targeting myeloid-derived suppressor cell, a promising strategy to overcome resistance to immune checkpoint inhibitors. *Front Immunol* 2020; 11: 783.

125. Liu G, Bi Y, Shen B, et al. SIRT1 limits the function and fate of myeloid-derived suppressor cells in tumors by orchestrating HIF-1α-dependent glycolysis. *Cancer Res* 2014; 74: 727–737.

126. Corzo CA, Condamine T, Lu L, et al. HIF-1α regulates function and differentiation of myeloid-derived suppressor cells in the tumor microenvironment. *J Exp Med* 2010; 207: 2439–2453.

127. Noman MZ, Janji B, Hu S, et al. Tumor-promoting effects of myeloid-derived suppressor cells are potentiated by hypoxia-induced expression of miR-210. *Cancer Res* 2015; 75: 3771–3787.

128. Zheng Y, Tian X, Wang T, et al. Long noncoding RNA PVT1 regulates the immunosuppression activity of granulocytic myeloid-derived suppressor cells in tumor-bearing mice. *Mol Cancer* 2019; 18: 61.

129. Wang Y, Yin K, Tian J, et al. Granulocytic myeloid-derived suppressor cells promote the stemness of colorectal cancer cells through exosomal S100A9. *Adv Sci* 2019; 6: 1901278.

130. Deng J, Li J, Sarde A, et al. Hypoxia-induced VISTA promotes the suppressive function of myeloid-derived suppressor cells in the tumor microenvironment. *Cancer Immunol Res* 2019; 7: 1079–1090.

131. Wang J, Yu F, Jia X, et al. MicroRNA-155 deficiency enhances the recruitment and functions of myeloid-derived suppressor cells in tumor microenvironment and promotes solid tumor growth. *Int J Cancer* 2015; 136: E602–E613.

132. Ogino T, Onishi H, Suzuki H, et al. Inclusive estimation of complex antigen presentation functions of monocyte-derived dendritic cells differentiated under normoxia and hypoxia conditions. *Cancer Immunol Immunother* 2012; 61: 409–424.

133. Monaci S, Coppola F, Giuntini G, et al. Hypoxia enhances the expression of RNASET2 in human monocyte-derived dendritic cells: role of PI3K/AKT pathway. *Int J Mol Sci* 2021; 22: 7564.

134. Pang L, Ng KT, Liu J, et al. Plasmacytoid dendritic cells recruited by HIF-1α/eADOID/ADORA1 signaling induce immunosuppression in hepatocellular carcinoma. *Cancer Lett* 2021; 522: 80–92.

135. Abou Khouzam R, Brodaczeskwa K, Filipiak A, et al. Tumor hypoxia regulates immune escape/invasion: influence on angiogenesis and potential impact of hypoxic biomarkers on cancer therapies. *Front Immunol* 2020; 11: 613114.

136. Liu J, Zhang X, Chen K, et al. CCR7 chemokine receptor-inducible Inc-dpf3 restrains dendritic cell migration by inhibiting HIF-1α-mediated glycolysis. *Immunity* 2019; 50: 600.e5–615.e5.

137. Chang WH and Lai AG. The hypoxic tumour microenvironment: a safe haven for immunosuppressive cells and a therapeutic barrier to overcome. *Cancer Lett* 2020; 487: 34–44.

138. Damgaci S, Ibrahim-Hashim A, Enzquez-Navas PM, et al. Hypoxia and acidosis: immune suppressors and therapeutic targets. *Immunology* 2018; 154: 354–362.

139. Jantsch J, Chakravortty D, Turza N, et al. Hypoxia and hypoxia-inducible factor-1 alpha module lipopolysaccharide-induced dendritic cell activation and function. *J Immunol* 2008; 180: 4697–4705.

140. Ni J, Wang X, Stojanovic A, et al. Single-cell RNA sequencing of tumor-infiltrating NK cells reveals that inhibition of transcription factor HIF-1α unleashes NK cell activity. *Immunity* 2020; 52: 1075.e8–1087.e8.

141. Balsamo M, Manzini C, Pietra G, et al. Hypoxia downregulates the expression of activating receptors involved in NK-cell-mediated target cell killing without affecting ADCC. *Eur J Immunol* 2013; 43: 2756–2764.

142. Gavert N, Conacci-Sorrell M, Gast D, et al. L1, a novel target of beta-catenin signaling, transforms cells and is expressed at the invasive front of colon cancers. *J Cell Biol* 2005; 168: 633–642.

143. Torres N, Regge MV, Secchiarli F, et al. Restoration of antitumor immunity through anti-MICA antibodies elicited with a chimeric protein. *J Immunother Cancer* 2020; 8: e000233.

144. Duan S, Guo W, Xu Z, et al. Natural killer group 2D receptor and its ligands in cancer immune escape. *Mol Cancer* 2019; 18: 29.

145. Leman JK, Sandford SK, Rhodes JL, et al. Multiparametric analysis of colorectal cancer immune responses. *World J Gastroenterol* 2018; 24: 2995–3005.
146. Zhang S, Zhong M, Wang C, et al. CCL5-deficiency enhances intratumoral infiltration of CD8(+) T cells in colorectal cancer. Cell Death Dis 2018; 9: 766.

147. Saleh R, Sasidharan Nair V, Toor SM, et al. Differential gene expression of tumor-infiltrating CD8(+) T cells in advanced versus early-stage colorectal cancer and identification of a gene signature of poor prognosis. J Immunother Cancer 2020; 8: e001294.

148. Kukita K, Tamura Y, Tanaka T, et al. Cancer-associated oxidase ERO1-α regulates the expression of MHC class I molecule via oxidative folding. J Immunol 2019; 194: 4988–4996.

149. de Almeida PE, Mak J, Hernandez G, et al. Anti-VEGF treatment enhances CD8(+) T-cell antitumor activity by amplifying hypoxia. Cancer Immunol Res 2020; 8: 806–818.

150. Gu M, Zhou X, Sohn JH, et al. NF-κB-inducing kinase maintains T cell metabolic fitness in antitumor immunity. Nat Immunol 2021; 22: 193–204.

151. Payen VL, Porporato PE, Baselet B, et al. Metabolic changes associated with tumor metastasis, part 1: tumor pH, glycolysis and the pentose phosphate pathway. Cell Mol Life Sci 2016; 73: 1333–1348.

152. Ding XC, Wang LL, Zhang XD, et al. The relationship between expression of PD-L1 and HIF-1α in glioma cells under hypoxia. J Hematol Oncol 2021; 14: 92.

153. Palsson-McDermott EM, Dyck L, Zaslona Z, et al. Pyruvate kinase M2 is required for the expression of the immune checkpoint PD-L1 in immune cells and tumors. Front Immunol 2017; 8: 1300.

154. Yang K, Zhang J and Bao C. Exosomal circEIF3K from cancer-associated fibroblasts promotes colorectal cancer (CRC) progression via miR-214/PD-L1 axis. BMC Cancer 2021; 21: 933.

155. Tamura R, Tanaka T, Akasaki Y, et al. The role of vascular endothelial growth factor in the hypoxic and immunosuppressive tumor microenvironment: perspectives for therapeutic implications. Med Oncol 2019; 37: 2.

156. Song Y, Fu Y, Xie Q, et al. Anti-angiogenic agents in combination with immune checkpoint inhibitors: a promising strategy for cancer treatment. Front Immunol 2020; 11: 1956.

157. Saurabh A, Chakraborty S, Kumar P, et al. Inhibiting HLA-G restores IFN-γ and TNF-α producing T cell in pleural tuberculosis. Tuberculosis 2018; 109: 69–79.

158. Mouillot G, Marcou C, Zidi I, et al. Hypoxia modulates HLA-G gene expression in tumor cells. Hum Immunol 2007; 68: 277–285.

159. Yaghi L, Poras I, Simoes RT, et al. Hypoxia inducible factor-1 mediates the expression of the immune checkpoint HLA-G in glioma cells through hypoxia response element located in exon 2. Oncotarget 2016; 7: 63690–63707.

160. Noman MZ, Hasmim M, Lequeux A, et al. Improving cancer immunotherapy by targeting the hypoxic tumor microenvironment: new opportunities and challenges. Cells 2019; 8: 1083.

161. Ge Y, Yoon SH, Jang H, et al. Decursin promotes HIF-1α proteasomal degradation and immune responses in hypoxic tumor microenvironment. Phytomedicine 2020; 78: 153318.

162. Wang Z, Li MY, Zhang ZH, et al. Panaxadiol inhibits programmed cell death-ligand 1 expression and tumour proliferation via hypoxia-inducible factor (HIF)-1α and STAT3 in human colon cancer cells. Pharmacol Res 2020; 155: 104727.

163. Bailey CM, Liu Y, Liu M, et al. Targeting HIF-1α abrogates PD-L1-mediated immune evasion in tumor microenvironment but promotes tolerance in normal tissues. J Clin Invest 2022; 132: e150846.

164. Wang Q, Gao J, Di W, et al. Anti-angiogenesis therapy overcomes the innate resistance to PD-1/PD-L1 blockade in VEGFA-overexpressed mouse tumor models. Cancer Immunol Immunother 2020; 69: 1781–1799.

165. Park S, Oh JH, Park DJ, et al. CU06-1004-induced vascular normalization improves immunotherapy by modulating tumor microenvironment via cytotoxic T cells. Front Immunol 2020; 11: 620166.

166. Zhu P, Wu Y, Yang A, et al. Catalpol suppressed proliferation, growth and invasion of CT26 colon cancer by inhibiting inflammation and tumor angiogenesis. Biomed Pharmacother 2017; 95: 68–76.

167. Wang Y, Wei B, Gao J, et al. Combination of fruquintinib and anti-PD-1 for the treatment of colorectal cancer. J Immunol 2020; 205: 2905–2915.

168. Chen G, Zhang S, Zhang R, et al. In situ tuning proangiogenic factor-mediated immunotolerance
synergizes the tumoricidal immunity via a hypoxia-triggerable liposomal bio-nanoreactor. *Theranostics* 2020; 10: 11998–12010.

169. Rahma OE, Tyan K, Giobbie-Hurder A, *et al.* Phase IB study of ziv-aflibercept plus pembrolizumab in patients with advanced solid tumors. *J Immunother Cancer* 2022; 10: e003569.

170. Li YC, Chen CH, Chang CL, *et al.* Melatonin and hyperbaric oxygen therapies suppress colorectal carcinogenesis through pleiotropic effects and multifaceted mechanisms. *Int J Biol Sci* 2021; 17: 3728–3744.

171. Ala M. The emerging role of metformin in the prevention and treatment of colorectal cancer: a game changer for management of colorectal cancer. *Curr Diabetes Rev* 2021; 18: e051121197762.

172. Fathi M, Bahmanpour S, Barshidi A, *et al.* Simultaneous blockade of TIGIT and HIF-1α induces synergistic anti-tumor effect and decreases the growth and development of cancer cells. *Int Immunopharmacol* 2021; 101: 108288.

173. Scharping NE, Menk AV, Whetstone RD, *et al.* Efficacy of PD-1 blockade is potentiated by metformin-induced reduction of tumor hypoxia. *Cancer Immunol Res* 2017; 5: 9–16.