BRITISH JOURNAL OF HOSPITAL MEDICINE

REVIEW

Advances in Immune Checkpoint Therapy in Hepatocellular Carcinoma

Yamei Huang[1], Weiping Yu[1,*]

[1]Department of Pathology and Pathophysiology, Medical School of Southeast University, Nanjing, Jiangsu, China
*Correspondence: wpylg@hotmail.com (Weiping Yu)

Abstract

The incidence and lethality of hepatocellular carcinoma (HCC) are increasing annually, and traditional treatments have been proven to be ineffective for patients with advanced stages of the disease. In recent years, immune checkpoint therapy has rapidly evolved, demonstrating promising results across a wide range of cancers and offering new hope for cancer treatment. However, the efficacy of immune checkpoint therapy in HCC varies greatly among individuals, with only a small proportion of HCC patients responding positively. A major cause of immune resistance and poor efficacy in HCC patients is immune evasion, which is often due to insufficient infiltration of immune cells. Understanding the mechanisms underlying immune evasion is crucial for enhancing the efficacy of immune therapies. In this review, we aim to summarize the mechanisms of immune evasion observed during immune checkpoint therapy and discuss future directions for this therapeutic approach. Our goal is to provide insights that could help overcome immune evasion, thereby improving the efficacy of immune therapies and extending patient survival time.

Key words: hepatocellular carcinoma (HCC); immune checkpoint therapy; immune evasion

Submitted: 11 June 2024 Revised: 16 July 2024 Accepted: 26 July 2024

Introduction

Liver cancer represents one of the most prevalent malignant tumours globally and ranks as the third leading cause of cancer-related mortality. Recent epidemiological data indicate an upward trend in both incidence and mortality rates of liver cancer. Annually, there are over 840,000 newly diagnosed cases and approximately 780,000 deaths attributed to liver cancer worldwide (Bray et al, 2024; Wangensteen and Chang, 2021). The pathological types of liver cancer include hepatocellular carcinoma (HCC), intrahepatic cholangiocarcinoma (ICC), and mixed types, of which HCC is the most common (Frager and Schwartz, 2020). Current treatments for HCC primarily include surgical resection, chemotherapy, and radiotherapy. However, these interventions typically extend patient survival by only three to six months, underscoring the urgent need for more effective therapeutic strategies to improve patient prognosis (Brown et al, 2023; Tang et al, 2020b). Over the past few decades, immunotherapy, particularly immune checkpoint therapy, has emerged as a promising avenue for cancer treatment, offering new hope for patients (Abbott and Ustoyev, 2019). Despite the significant advancements in immune checkpoint therapy for various tumour types, its efficacy in HCC remains limited, with only a small

How to cite this article:
Huang Y, Yu W. Advances in Immune Checkpoint Therapy in Hepatocellular Carcinoma. Br J Hosp Med. 2024. https://doi.org/10.12968/hmed.2024.0375

subset of patients exhibiting favourable responses. Immune evasion is a primary factor contributing to this limited efficacy (Yu, 2023). The precise mechanisms underlying immune evasion during immune checkpoint therapy in HCC patients are currently unknown. Therefore, it is necessary to investigate the molecular mechanisms driving immune evasion in HCC to develop more effective immunotherapy strategies.

Immunotherapy

Conventional treatment modalities for HCC have demonstrated efficacy in early-stage patients, but their effectiveness is limited in advanced disease, and patients may experience significant adverse effects during treatment (Liu et al, 2023; Yu, 2023). In recent years, immunotherapy has shown promising results in clinical trials, offering novel therapeutic options for HCC patients (Yu, 2023). Immunotherapy represents a paradigm shift in cancer treatment strategies by restoring immune surveillance, reactivating the anti-tumour activity of immune cells, and subsequently eliminating tumour cells. Compared to traditional treatments, immunotherapy offers several advantages, including precise targeting, fast efficacy, potential for cancer cure, minimal toxic side effects, and negligible damage to other organs in the body (Chen and Mellman, 2017; Li et al, 2023). Current immunotherapies can be categorized into four primary modalities:

(1) Immune checkpoint therapy: This strategy involves the use of monoclonal antibodies to block immune checkpoint proteins, such as programmed cell death protein-1 (PD-1) expressed on T cells, its ligand programmed cell death-ligand 1 (PD-L1) expressed on tumour cells, and cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) expressed on T cells. By disrupting these inhibitory signalling pathways, these therapies aim to reinvigorate exhausted T cells, particularly $CD8^+$ cytotoxic T lymphocytes, thereby enhancing their tumour-specific cytolytic function (Huang et al, 2024; Ning et al, 2023).

(2) Cell therapy: This approach encompasses various strategies, including chimeric antigen receptor T cell (CAR-T) therapy and T cell receptor-engineered T cell (TCR-T) therapy. These methods involve *ex vivo* expansion and genetic modification of autologous T cells to enhance their tumour-targeting capabilities, followed by reinfusion into the patient to elicit a robust anti-tumour response (Chen et al, 2024; June et al, 2018).

(3) Cancer vaccines: This modality utilizes tumour-associated antigens, delivered by various platforms such as nucleic acids or peptides, to stimulate and amplify tumour-specific T-cell responses *in vivo* (Pan et al, 2023).

(4) Cytokine therapy: This approach leverages the immunomodulatory properties of specific cytokines, including interleukin-2 (IL-2), interleukin-12 (IL-12), and interferon-gamma (IFN-γ). These cytokines can enhance the proliferation and activation of immune effector cells, promote the secretion of cytolytic molecules, and augment the overall anti-tumour immune response (Gladow et al, 2024).

Immunotherapy has demonstrated clinical efficacy in treating various cancers. For example, immune checkpoint therapy can significantly inhibit the progression

of cancers such as liver cancer, kidney cancer, melanoma, non-small cell lung cancer, and skin cancer, leading to substantial tumour reduction or even complete remission (Ren et al, 2024; Fan et al, 2023). Additionally, cell therapy has greatly relieved the symptoms in patients with haematological tumours such as acute lymphoblastic leukaemia and multiple myeloma, and reduced the relapse rate (Ravich et al, 2022; Ma et al, 2023). The rapid advancement of immunotherapy, despite demonstrating significant efficacy, has been accompanied by several challenges. These include the high costs associated with development and treatment, substantial individual variability in therapeutic response, the absence of specific biomarkers for efficacy monitoring, and the emergence of immune-related AEs (irAEs) (Kim et al, 2024; Pinter et al, 2023). Addressing these issues is imperative to enhance the effectiveness of immunotherapy and to provide hope for cancer patients.

Immune Checkpoint Therapy

Immune checkpoint therapy is a prominent immunotherapeutic approach for the treatment of HCC. By inhibiting the activity of immune checkpoints such as PD-1, PD-L1, and CTLA-4, this therapy releases "abducted" immune cells and increases the extent of immune infiltration. Consequently, it promotes the body's immune surveillance and cytotoxic response (Fig. 1) (Postow et al, 2015).

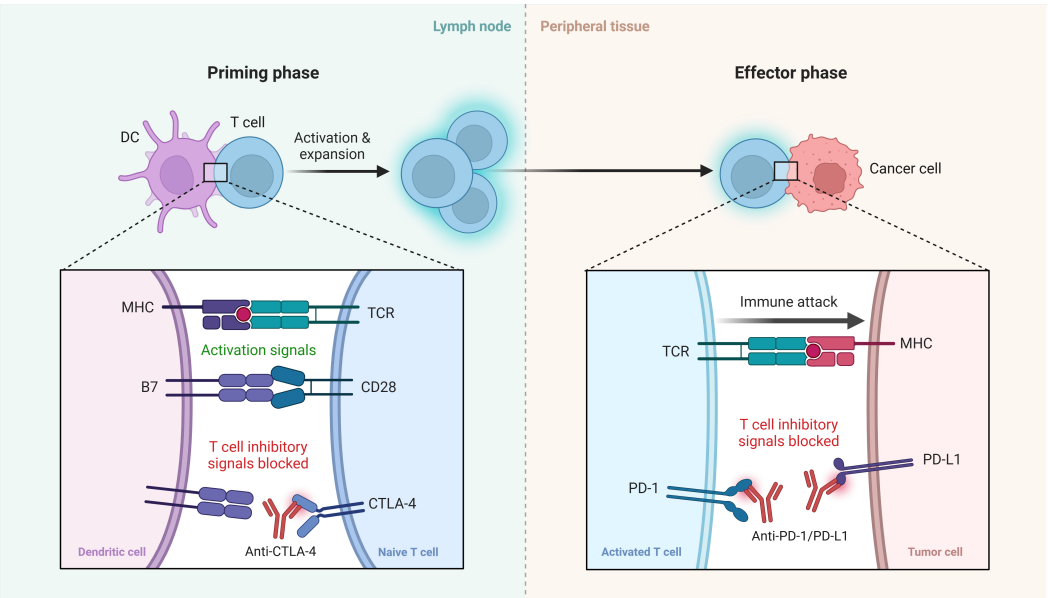

Fig. 1. Immune checkpoint therapy. This figure was drawn using BioRender software (BioRender-ing 101, DBA BioRender, Toronto Ontario, Canada). Abbreviations: PD-1, programmed cell death protein-1; PD-L1, programmed cell death-ligand 1; CTLA-4, cytotoxic T lymphocyte-associated antigen-4; TCR, T cell receptor; DC, dendritic cell; MHC, major histocompatibility complex; B7, hypothetical protein B7; CD28, CD28 molecule.

PD-1/PD-L1 Blockade Therapy

PD-1 is an immunomodulatory transmembrane receptor expressed on various immune cells, including T cells, dendritic cells (DCs), B cells, and natural killer (NK) cells. Its primary ligand, PD-L1, is predominantly expressed in tumour cells (Gao et al, 2024). The interaction between PD-1 and PD-L1 results in the suppression of T cell-mediated immune recognition and surveillance, thereby enabling PD-L1-expressing tumour cells to evade immune detection and proliferate rapidly (Han et al, 2020). PD-1 or PD-L1 antibodies can specifically disrupt this interaction, eliminating the immunosuppression of T cells. This intervention allows for the restoration of T cell-mediated recognition and cytotoxicity against tumour cells, ultimately reestablishing immune homeostasis (Salmaninejad et al, 2017; Bruni et al, 2020).

CTLA-4 Blockade Therapy

CTLA-4 is a negatively regulated transmembrane receptor predominantly expressed in T cells, particularly regulatory T cells (Tregs). It exhibits a high degree of genetic and structural homology with the co-stimulatory molecule receptor CD28 molecule (CD28), with which it competes for binding to the hypothetical protein B7 (B7) ligands (Van Coillie et al, 2020). In contrast to the activating function of B7/CD28 interactions, CTLA-4/CD28 inhibits the proliferation and differentiation of T cells, thereby attenuating the body's anti-tumour immune response. CTLA-4 antibody can block the competitive binding of CTLA-4 to B7 ligands, mitigating the immunosuppressive function of Tregs, increasing $CD8^+$ T cell infiltration, and consequently enhancing the overall immune response (Watanabe et al, 2023).

Clinical Advances in Immune Checkpoint Therapy

Currently, immune checkpoint therapy has shown promising results in clinical trials for HCC and has demonstrated superior efficacy compared to traditional treatment options in clinical applications. Consequently, it has been incorporated into first- and second-line therapeutic regimens for HCC. In 2017, the Food and Drug Administration (FDA) approved nivolumab, a PD-1 antibody, for clinical use. This approval has greatly improved the survival rates of patients with advanced HCC, including those resistant to sorafenib, and has exhibited a favourable safety profile (El-Khoueiry et al, 2017). Pembrolizumab, a PD-1 antibody, has demonstrated notable efficacy in patients with HCC who were previously treated with oxaliplatin or sorafenib but were intolerant to these therapies. It has been effective in improving symptoms, prolonging survival time, and enhancing the quality of life in patients with advanced disease (Zhu et al, 2018). Similarly, camrelizumab, another PD-1 antibody, has shown significant efficacy and improved survival outcomes in patients with unresectable HCC who were unable to undergo surgery. The PD-L1 inhibitor durvalizumab has shown promising efficacy, tolerability, and safety in patients with untreated unresectable HCC. In comparison to sorafenib, the PD-L1 inhibitor avelumab significantly prolonged overall survival in HCC patients without exacerbating hepatotoxicity. Atelizumab, another PD-L1 inhibitor, effectively improved symptoms and significantly extended overall survival in patients with in-

termediate to advanced HCC. The CTLA-4 antibody ipilimumab exhibited a high safety profile in patients with advanced HCC who continued to develop after sorafenib treatment, inhibiting tumour progression, alleviating symptoms, and prolonging overall survival (He et al, 2020; Hsu et al, 2019; Kudo et al, 2021; Kelley et al, 2021; Qin et al, 2020).

Limitations of Immune Checkpoint Therapy

Recent clinical applications of immune checkpoint therapy demonstrate significant clinical efficacy in advanced-stage HCC, especially in patients refractory to conventional treatment modalities. This therapeutic approach has shown promise in substantially delaying disease progression and markedly improving patients' quality of life (Nikoo et al, 2023). Immune checkpoint therapy has demonstrated significant efficacy; however, several challenges have emerged in its implementation. First, the high cost of this therapeutic approach presents a substantial economic barrier, rendering it inaccessible to a large proportion of the population (Li et al, 2023). Second, the lack of standardized protocols for the prevention and management of irAEs associated with immunotherapy may compromise treatment efficacy and patient outcomes. Most importantly, significant inter-individual variability exists in the efficacy of immune checkpoint therapy among HCC patients. Clinical data suggest that the overall response rate to this therapy in HCC patients is approximately 20% or lower, which is primarily attributed to immune evasion and drug resistance mechanisms, which are often associated with the lack of immune effector cells, particularly $CD8^+$ T lymphocytes, in the patient's body. Consequently, this leads to inadequate immune cell infiltration within the tumour microenvironment (TME) (Hegde and Chen, 2020). Therefore, the molecular mechanisms underlying immune evasion and drug resistance phenomena that affect the efficacy of immune checkpoint therapy are essential for improving immunotherapeutic outcomes and extending the benefits to a broader cohort of HCC patients.

Mechanisms of Immune Evasion and Immune Resistance

Deficiency of Tumour Antigens

Tumour antigens are novel antigenic components present on tumour cells that distinguish them from normal cells. These antigens arise from various mechanisms during HCC development, including genetic mutations, aberrant glycosylation, and viral infections. Tumour antigens can be broadly classified into two categories: tumour-specific antigens (TSAs) and tumour-associated antigens (TAAs). TSAs are unique to tumour cells absent in normal tissues, while TAAs are expressed in both tumour cells and normal cells (Sharma et al, 2017). T cells recognize tumour cells through these antigens, subsequently initiating an immune response. The effectiveness of immunotherapy in HCC patients is limited by insufficient tumour antigen presentation, which impairs T cell recognition of tumour cells and subsequent anti-tumour immune response induction. This contributes to the lack of therapeutic response observed in some patients undergoing immunotherapy (Havel

et al, 2019). Tumour mutation burden (TMB) is defined as the quantity of non-synonymous single nucleotide mutations in somatic cells within a specific genomic region of tumour tissue, as determined by whole-exon or targeted sequencing (Chan et al, 2019). TMB serves as an indicator of both tumour antigen expression and immune cell infiltration within tumour tissues (Hegde et al, 2016). There was a study that demonstrated a positive correlation between TMB and the production of tumour antigens, as well as the efficacy of immunotherapy (Mouw et al, 2017). In HCC, tumour antigens and TMB have been strongly associated with the effectiveness of immune checkpoint therapy, suggesting their potential as novel biomarkers for monitoring immunotherapeutic efficacy (Samstein et al, 2019).

Defects in Tumour Antigen Processing and Presentation Mechanisms

Upon recognition of tumour antigens produced by HCC cells, antigen-presenting cells (APCs) process these antigens through the major histocompatibility complex (MHC) class I pathway. The processed antigens are then loaded onto MHC class I molecules, which form a complex with β-2 microglobulin and calreticulin. This results in the formation of an antigenic peptide-MHC class I complex (Chen and Mellman, 2013). TCRs are specifically designed to recognize these antigenic peptide-MHC class I complexes. When T cells interact with APCs, they recognize the antigenic peptide-MHC class I complex and identify tumour cells through tumour-associated antigens, thereby initiating the body's immune response. However, the absence or reduced expression of MHC class I molecules, β-2 microglobulin, calreticulin, and other related proteins can impair the processing of tumour antigens by APCs, preventing the formation of effective complexes. This interference with T cell recognition function inhibits the immune response (Torrejon et al, 2020). In addition, reduced proteasome activity disrupts the antigen processing pathway mediated by MHC class I molecules, leading to decreased T-cell infiltration (Yamamoto et al, 2020). In summary, abnormalities in any component of the antigen processing, trafficking, and presentation cascade within tumour tissue can result in the attenuation of T cell function and a reduction in T cell population density. Consequently, these alterations may diminish the therapeutic efficacy of immune checkpoint inhibition in patients with HCC.

Dysfunction in DCs-T Cell Interactions

DCs represent the most specialized and efficient APC in the organism. They exhibit an abundance of antigen-presenting molecules, co-stimulatory molecules, and adhesion factors on the cell surface. These characteristics enable DCs to effectively internalize, process, and present antigens, while also processing intrinsic immunostimulatory capabilities to induce T cell proliferation and activation (Walsh et al, 2013). DCs provide critical signals necessary for T cell proliferation and activation through the expression of co-stimulatory molecules, including CD80 molecule (CD80), CD86 molecule (CD86), and CD40 molecule (CD40), which are highly expressed on their surface. This molecular interplay is essential for initiating and modulating the body's immune response (Logue and Sha, 2004). However, HCC cells competitively bind to the co-stimulatory molecules on DCs, thereby blocking

the signals necessary for T-cell activation and facilitating immune evasion (Lin et al, 2021). DCs play a crucial role in the TME by secreting chemokines that promote the aggregation of T cells, increasing immune infiltration in tumour tissues, and inducing an anti-tumour immune response (Haniffa et al, 2015). DCs have been demonstrated to secrete and produce chemokines, including C-C motif chemokine ligand 4 (CCL4), C-X-C motif chemokine ligand 9 (CXCL9), and C-X-C motif chemokine ligand 10 (CXCL10). These chemokines facilitate T cell recruitment into the TME by binding to their respective receptors expressed on T cells. DCs play an essential role in enhancing T-cell infiltration within the TME through the production of these chemokines (Spranger et al, 2017; Spranger and Gajewski, 2018). In addition, FMS-like tyrosine kinase 3 ligand (FLT3L) and granulocyte-macrophage colony-stimulating factor (GM-CSF) have been shown to significantly contribute to DC-mediated T cell recruitment (Demaria et al, 2019). High expression of FLT3L and GM-CSF enhances the ability of DCs to recruit T cells, while deficiency in either FLT3L or GM-CSF leads to a decrease in the number of DCs and impairs the recruitment of DCs (Lai et al, 2020). The interaction between DCs and T cells triggers the secretion of cytokines, including IFN-γ, interleukin-10 (IL-10), and IL-12. These cytokines not only promote the proliferation and activation of $CD8^+$ T cells but also inhibit tumour angiogenesis. Consequently, the proper interaction between DCs and T cells plays a crucial role in T cell proliferation and activation, as well as in the stimulation of antitumour immune response.

Immunosuppressive Properties of TME

TME is a complex and integrated system formed by the interaction of tumour cells, immune cells, mesenchymal cells, blood vessels, fibroblasts, and endothelial cells. The TME not only promotes the proliferation and migration of tumour cells but also helps tumour tissues escape from the surveillance of the immune system. This immunosuppressive function of the TME is a significant factor contributing to the development of therapeutic resistance and diminished efficacy of immunotherapy in HCC patients (Joyce and Fearon, 2015). The TME contains a variety of immune cells with immunosuppressive functions, which attenuate the body's immune response (Hegde et al, 2016). For example, Tregs, myeloid-derived suppressor cells (MDSCs), and M2 macrophages inhibit the activation of $CD8^+$ T cells and NK cells by secreting inhibitory molecules such as transforming growth factor-β (TGF-β), IL-10, and PD-L1. These molecules consequently limit the cytotoxic effects of $CD8^+$ T cells and NK cells on tumour tissues (Tauriello et al, 2018; Mariathasan et al, 2018). MDSCs and M2 macrophages inhibit the maturation and antigen presentation of DCs, disrupting the interactions between DCs and T cells, which are already limited in number and function. This interference reduces the immune system's ability to monitor and destroy tumour tissue (Li et al, 2019). Cancer-associated fibroblasts (CAFs) further inhibit immune cell function through the secretion of vascular endothelial growth factor (VEGF), C-X-C motif chemokine ligand 12 (CXCL12), and TGF-β, which diminishes the efficacy of immunotherapy and promotes tumour progression. Additionally, CAFs secrete substantial amounts of fibronectin and collagen, which fortify the extracellular matrix

(ECM) and reduce the penetration of immunotherapeutic drugs, thereby preventing these drugs from effectively reaching and acting on tumour tissues (Holmgaard et al, 2015). In addition, the hypoxic acidic environment of TME and metabolites such as lactic acid and pyruvic acid inhibit immune cell activity, leading to reduced immune infiltration in the TME and diminished efficacy of immunotherapy (Sahai et al, 2020; Stylianopoulos et al, 2018). Therefore, in HCC, the highly immunosuppressive TME is closely associated with poor prognosis in patients receiving immunotherapy.

Activation of Oncogenic Signalling Pathways

Numerous studies have shown a strong correlation between the activation of oncogenic signalling pathways in tumours and the development of immune evasion mechanisms and resistance to immunotherapy. For example, in melanoma, the activated Wnt/β-catenin signalling pathway negatively regulates the chemokine CCL4 (Xuan et al, 2021; Dong et al, 2024). This regulation occurs through the modulation of the transcriptional repressor activating transcription factor 3 (AFT3), leading to reduced expression of CCL4. The diminished CCL4 levels subsequently impair the recruitment of DCs into the TME. Furthermore, this cascade leads to decreased secretion of CXCL9 and CXCL10 by DCs within the TME. Ultimately, these alterations lead to a poor infiltration of cytotoxic T lymphocytes (CTLs) into the TME (Dieckmann et al, 2016). In addition, analysis of clinical data from melanoma patients receiving immunotherapy revealed an inverse correlation between β-catenin expression and several key immunological parameters, including DC infiltration, $CD8^+$ T cell levels, and patient survival outcomes (Spranger et al, 2015). By injecting DCs, a significant increase in CTL infiltration was observed in β-catenin-positive mice, leading to inhibited tumour growth and prolonged survival (Grasso et al, 2018). This study indicates that the activation of the Wnt/β-catenin signalling pathway and the absence of DCs are interacting factors contributing to melanoma's resistance to immunotherapy. In HCC, deletion of the phosphatase and tensin homolog (*PTEN*) gene results in constitutive activation of the phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) signalling pathway. This aberrant activation leads to enhanced lipoylation of microtubule-associated protein 1A/1B-light chain 3 (LC3), a key autophagy marker. Consequently, LC3 activity is attenuated, which in turn inhibits the T cell proliferation and activation. This cascade of events ultimately compromises the efficacy of immunotherapy in HCC. Analysis of The Cancer Genome Atlas (TCGA) database showed that HCC patients with low *PTEN* expression exhibited significantly reduced expression of effector T cell markers, such as IFN-γ and Granzyme B, compared to those with high *PTEN* expression. Additionally, these patients demonstrated markedly decreased $CD8^+$ T cell infiltration. These findings suggest that activation of the PI3K/AKT signalling pathway is a crucial factor in attenuating the anti-tumour immune response in HCC patients (Peng et al, 2016). Mutations in the Kirsten rat sarcoma viral oncogene homolog (*KRAS*) gene lead to the constitutive activation of the mitogen-activated protein kinase (MAPK) signalling pathway. This activation results in increased secretion of pro-inflammatory cytokines, including interleukin-6 (IL-6),

interleukin-8 (IL-8), and interleukin-23 (IL-23). Additionally, it triggers the activation of Nucleotide oligomerization domain (NOD), leucine-rich repeat (LRR) and pyrin domain-containing protein 3 (NLRP3) inflammatory vesicles. These molecular events collectively mediate inflammatory responses within the TME, ultimately promoting tumour progression (Hamarsheh et al, 2020). Co-activation of myelocytomatosis (MYC) and KRAS increases the expression of PD-L1 and CD47 in tumour cells. This upregulation disrupts antigen processing by DCs and leads to reduced immune infiltration within the TME. The mechanism involves competitive binding of CD47 to signal regulatory protein α (SIRPα) receptors on APCs (Jaiswal et al, 2009). The co-activation of MYC and KRAS induces the release of chemokines such as C-C motif chemokine 5 (CCL5) and C-C motif chemokine 9 (CCL9), which mediate the reprogramming of the TME and promote tumour angiogenesis. This process results in the exclusion of immune cells, such as DCs, NK cells, B cells, and T cells, from the TME (Kortlever et al, 2017). Additionally, the co-activation of signal transducer and activator of transcription 3 (STAT3) and Cyclin-Dependent Kinase 4/6 (CDK4/6) causes the activation of the janus kinase/signal transducer and activator of transcription (JAK/STAT) signalling pathway, which subsequently diminishes the number of T cells and inhibits their function through inflammatory pathways (Schaer et al, 2018). In summary, the activation of different oncogenic cell signalling pathways not only contributes to the pathogenesis of HCC but also affects the efficacy of immune checkpoint therapy.

Chemokines and Epigenetic Regulation

The interaction of chemokines with their corresponding receptors plays a crucial role in the recruitment of immune cells, including B cells, DCs, and T cells, to the TME, thereby increasing the extent of immune infiltration. There is a study that reports that insufficient secretion of specific chemokines, such as CCL4, CCL5, CXCL9, CXCL10, C-X-C motif chemokine ligand 16 (CXCL16), and C-X3-C motif chemokine ligand 1 (CX3CL1), is strongly correlated with reduced infiltration of DCs and T cells (Maimela et al, 2018). Low expression of these chemokines interferes with their binding to receptors expressed on different immune cells, attenuating the recruitment function of chemokines and consequently reducing immune cell infiltration within the TME. This alteration in the immune landscape potentially compromises the efficacy of immunotherapy (van der Woude et al, 2017). Furthermore, aberrant epigenetic regulation of tumours has been implicated in the modulation of chemokine secretion and expression. DNA methyltransferases (DNMTs) and zambian peer educators for human immunodeficiency virus (HIV) self-testing (ZEST) homologous enhancers 2 reduce the expression of CXCL9 and CXCL10 through DNA methylation and histone lysine methylation pathways (Peng et al, 2015). Similarly, the expression level of CCL5 is positively correlated with the number of $CD8^+$ T cells. However, DNA methylation also inhibits the secretion and expression of CCL5, thereby reducing its recruitment of $CD8^+$ T cells via the C-C chemokine receptor type 5 (CCR5) receptor, and consequently decreasing the infiltration of $CD8^+$ T cells in the TME (Dangaj et al, 2019). The combined application of DNMT inhibitors and histone deacetylase inhibitors can improve im-

mune resistance through the suppression of the MYC-activated MAPK signalling pathway. This inhibition leads to increased expression of CCL5 and stimulation of IFN-γ secretion, resulting in substantial T cell infiltration within the TME and improved immune resistance (Topper et al, 2017). However, certain chemokines exhibit inhibitory effects on T-cell recruitment. For instance, tumour tissues with high C-X-C motif chemokine ligand 8 (CXCL8) expression demonstrate a significant increase in neutrophil and monocyte populations, accompanied by a marked reduction in T cell numbers (Mortezaee, 2020). The chemokine CXCL12, secreted by CAFs, plays a crucial role in T cell exclusion from tumour tissue, thereby limiting their access to the tumour tissue and impeding their cytotoxic function (Schalper et al, 2020). In summary, chemokines and their epigenetic modulation are considered key factors influencing immune infiltration in tumour tissues and significantly influence the efficacy of immune checkpoint therapy in HCC patients.

Abnormalities of the Vascular System and Hypoxia

The vasculature of the tumour tissue plays a crucial role in facilitating T cell's entry into the TME. Consequently, the degree of T cell infiltration within the TME is significantly influenced by the characteristics of the tumour's vascular system. After entering the circulatory system through the tumour vasculature, T cells bind to vascular endothelial cells. This adhesion is mediated by surface adhesion molecules on the endothelial cells, including P-selectin, E-selectin, intercellular adhesion molecules (ICAMs), and vascular cell adhesion molecules (VCAMs). These interactions enable T cells to traverse the vascular wall and migrate into the tumour tissue effectively. When the expression of surface adhesion molecules is decreased or absent, the interaction of T cells with these adhesion molecules is impaired, resulting in a reduced number of T cells successfully migrating into the TME (Huang et al, 2018). Additionally, the binding of the endothelin B receptor to endothelin decreases the expression of intercellular adhesion molecule-1 (ICAM-1), which further hinders the infiltration of T cells through the vessel wall into the tumour tissue. VEGF secreted by tumours not only promotes the proliferation of vascular endothelial cells and angiogenesis, leading to impaired tissue perfusion and increased vascular permeability but also downregulates the expression of endothelial cell surface adhesion molecules, such as VCAM-1. This downregulation blocks the migration of T cells into the interior of the tumour tissue, consequently inhibiting T cell infiltration and accumulation within the TME (Apte et al, 2019). The cooperative action of VEGF, prostaglandin E2 (PGE2), and IL-10 induces the upregulation of Fas ligand (FasL) expression on endothelial cells, resulting in the apoptosis of T cells as they enter the vasculature. This mechanism directly contributes to a reduction in T-cell infiltration into tumour tissues. Inhibition of FasL signalling can reverse this T cell depletion effect, thereby facilitating T cell extravasation into the TME and enabling their immunological functions (Tang et al, 2020a). Cyclooxygenase (COX) and PGE2 inhibitors, such as acetylsalicylic acid, have been shown to increase T cell infiltration in the TME, potentially leading to improved patient survival prognosis (Sautès-Fridman et al, 2019). In addition, impaired vascular connectivity and increased permeability within the TME result in hypoxic and acidotic,

as well as necrosis. These factors collectively contribute to the suppression of T cell function and impairment of anti-tumour immune responses. Hypoxia is one of the characteristic hallmarks of cancer, primarily driven by two key factors: the rapid proliferation of cancer cells, which leads to excessive oxygen consumption and a substantial increase in oxygen demand, and an inadequate blood supply due to insufficient vasculogenesis within the TME (McDonald et al, 2016). The hypoxic TME inhibits T cell infiltration through multiple mechanisms. First, hypoxia-inducible factor 1 (HIF-1), activated under hypoxic conditions, facilitates the recruitment of immunosuppressive cells such as MDSCs, and Tregs into the tumour tissue, thereby enhancing immunosuppression within the TME (Damgaci et al, 2018). Second, hypoxia-induced secretion of C-C motif chemokine ligand 28 (CCL28) and VEGF by tumour cells not only promotes angiogenesis and supports cancer cell proliferation and migration but also interferes with T cell proliferation and migration (Allard et al, 2020). Third, under hypoxic conditions, TGF-β upregulates the expression of ectonucleoside triphosphate diphosphohydrolase 1 (ENTPD1) and 5'-nucleotidase ecto (NT5E) which catalyze the conversion of adenosine 5'-triphosphate (ATP) to adenosine (ADO). ADO binds to the adenosine A2A receptor (ADORA2A), inhibiting cytokine secretion (e.g., IL-2) and reducing T cell proliferation and activation, thus attenuating the anti-tumour immune response. In animal experiments, inhibition of the A2A receptor has been shown to significantly increase the number of T cells in mice, thereby effectively inhibiting tumour growth and prolonging survival time (Sek et al, 2018). ADO also inhibits the activity of NK cells and DCs, promotes the proliferation of MDSCs, and inhibits tumour-killing functions through an immunosuppressive milieu (Leone and Emens, 2018). In conclusion, abnormalities in the vascular system and a hypoxic environment can significantly influence the efficacy of immune checkpoint therapy in patients with HCC.

The Future of Immune Checkpoint Therapy

Strategies for Combination Therapy

The future development of immune checkpoint therapy for HCC patients should focus on combination strategies with other treatment modalities, including radiotherapy, chemotherapy, and targeted therapies. This approach aims to overcome the limitations of monotherapy by leveraging complementary mechanisms of action, to improve the efficacy of the therapy, reduce drug-resistant reactions, prolong patient survival, and improve quality of life (Galon and Bruni, 2019). For example, the combination of immune checkpoint therapy and CAR-T therapy can use antibodies to block the binding of negative signals, improve the immune infiltration in the TME, enhance the activity and efficacy of CAR-T, and contribute to more effective targeted elimination of tumour cells by CAR-T therapy (Adachi et al, 2018). The combined application of immune checkpoint therapy and chemotherapy can inhibit the proliferation and migration of tumour cells and promote the secretion of tumour antigens by altering the TME through chemotherapeutic agents, thus improving the recognition of tumour cells by T cells and enhancing the tumour-killing effect of immune checkpoint therapy on tumours (Galluzzi et al, 2020). The combination of

immune checkpoint inhibitors and radiotherapy demonstrates synergistic effects, inducing tumour cell apoptosis and promoting the development of immunological memory. This dual action not only enhances the immediate therapeutic efficacy but also reduces the probability of recurrence by establishing long-term immune surveillance (Li et al, 2020). Furthermore, the integration of immune checkpoints with targeted therapies can potentially counteract the immunosuppressive effects of oncogenic signalling pathways. This approach aims to restore immune surveillance, activate anti-tumour immune responses, and ultimately enhance the efficacy of immunotherapy (Kang et al, 2022; Luo et al, 2022).

Mechanisms for Blocking Immune Evasion

Immune evasion significantly influences the effectiveness of immune checkpoint therapy. Therefore, future advancements will focus on developing drugs and innovative therapies that address the molecular mechanisms underlying immune evasion in HCC patients. This approach aims to restore the body's immune surveillance capabilities against tumour cells and enhance the efficacy of immune checkpoint therapies in targeting and eliminating tumours. For instance, by inhibiting the activation of oncogenic signalling pathways, it is possible to mitigate the negative regulation exerted by certain cells on immune genes, thereby increasing the infiltration of immune cells in tumour tissues. Enhancing the expression of MHC class I molecules during immune checkpoint therapy can facilitate the proper recognition, processing, and presentation of tumour antigens, thereby augmenting the capacity of T cells to recognize and destroy tumour cells (Luo et al, 2018; Wallin et al, 2016). The development of inhibitors targeting immunosuppressive factors such as TGF-β and IL-10 (Vanpouille-Box et al, 2015), or reducing the number of immunosuppressive cells like Tregs and MDSCs, can mitigate the immunosuppressive properties of the TME. These approaches can potentially restore the proliferation and activity of DCs and T cells, ultimately enhancing the body's immune response (Bockorny et al, 2020). In addition, by improving hypoxia within tumour tissue, inhibiting angiogenesis, and promoting the secretion of chemokines, the nutrient supply of the tumour tissue can be disrupted. This, in turn, inhibits tumour proliferation and spread of the tumour while enhancing the ability of the immune cells to target and destroy tumour cells (Kumar and Chamoto, 2021).

Exploring New Immune Checkpoints

Due to the complexity of HCC pathogenesis and the diversity of factors affecting the efficacy of immune checkpoint therapy, future research must prioritize the identification and exploration of novel immune checkpoints. This approach is expected to benefit patients with HCC who exhibit resistance to current immune checkpoint therapies, such as PD-1, PD-L1, and CTLA-4 inhibitors. For instance, lymphocyte activation gene-3 (*LAG-3*), a member of the immunoglobulin superfamily, negatively regulates T cell proliferation and function. *LAG-3* plays an important role in tumour evasion from immune system surveillance (Paik, 2022). T cell immunoreceptor with Ig and ITIM domain (TIGIT), a member of the Ig superfamily, attenuates the immune response by inhibiting the cytotoxic activity of

NK cells and T cells against tumour cells, while simultaneously augmenting the immunosuppressive properties of Tregs (Harjunpää and Guillerey, 2020). T cell immunoglobulin and mucin domain-3 (TIM-3) are highly expressed on Tregs and stimulate their secretion of IL-10, which restricts the proliferation and activation of $CD8^+$ T cells, thus allowing tumour cells to escape from the immune system (Zhao et al, 2021). V-domain Ig suppressor of T cell activation (VISTA), which shares significant homology with PD-L1, inhibits cytokine secretion and T cell activation, thus promoting tumour progression (Huang et al, 2020). B and T lymphocyte attenuator (BTLA), a receptor structurally similar to PD-1, reduces the number of $CD8^+$ T-cells and attenuates the anti-tumour immune response by inhibiting the secretion of IFN-γ, tumour necrosis factor-α (TNF-α), and IL-2 (Ning et al, 2021). The development of therapeutic strategies targeting novel immune checkpoints could provide promising alternatives for HCC patients who exhibited limited responsiveness to current treatment modalities.

Biomarker Development

To assess the efficacy of therapy in HCC patients, it is crucial to observe whether treatment can effectively inhibit tumour growth. Biomarker testing can dynamically monitor the efficacy of the therapy and assess its effectiveness. For example, the expression level of PD-L1 on the surface of tumour cells indicates the proportion of tumour cell positivity, providing a more accurate reflection of the efficacy of immune checkpoint therapy. This makes PD-L1 a biomarker of considerable clinical value. Additionally, the degree of infiltration of DCs and $CD8^+$ T cells is positively correlated with the efficacy of immunotherapy and the patient's survival time. Immune cell markers, such as integrin subunit alpha X (CD11c) and cluster of differentiation 8 subunit alpha (CD8), can be used as biomarkers to predict and monitor immune efficacy (Peng et al, 2024). Although there are few studies on the application of TSA and TMB in HCC, it has been demonstrated in other cancer studies that the number of TSA and the degree of TMB are positively correlated with immunotherapy efficacy. Higher TSA counts and elevated TMB have been associated with an increased likelihood of favourable immunotherapy outcomes and improved clinical responses. These findings underscore the potential of TSA and TMB as valuable biomarkers for studying immunotherapy efficacy in HCC (Greten et al, 2023). The application of biomarkers for HCC immunotherapy is still in the exploratory and developmental stages. In future research, it is necessary to conduct comprehensive investigations into a broader spectrum of biomarkers, elucidating their potential and validating their clinical applicability. Concurrently, efforts should be directed towards the identification and development of novel biomarkers through single-cell sequencing, artificial intelligence, and other technologies to improve the accuracy of efficacy monitored through the detection of multiple markers.

Precision Medicine and Individualized Treatment Strategies

With the in-depth exploration of the pathogenesis of cancer and the molecular mechanism of immune resistance, future cancer treatment strategies will increas-

ingly adopt precision medicine and personalized approaches. High-throughput sequencing technologies, including genomics, proteomics, and metabolomics, will be employed to analyze patients' mutated genes and elucidate disease mechanisms, thereby identifying specific therapeutic targets. Individualized treatment plans will be developed for patients through imaging examinations, assessment of the immune microenvironment in the patient's body, genetic testing, and lifestyle analysis. During the treatment period, ctDNA, TMB, TSA, and other biomarkers are detected to dynamically monitor the efficacy of the treatment and adjust the treatment plan (Pauken et al, 2022; Tang et al, 2023). With the rapid advancements in sequencing technology and the era of big data, precision medicine and individualized treatment strategies are becoming integral to immunotherapy. These approaches aim to improve the efficacy of immunotherapy in HCC patients, reduce adverse effects, and improve disease outcomes.

Prevention and Management of irAEs

The adverse effects associated with immune checkpoint therapy, while generally less severe than those of conventional treatment modalities, can still significantly affect the prognosis and quality of life of patients with HCC. Therefore, before immunotherapy, the medical team should communicate fully with the patient to ensure a thorough understanding of the expected therapeutic outcomes and potential adverse effects. Additionally, a personalized nutritional plan should be developed, taking into account the patient's circumstances and physiological status. During the treatment period, patients should closely monitor changes in physical symptoms and promptly communicate any detected discomfort to the healthcare team. Adherence to prescribed nutritional supplementation is crucial, as is the avoidance of environments or substances that may increase the risk of infections. Regular clinical checkups and evaluations should be conducted to ensure optimal treatment efficacy and safety. Multidisciplinary healthcare teams should strengthen their cooperation and communication to effectively prevent, manage, and treat irAEs, mitigate patient discomfort and maintain quality of life (Blum et al, 2023; Esfahani et al, 2020).

Conclusion

Immune checkpoint inhibitors have emerged as a significant advancement in the treatment of HCC, establishing themselves as first-line and second-line treatment options for HCC patients. This approach has demonstrated considerable efficacy in managing disease progression for patients who are ineligible for surgical intervention and have shown resistance to chemotherapy and other drugs. The potential of immune checkpoints in achieving long-term disease control is particularly noteworthy. Despite its promise, immune checkpoint therapy faces several challenges. These include substantial inter-patient variability in treatment response, high associated costs, and the risk of irAEs. Nonetheless, this therapeutic strategy has exhibited significant superiority in extending overall survival and improving the quality of life for HCC patients compared to the previous standard-of-care treatments. In this review, we summarize the application of immune checkpoint therapy

in HCC, delve into the mechanisms of immune evasion, and propose future directions for the development of immune checkpoint therapy. With in-depth scientific research and rapid technological advances, a deeper understanding of the pathogenesis of cancer and the mechanism of immune evasion has been achieved. The efficacy of immune checkpoint therapy has been continuously optimized through combination therapies, the development of new immune checkpoints, the monitoring of biomarkers of immune efficacy, and precision medicine, thereby enabling more HCC patients to benefit from the therapy.

Key Points

- Immune evasion represents a primary factor contributing to the limited efficacy of immune checkpoint therapy in patients with HCC.
- The mechanisms underlying immune evasion in HCC are multifaceted and complex, mainly including insufficient tumour antigen presentation, impaired DC-T cell interactions, and activation of oncogenic signalling pathways.
- Improving the efficacy of immune checkpoint therapy in HCC necessitates multimodal approaches, with a particular focus on combination treatment strategies.

Availability of Data and Materials

All the data of this study are included in this article.

Author Contributions

YMH and WPY designed the manuscript. YMH drafted the manuscript. WPY revised the manuscript. Both authors contributed to the important editorial changes in the manuscript. Both authors read and approved the final manuscript. Both authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgement

Not applicable.

Funding

This research received no external funding.

Conflict of Interest

The authors declare no conflict of interest.

References

Abbott M, Ustoyev Y. Cancer and the Immune System: The History and Background of Immunotherapy. Seminars in Oncology Nursing. 2019; 35: 150923. https://doi.org/10.1016/j.soncn.2019.08.002

Adachi K, Kano Y, Nagai T, Okuyama N, Sakoda Y, Tamada K. IL-7 and CCL19 expression in CAR-T cells improves immune cell infiltration and CAR-T cell survival in the tumor. Nature Biotechnology. 2018; 36: 346–351. https://doi.org/10.1038/nbt.4086

Allard B, Allard D, Buisseret L, Stagg J. The adenosine pathway in immuno-oncology. Nature Reviews. Clinical Oncology. 2020; 17: 611–629. https://doi.org/10.1038/s41571-020-0382-2

Apte RS, Chen DS, Ferrara N. VEGF in Signaling and Disease: Beyond Discovery and Development. Cell. 2019; 176: 1248–1264. https://doi.org/10.1016/j.cell.2019.01.021

Blum SM, Rouhani SJ, Sullivan RJ. Effects of immune-related adverse events (irAEs) and their treatment on antitumor immune responses. Immunological Reviews. 2023; 318: 167–178. https://doi.org/10.1111/imr.13262

Bockorny B, Semenisty V, Macarulla T, Borazanci E, Wolpin BM, Stemmer SM, et al. BL-8040, a CXCR4 antagonist, in combination with pembrolizumab and chemotherapy for pancreatic cancer: the COMBAT trial. Nature Medicine. 2020; 26: 878–885. https://doi.org/10.1038/s41591-020-0880-x

Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal for Clinicians. 2024; 74: 229–263. https://doi.org/10.3322/caac.21834

Brown ZJ, Tsilimigras DI, Ruff SM, Mohseni A, Kamel IR, Cloyd JM, et al. Management of Hepatocellular Carcinoma: A Review. JAMA Surgery. 2023; 158: 410–420. https://doi.org/10.1001/jamasurg.2022.7989

Bruni D, Angell HK, Galon J. The immune contexture and Immunoscore in cancer prognosis and therapeutic efficacy. Nature Reviews. Cancer. 2020; 20: 662–680. https://doi.org/10.1038/s41568-020-0285-7

Chan TA, Yarchoan M, Jaffee E, Swanton C, Quezada SA, Stenzinger A, et al. Development of tumor mutation burden as an immunotherapy biomarker: utility for the oncology clinic. Annals of Oncology. 2019; 30: 44–56. https://doi.org/10.1093/annonc/mdy495

Chen A, Yu Z, Ma N, Lu X, Zhang Y, Xu W, et al. Atovaquone enhances antitumor efficacy of TCR-T therapy by augmentation of ROS-induced ferroptosis in hepatocellular carcinoma. Cancer Immunology, Immunotherapy. 2024; 73: 49. https://doi.org/10.1007/s00262-024-03628-2

Chen DS, Mellman I. Elements of cancer immunity and the cancer-immune set point. Nature. 2017; 541: 321–330. https://doi.org/10.1038/nature21349

Chen DS, Mellman I. Oncology meets immunology: the cancer-immunity cycle. Immunity. 2013; 39: 1–10. https://doi.org/10.1016/j.immuni.2013.07.012

Damgaci S, Ibrahim-Hashim A, Enriquez-Navas PM, Pilon-Thomas S, Guvenis A, Gillies RJ. Hypoxia and acidosis: immune suppressors and therapeutic targets. Immunology. 2018; 154: 354–362. https://doi.org/10.1111/imm.12917

Dangaj D, Bruand M, Grimm AJ, Ronet C, Barras D, Duttagupta PA, et al. Cooperation between Constitutive and Inducible Chemokines Enables T Cell Engraftment and Immune Attack in Solid Tumors. Cancer Cell. 2019; 35: 885–900.e10. https://doi.org/10.1016/j.ccell.2019.05.004

Dong H, Zhou S, Chen X, Deng X, Fang A. Pan-cancer analysis of the prognostic significance of *ACKR2* expression and the related genetic/epigenetic dysregulations. Expert Review of Clinical Immunology. 2024; 20: 225–236. https://doi.org/10.1080/1744666X.2023.2274361

Demaria O, Cornen S, Daëron M, Morel Y, Medzhitov R, Vivier E. Harnessing innate immunity in cancer therapy. Nature. 2019; 574: 45–56. https://doi.org/10.1038/s41586-019-1593-5

Dieckmann NMG, Frazer GL, Asano Y, Stinchcombe JC, Griffiths GM. The cytotoxic T lymphocyte immune synapse at a glance. Journal of Cell Science. 2016; 129: 2881–2886. https://doi.org/10.1242/jcs.186205

El-Khoueiry AB, Sangro B, Yau T, Crocenzi TS, Kudo M, Hsu C, et al. Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): an open-label, non-comparative, phase 1/2 dose escalation and expansion trial. Lancet. 2017; 389: 2492–2502. https://doi.org/10.1016/S0140-6736(17)31046-2

Esfahani K, Elkrief A, Calabrese C, Lapointe R, Hudson M, Routy B, et al. Moving towards personalized treatments of immune-related adverse events. Nature Reviews. Clinical Oncology. 2020; 17: 504–515. https://doi.org/10.1038/s41571-020-0352-8

Fan Q, Li Z, Yin J, Xie M, Cui M, Fan C, et al. Inhalable pH-responsive DNA tetrahedron nanoplatform for boosting anti-tumor immune responses against metastatic lung cancer. Biomaterials. 2023; 301: 122283. https://doi.org/10.1016/j.biomaterials.2023.122283

Frager SZ, Schwartz JM. Hepatocellular carcinoma: epidemiology, screening, and assessment of hepatic reserve. Current Oncology. 2020; 27: S138–S143. https://doi.org/10.3747/co.27.7181

Gao M, Shi J, Xiao X, Yao Y, Chen X, Wang B, et al. PD-1 regulation in immune homeostasis and immunotherapy. Cancer Letters. 2024; 588: 216726. https://doi.org/10.1016/j.canlet.2024.216726

Galluzzi L, Humeau J, Buqué A, Zitvogel L, Kroemer G. Immunostimulation with chemotherapy in the era of immune checkpoint inhibitors. Nature Reviews. Clinical Oncology. 2020; 17: 725–741. https://doi.org/10.1038/s41571-020-0413-z

Galon J, Bruni D. Approaches to treat immune hot, altered and cold tumours with combination immunotherapies. Nature Reviews. Drug Discovery. 2019; 18: 197–218. https://doi.org/10.1038/s41573-018-0007-y

Gladow N, Hollmann C, Weirather J, Ding X, Burkard M, Uehlein S, et al. Role of $CD4^+$ T-cells for regulating splenic myelopoiesis and monocyte differentiation after experimental myocardial infarction. Basic Research in Cardiology. 2024; 119: 261–275. https://doi.org/10.1007/s00395-024-01035-3

Grasso CS, Giannakis M, Wells DK, Hamada T, Mu XJ, Quist M, et al. Genetic Mechanisms of Immune Evasion in Colorectal Cancer. Cancer Discovery. 2018; 8: 730–749. https://doi.org/10.1158/2159-8290.CD-17-1327

Greten TF, Villanueva A, Korangy F, Ruf B, Yarchoan M, Ma L, et al. Biomarkers for immunotherapy of hepatocellular carcinoma. Nature Reviews. Clinical Oncology. 2023; 20: 780–798. https://doi.org/10.1038/s41571-023-00816-4

Hamarsheh S, Groß O, Brummer T, Zeiser R. Immune modulatory effects of oncogenic KRAS in cancer. Nature Communications. 2020; 11: 5439. https://doi.org/10.1038/s41467-020-19288-6

Han Y, Liu D, Li L. PD-1/PD-L1 pathway: current researches in cancer. American Journal of Cancer Research. 2020; 10: 727–742.

Haniffa M, Bigley V, Collin M. Human mononuclear phagocyte system reunited. Seminars in Cell & Developmental Biology. 2015; 41: 59–69. https://doi.org/10.1016/j.semcdb.2015.05.004

Harjunpää H, Guillerey C. TIGIT as an emerging immune checkpoint. Clinical and Experimental Immunology. 2020; 200: 108–119. https://doi.org/10.1111/cei.13407

Havel JJ, Chowell D, Chan TA. The evolving landscape of biomarkers for checkpoint inhibitor immunotherapy. Nature Reviews. Cancer. 2019; 19: 133–150. https://doi.org/10.1038/s41568-019-0116-x

He AR, Yau T, Hsu C, Kang Y, Kim T, Santoro A, et al. Nivolumab (NIVO) + ipilimumab (IPI) combination therapy in patients (pts) with advanced hepatocellular carcinoma (aHCC): Subgroup analyses from CheckMate 040. Journal of Clinical Oncology. 2020; 38. https://doi.org/10.1200/JCO.2020.38.4_suppl.512

Hegde PS, Chen DS. Top 10 Challenges in Cancer Immunotherapy. Immunity. 2020; 52: 17–35. https://doi.org/10.1016/j.immuni.2019.12.011

Hegde PS, Karanikas V, Evers S. The Where, the When, and the How of Immune Monitoring for Cancer Immunotherapies in the Era of Checkpoint Inhibition. Clinical Cancer Research. 2016; 22: 1865–1874. https://doi.org/10.1158/1078-0432.CCR-15-1507

Holmgaard RB, Zamarin D, Li Y, Gasmi B, Munn DH, Allison JP, et al. Tumor-Expressed IDO Recruits and Activates MDSCs in a Treg-Dependent Manner. Cell Reports. 2015; 13: 412–424. https://doi.org/10.1016/j.celrep.2015.08.077

Hsu CH, Lee MS, Lee KH, Numata K, Stein S, Verret W, et al. Randomised efficacy and safety results for atezolizumab (Atezo)+ bevacizumab (Bev) in patients (pts) with previously untreated, unresectable hepatocellular carcinoma (HCC). Annals of Oncology. 2019; 30: ix187.

Huang X, Zhang X, Li E, Zhang G, Wang X, Tang T, et al. VISTA: an immune regulatory protein checking tumor and immune cells in cancer immunotherapy. Journal of Hematology & Oncology. 2020; 13: 83. https://doi.org/10.1186/s13045-020-00917-y

Huang Y, Chen Z, Shen G, Fang S, Zheng J, Chi Z, et al. Immune regulation and the tumor microenvironment in anti-PD-1/PDL-1 and anti-CTLA-4 therapies for cancer immune evasion: A bibliometric analysis. Human Vaccines & Immunotherapeutics. 2024; 20: 2318815. https://doi.org/10.1080/21645515.2024.2318815

Huang Y, Kim BYS, Chan CK, Hahn SM, Weissman IL, Jiang W. Improving immune-vascular crosstalk for cancer immunotherapy. Nature Reviews. Immunology. 2018; 18: 195–203. https://doi.org/10.1038/nri.2017.145

Jaiswal S, Jamieson CHM, Pang WW, Park CY, Chao MP, Majeti R, et al. CD47 is upregulated on circulating hematopoietic stem cells and leukemia cells to avoid phagocytosis. Cell. 2009; 138: 271–285. https://doi.org/10.1016/j.cell.2009.05.046

Joyce JA, Fearon DT. T cell exclusion, immune privilege, and the tumor microenvironment. Science. 2015; 348: 74–80. https://doi.org/10.1126/science.aaa6204

June CH, O'Connor RS, Kawalekar OU, Ghassemi S, Milone MC. CAR T cell immunotherapy for human cancer. Science. 2018; 359: 1361–1365. https://doi.org/10.1126/science.aar6711

Kang YK, Chen LT, Ryu MH, Oh DY, Oh SC, Chung HC, et al. Nivolumab plus chemotherapy versus placebo plus chemotherapy in patients with HER2-negative, untreated, unresectable advanced or recurrent gastric or gastro-oesophageal junction cancer (ATTRACTION-4): a randomised, multicentre, double-blind, placebo-controlled, phase 3 trial. The Lancet. Oncology. 2022; 23: 234–247. https://doi.org/10.1016/S1470-2045(21)00692-6

Kelley RK, Sangro B, Harris W, Ikeda M, Okusaka T, Kang YK, et al. Safety, Efficacy, and Pharmacodynamics of Tremelimumab Plus Durvalumab for Patients With Unresectable Hepatocellular Carcinoma: Randomized Expansion of a Phase I/II Study. Journal of Clinical Oncology. 2021; 39: 2991–3001. https://doi.org/10.1200/JCO.20.03555

Kim EJ, Kim YJ, Heo JY, Kim M, Lee S, Seo S, et al. Detection and evaluation of signals for immune-related adverse events: a nationwide, population-based study. Frontiers in Oncology. 2024; 13: 1295923. https://doi.org/10.3389/fonc.2023.1295923

Kortlever RM, Sodir NM, Wilson CH, Burkhart DL, Pellegrinet L, Brown Swigart L, et al. Myc Cooperates with Ras by Programming Inflammation and Immune Suppression. Cell. 2017; 171: 1301–1315.e14. https://doi.org/10.1016/j.cell.2017.11.013

Kudo M, Motomura K, Wada Y, Inaba Y, Sakamoto Y, Kurosaki M, et al. Avelumab in Combination with Axitinib as First-Line Treatment in Patients with Advanced Hepatocellular Carcinoma: Results from the Phase 1b VEGF Liver 100 Trial. Liver Cancer. 2021; 10: 249–259. https://doi.org/10.1159/000514420

Kumar A, Chamoto K. Immune metabolism in PD-1 blockade-based cancer immunotherapy. International Immunology. 2021; 33: 17–26. https://doi.org/10.1093/intimm/dxaa046

Lai J, Mardiana S, House IG, Sek K, Henderson MA, Giuffrida L, et al. Adoptive cellular therapy with T cells expressing the dendritic cell growth factor Flt3L drives epitope spreading and antitumor immunity. Nature Immunology. 2020; 21: 914–926. https://doi.org/10.1038/s41590-020-0676-7

Li J, Zeng H, Li L, Yang Q, He L, Dong M. Advanced Generation Therapeutics: Biomimetic Nanodelivery System for Tumor Immunotherapy. ACS Nano. 2023; 17: 24593–24618. https://doi.org/10.1021/acsnano.3c10212

Li X, Lovell JF, Yoon J, Chen X. Clinical development and potential of photothermal and photodynamic therapies for cancer. Nature Reviews. Clinical Oncology. 2020; 17: 657–674. https://doi.org/10.1038/s41571-020-0410-2

Li X, Wenes M, Romero P, Huang SCC, Fendt SM, Ho PC. Navigating metabolic pathways to enhance antitumour immunity and immunotherapy. Nature Reviews. Clinical Oncology. 2019; 16: 425–441. https://doi.org/10.1038/s41571-019-0203-7

Lin H, Kryczek I, Li S, Green MD, Ali A, Hamasha R, et al. Stanniocalcin 1 is a phagocytosis checkpoint driving tumor immune resistance. Cancer Cell. 2021; 39: 480–493.e6. https://doi.org/10.1016/j.ccell.2020.12.023

Liu J, Park K, Shen Z, Lee H, Geetha P, Pakyari M, et al. Immunotherapy, targeted therapy, and their cross talks in hepatocellular carcinoma. Frontiers in Immunology. 2023; 14: 1285370. https://doi.org/10.3389/fimmu.2023.1285370

Logue EC, Sha WC. CD28-B7 bidirectional signaling: a two-way street to activation. Nature Immunology. 2004; 5: 1103–1105. https://doi.org/10.1038/ni1104-1103

Luo J, Li L, Zhu Z, Chang B, Deng F, Wang D, et al. Fucoidan inhibits EGFR redistribution and potentiates sorafenib to overcome sorafenib-resistant hepatocellular carcinoma. Biomedicine & Pharmacotherapy. 2022; 154: 113602. https://doi.org/10.1016/j.biopha.2022.113602

Luo N, Nixon MJ, Gonzalez-Ericsson PI, Sanchez V, Opalenik SR, Li H, et al. DNA methyltransferase inhibition upregulates MHC-I to potentiate cytotoxic T lymphocyte responses in breast cancer. Nature Communications. 2018; 9: 248. https://doi.org/10.1038/s41467-017-02630-w

Leone RD, Emens LA. Targeting adenosine for cancer immunotherapy. Journal for Immunotherapy of Cancer. 2018; 6: 57. https://doi.org/10.1186/s40425-018-0360-8

Ma XC, Lv X, Li Y. Development of CD30 CAR-T cells in refractory or relapsed Hodgkin's lymphoma. Expert Review of Hematology. 2023; 16: 1017–1023. https://doi.org/10.1080/17474086.2023.2276210

Maimela NR, Liu S, Zhang Y. Fates of CD8+ T cells in Tumor Microenvironment. Computational and Structural Biotechnology Journal. 2018; 17: 1–13. https://doi.org/10.1016/j.csbj.2018.11.004

Mariathasan S, Turley SJ, Nickles D, Castiglioni A, Yuen K, Wang Y, et al. TGFβ attenuates tumour response to PD-L1 blockade by contributing to exclusion of T cells. Nature. 2018; 554: 544–548. https://doi.org/10.1038/nature25501

McDonald PC, Chafe SC, Dedhar S. Overcoming Hypoxia-Mediated Tumor Progression: Combinatorial Approaches Targeting pH Regulation, Angiogenesis and Immune Dysfunction. Frontiers in Cell and Developmental Biology. 2016; 4: 27. https://doi.org/10.3389/fcell.2016.00027

Mortezaee K. CXCL12/CXCR4 axis in the microenvironment of solid tumors: A critical mediator of metastasis. Life Sciences. 2020; 249: 117534. https://doi.org/10.1016/j.lfs.2020.117534

Mouw KW, Goldberg MS, Konstantinopoulos PA, D'Andrea AD. DNA Damage and Repair Biomarkers of Immunotherapy Response. Cancer Discovery. 2017; 7: 675–693. https://doi.org/10.1158/2159-8290.CD-17-0226

Nikoo M, Hassan ZF, Mardasi M, Rostamnezhad E, Roozbahani F, Rahimi S, et al. Hepatocellular carcinoma (HCC) immunotherapy by anti-PD-1 monoclonal antibodies: A rapidly evolving strategy. Pathology, Research and Practice. 2023; 247: 154473. https://doi.org/10.1016/j.prp.2023.154473

Ning J, Hou X, Hao J, Zhang W, Shi Y, Huang Y, et al. METTL3 inhibition induced by M2 macrophage-derived extracellular vesicles drives anti-PD-1 therapy resistance via M6A-CD70-mediated immune suppression in thyroid cancer. Cell Death and Differentiation. 2023; 30: 2265–2279. https://doi.org/10.1038/s41418-023-01217-x

Ning Z, Liu K, Xiong H. Roles of BTLA in Immunity and Immune Disorders. Frontiers in Immunology. 2021; 12: 654960. https://doi.org/10.3389/fimmu.2021.654960

Paik J. Nivolumab Plus Relatlimab: First Approval. Drugs. 2022; 82: 925–931. https://doi.org/10.1007/s40265-022-01723-1

Pan S, Fan R, Han B, Tong A, Guo G. The potential of mRNA vaccines in cancer nanomedicine and immunotherapy. Trends in Immunology. 2023; 45: 20–31. https://doi.org/10.1016/j.it.2023.11.003

Pauken KE, Lagattuta KA, Lu BY, Lucca LE, Daud AI, Hafler DA, et al. TCR-sequencing in cancer and autoimmunity: barcodes and beyond. Trends in Immunology. 2022; 43: 180–194. https://doi.org/10.1016/j.it.2022.01.002

Peng D, Kryczek I, Nagarsheth N, Zhao L, Wei S, Wang W, et al. Epigenetic silencing of TH1-type chemokines shapes tumour immunity and immunotherapy. Nature. 2015; 527: 249–253. https://doi.org/10.1038/nature15520

Peng W, Chen JQ, Liu C, Malu S, Creasy C, Tetzlaff MT, et al. Loss of PTEN Promotes Resistance to T Cell-Mediated Immunotherapy. Cancer Discovery. 2016; 6: 202–216. https://doi.org/10.1158/2159-8290.CD-15-0283

Peng X, Lu X, Yang D, Liu J, Wu H, Peng H, et al. A novel CD8+ T cell-related gene signature as a prognostic biomarker in hepatocellular carcinoma. Medicine. 2024; 103: e37496. https://doi.org/10.1097/MD.0000000000037496

Pinter M, Scheiner B, Pinato DJ. Immune checkpoint inhibitors in hepatocellular carcinoma: emerging challenges in clinical practice. The Lancet. Gastroenterology & Hepatology. 2023; 8: 760–770. https://doi.org/10.1016/S2468-1253(23)00147-4

Postow MA, Callahan MK, Wolchok JD. Immune Checkpoint Blockade in Cancer Therapy. Journal of Clinical Oncology. 2015; 33: 1974–1982. https://doi.org/10.1200/JCO.2014.59.4358

Qin S, Ren Z, Meng Z, Chen Z, Chai X, Xiong J, et al. Camrelizumab in patients with previously treated advanced hepatocellular carcinoma: a multicentre, open-label, parallel-group, randomised, phase 2 trial. The Lancet. Oncology. 2020; 21: 571–580. https://doi.org/10.1016/S1470-2045(20)30011-5

Ravich JW, Huang S, Zhou Y, Brown P, Pui CH, Inaba H, et al. Impact of High Disease Burden on Survival in Pediatric Patients with B-ALL Treated with Tisagenlecleucel. Transplantation and Cellular Therapy. 2022; 28: 73.e1–73.e9. https://doi.org/10.1016/j.jtct.2021.11.019

Ren H, Zhang Y, Huang W, Xu H, He W, Hao N, et al. Tumor-targeted nanodrug FSGG/siGal-9 for transdermal photothermal immunotherapy of melanoma. Communications Biology. 2024; 7: 188. https://doi.org/10.1038/s42003-024-05891-6

Sahai E, Astsaturov I, Cukierman E, DeNardo DG, Egeblad M, Evans RM, et al. A framework for advancing our understanding of cancer-associated fibroblasts. Nature Reviews. Cancer. 2020; 20: 174–186. https://doi.org/10.1038/s41568-019-0238-1

Salmaninejad A, Khoramshahi V, Azani A, Soltaninejad E, Aslani S, Zamani MR, et al. PD-1 and cancer: molecular mechanisms and polymorphisms. Immunogenetics. 2017; 70: 73–86. https://doi.org/10.1007/s00251-017-1015-5

Samstein RM, Lee CH, Shoushtari AN, Hellmann MD, Shen R, Janjigian YY, et al. Tumor mutational load predicts survival after immunotherapy across multiple cancer types. Nature Genetics. 2019; 51: 202–206. https://doi.org/10.1038/s41588-018-0312-8

Sautès-Fridman C, Petitprez F, Calderaro J, Fridman WH. Tertiary lymphoid structures in the era of cancer immunotherapy. Nature Reviews Cancer. 2019; 19: 307–325. https://doi.org/10.1038/s41568-019-0144-6

Schaer DA, Beckmann RP, Dempsey JA, Huber L, Forest A, Amaladas N, et al. The CDK4/6 Inhibitor Abemaciclib Induces a T Cell Inflamed Tumor Microenvironment and Enhances the Efficacy of PD-L1 Checkpoint Blockade. Cell Reports. 2018; 22: 2978–2994. https://doi.org/10.1016/j.celrep.2018.02.053

Schalper KA, Carleton M, Zhou M, Chen T, Feng Y, Huang SP, et al. Elevated serum interleukin-8 is associated with enhanced intratumor neutrophils and reduced clinical benefit of immune-checkpoint inhibitors. Nature Medicine. 2020; 26: 688–692. https://doi.org/10.1038/s41591-020-0856-x

Sek K, Mølck C, Stewart GD, Kats L, Darcy PK, Beavis PA. Targeting Adenosine Receptor Signaling in Cancer Immunotherapy. International Journal of Molecular Sciences. 2018; 19: 3837. https://doi.org/10.3390/ijms19123837

Sharma P, Hu-Lieskovan S, Wargo JA, Ribas A. Primary, Adaptive, and Acquired Resistance to Cancer Immunotherapy. Cell. 2017; 168: 707–723. https://doi.org/10.1016/j.cell.2017.01.017

Spranger S, Bao R, Gajewski TF. Melanoma-intrinsic β-catenin signalling prevents anti-tumour immunity. Nature. 2015; 523: 231–235. https://doi.org/10.1038/nature14404

Spranger S, Dai D, Horton B, Gajewski TF. Tumor-Residing Batf3 Dendritic Cells Are Required for Effector T Cell Trafficking and Adoptive T Cell Therapy. Cancer Cell. 2017; 31: 711–723.e4. https://doi.org/10.1016/j.ccell.2017.04.003

Spranger S, Gajewski TF. Impact of oncogenic pathways on evasion of antitumour immune responses. Nature Reviews. Cancer. 2018; 18: 139–147. https://doi.org/10.1038/nrc.2017.117

Stylianopoulos T, Munn LL, Jain RK. Reengineering the Physical Microenvironment of Tumors to Improve Drug Delivery and Efficacy: From Mathematical Modeling to Bench to Bedside. Trends in Cancer. 2018; 4: 292–319. https://doi.org/10.1016/j.trecan.2018.02.005

Tang R, Xu J, Zhang B, Liu J, Liang C, Hua J, et al. Ferroptosis, necroptosis, and pyroptosis in anticancer immunity. Journal of Hematology & Oncology. 2020a; 13: 110. https://doi.org/10.1186/s13045-020-00946-

7

Tang W, Chen Z, Zhang W, Cheng Y, Zhang B, Wu F, et al. The mechanisms of sorafenib resistance in hepatocellular carcinoma: theoretical basis and therapeutic aspects. Signal Transduction and Targeted Therapy. 2020b; 5: 87. https://doi.org/10.1038/s41392-020-0187-x

Tang W, Sun G, Ji GW, Feng T, Zhang Q, Cao H, et al. Single-cell RNA-sequencing atlas reveals an FABP1-dependent immunosuppressive environment in hepatocellular carcinoma. Journal for Immunotherapy of Cancer. 2023; 11: e007030. https://doi.org/10.1136/jitc-2023-007030

Tauriello DVF, Palomo-Ponce S, Stork D, Berenguer-Llergo A, Badia-Ramentol J, Iglesias M, et al. TGFβ drives immune evasion in genetically reconstituted colon cancer metastasis. Nature. 2018; 554: 538–543. https://doi.org/10.1038/nature25492

Topper MJ, Vaz M, Chiappinelli KB, DeStefano Shields CE, Niknafs N, Yen RWC, et al. Epigenetic Therapy Ties MYC Depletion to Reversing Immune Evasion and Treating Lung Cancer. Cell. 2017; 171: 1284–1300.e21. https://doi.org/10.1016/j.cell.2017.10.022

Torrejon DY, Abril-Rodriguez G, Champhekar AS, Tsoi J, Campbell KM, Kalbasi A, et al. Overcoming Genetically Based Resistance Mechanisms to PD-1 Blockade. Cancer Discovery. 2020; 10: 1140–1157. https://doi.org/10.1158/2159-8290.CD-19-1409

Van Coillie S, Wiernicki B, Xu J. Molecular and Cellular Functions of CTLA-4. Advances in Experimental Medicine and Biology. 2020; 1248: 7–32. https://doi.org/10.1007/978-981-15-3266-5_2

van der Woude LL, Gorris MAJ, Halilovic A, Figdor CG, de Vries IJM. Migrating into the Tumor: a Roadmap for T Cells. Trends in Cancer. 2017; 3: 797–808. https://doi.org/10.1016/j.trecan.2017.09.006

Vanpouille-Box C, Diamond JM, Pilones KA, Zavadil J, Babb JS, Formenti SC, et al. TGFβ Is a Master Regulator of Radiation Therapy-Induced Antitumor Immunity. Cancer Research. 2015; 75: 2232–2242. https://doi.org/10.1158/0008-5472.CAN-14-3511

Wallin JJ, Bendell JC, Funke R, Sznol M, Korski K, Jones S, et al. Atezolizumab in combination with bevacizumab enhances antigen-specific T-cell migration in metastatic renal cell carcinoma. Nature Communications. 2016; 7: 12624. https://doi.org/10.1038/ncomms12624

Walsh D, McCarthy J, O'Driscoll C, Melgar S. Pattern recognition receptors–molecular orchestrators of inflammation in inflammatory bowel disease. Cytokine & Growth Factor Reviews. 2013; 24: 91–104. https://doi.org/10.1016/j.cytogfr.2012.09.003

Wangensteen KJ, Chang KM. Multiple Roles for Hepatitis B and C Viruses and the Host in the Development of Hepatocellular Carcinoma. Hepatology. 2021; 73: 27–37. https://doi.org/10.1002/hep.31481

Watanabe T, Ishino T, Ueda Y, Nagasaki J, Sadahira T, Dansako H, et al. Activated CTLA-4-independent immunosuppression of Treg cells disturbs CTLA-4 blockade-mediated antitumor immunity. Cancer Science. 2023; 114: 1859–1870. https://doi.org/10.1111/cas.15756

Xuan X, Wang Y, Sun Y, Huang C. Identification of Genes Potentially Associated with Melanoma Tumorigenesis Through Co-Expression Network Analysis. International Journal of General Medicine. 2021; 14: 8495–8508. https://doi.org/10.2147/IJGM.S336295

Yamamoto K, Venida A, Yano J, Biancur DE, Kakiuchi M, Gupta S, et al. Autophagy promotes immune evasion of pancreatic cancer by degrading MHC-I. Nature. 2020; 581: 100–105. https://doi.org/10.1038/s41586-020-2229-5

Yu SJ. Immunotherapy for hepatocellular carcinoma: Recent advances and future targets. Pharmacology & Therapeutics. 2023; 244: 108387. https://doi.org/10.1016/j.pharmthera.2023.108387

Zhao L, Cheng S, Fan L, Zhang B, Xu S. TIM-3: An update on immunotherapy. International Immunopharmacology. 2021; 99: 107933. https://doi.org/10.1016/j.intimp.2021.107933

Zhu AX, Finn RS, Edeline J, Cattan S, Ogasawara S, Palmer D, et al. Pembrolizumab in patients with advanced hepatocellular carcinoma previously treated with sorafenib (KEYNOTE-224): a non-randomised, open-label phase 2 trial. The Lancet. Oncology. 2018; 19: 940–952. https://doi.org/10.1016/S1470-2045(18)30351-6