Clinical Epigenetics

REVIEW

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Epigenetic regulators combined with tumour immunotherapy: current status and perspectives

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Abstract

Immunotherapy, particularly immune checkpoint inhibitor therapy, has demonstrated clinical benefits in solid tumours. Despite its satisfactory clinical efficacy, it still faces several issues, such as limited eligibility, low response rates and cytotoxicity. Cancer epigenetics implies that tumour cells exhibit unique phenotypes because of their unique characteristics, thus reprogramming of the epigenome holds promise for cancer therapy. Epigenetic regulation plays an important role in regulating gene expression during tumour development and maintenance. Epigenetic regulators induce cancer cell cycle arrest, apoptosis and differentiation of cancer cells, thereby exerting anti-tumour effects. Recent studies have revealed a significant correlation between epigenetic regulatory factors and immune checkpoint therapy. Epigenetics can modulate various aspects of the tumour immune microenvironment and immune response to enhance the sensitivity of immunotherapy, such as lowering the concentration required and mitigating cytotoxicity. This review primarily discusses DNA methyltransferase inhibitors, histone deacetylase inhibitors, enhancer of zeste homolog 2 inhibitors and lysine-specific demethylase 1 inhibitors, which are associated with transcriptional repression. This repression alters the expression of genes involved in the immune checkpoint, thereby enhancing the effectiveness of immunotherapy. We also discuss the potential and challenges of tumour immunotherapy and highlight its advantages, application challenges and clinical research on integrating epigenetic regulatory factors with tumour immunotherapy.

Keywords Tumour immunotherapy, Epigenetic regulation, Immune check inhibitor, DNA methyltransferase inhibitor, Histone deacetylase inhibitors

Introduction

Recently, tumour immunotherapy has emerged, in which the immune system is activated to generate a durable anti-tumour immune response [1]. Immune checkpoint inhibitors (ICIs) have shown remarkable efficacy in the treatment of various solid tumours, including lung, hepatocellular, and breast cancers [2]. Although many ICIs, including programmed cell death ligand 1 (PD-L1) or cytotoxic T lymphocyte-associated protein 4 (CTLA-4) antibodies and co-stimulatory molecular agonists, have shown clinically satisfactory results in the treatment of various cancers, many issues remain, including low response rates and non-specific toxicities [3].

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The epigenetic landscape of cancer imparts tumour cells with distinct phenotypes, which may initiate carcinogenesis, and subsequently lead to genetic alterations that promote tumourigenesis. However, unlike genetic mutations, epigenetic changes are potentially reversible and highly adaptable, as the epigenome is inherently programmable [4]. The potential to reprogramme the epigenome and alter the cellular landscape offers a novel and promising therapeutic strategy. Epigenetic regulators, such as histone deacetylase (HDAC) inhibitors (HDACis), DNA methyltransferase (DNMT) inhibitors (DNMTis), enhancer of zeste homolog 2 (EZH2) inhibitors (EZH2is) and lysine-specific demethylase 1 (LSD1) inhibitors (LSD1is), serve as novel anti-tumour drugs that induce cancer cell dysfunction such as cell cycle arrest, apoptosis and differentiation, which result in anti-tumour effects [5]. Recent studies have indicated a significant correlation between epigenetic regulators and the enhancement of immune checkpoint therapy. The effects of DNMTs and HDACs, which are commonly linked to transcriptional inhibition, alter the expression of genes involved in immune checkpoint pathways, thereby enhancing the efficacy of immunotherapy [6]. They enhance $CD8^+$ T cell killing or increase the level of programmed cell death protein 1 (PD-1), and when combined with PD-1-blocking antibodies, this treatment inhibits tumour progression [7]. The use of epigenetic regulators in combination with tumour immunotherapy is a promising strategy for tumour immunotherapy. In this review, we discuss the therapeutic effects of immunotherapy in tumours along with its limitations, such as immune evasion, and summarise epigenomodulator-based approaches to tumour therapy. We highlight the advantages of integrating epigenetic regulatory factors with tumour immunotherapy, including the combination of epigenetic regulators combined with ICIs, and provide specific examples of combining epigenetic regulators and cytokine therapy for cancer treatment. Finally, we discuss the prospects and potential future challenges of using epigenetic regulators and tumour immunotherapy for various tumour types currently under investigation (Fig. 1).

Tumour and immunity

Abnormal immune function, particularly when low or compromised, can facilitate tumour development and progression [8]. The immune system identifies, monitors and eliminates most tumour cells. Nevertheless, some tumour cells consistently evade immune surveillance, and enter a state of equilibrium. During these processes, any hindrance to progression is a common characteristic of tumour-immune surveillance failure, which results in tolerance to cancer growth and renders the immune system incapable of controlling tumour progression [9]. Consequently, by modifying tumour cells and the microenvironment, malignant tumours ultimately develop. The tumour microenvironment (TME) encompasses a variety of complex components that suppress the cytotoxic immune response, including lymphocytes and bone marrow cells [10]. These cells impair innate and adaptive immunity through interactions with other immune cells in the TME, such as neutrophils and dendritic cells (DCs) (Fig. 2) [11]. Elucidating the role of these immune cells in tumour immune evasion is essential for guiding immunotherapy.

T cells

T cells constitute a principal element of the adaptive immune system and provide an effective defence against pathogens and cancer. Moreover, they profoundly influence tumour immunosuppression and immune evasion. $CD4^+$ T cells eliminate self-derived tumours in an MHC-II-dependent manner and contribute to the anti-tumour immune response [12, 13]. $CD8^+$ T cells primarily enhance anti-tumour immunity. Insufficient nutrients, such as glucose and amino acids, within the TME, disrupt amino acid metabolism in $CD8^+$ T cells and impair the immune response, thereby suppressing $CD8^+$ T activity and promoting tumour growth [13, 14]. Naive $CD4^+$ T cells differentiate into helper T cells, including TH1, TH2, TH9 and TFH cells, after antigen stimulation [15]. TH2 lymphocytes are ubiquitous in the TME and contribute to tumourigenesis by secreting anti-inflammatory cytokines.

B cells

B lymphocyte subsets can secrete immunosuppressive cytokines, thereby facilitating immune evasion [16]. Plasma cells utilize two mechanisms to induce tumour immune evasion. One mechanism involves the release of immunosuppressive cytokines that dampen the activity of effector T cells, thus diminishing tumour destruction. The other is the secretion of transforming growth factor (TGF)-β and gamma-aminobutyric acid by plasma cells to promote the production of regulatory T cells (Tregs) and M2 macrophages, thus inducing immune evasion from the tumour [17]. Breg cells mediate immune tolerance through a mechanism akin to that of plasma cells, promoting immune tolerance by secreting inhibitory cytokines, such as interleukin (IL)-10, IL-35 and TGF-β [18].

Natural killer (NK) cells

NK cells serve as the primary line of defence against tumours, destroying tumour antigens (TAs) through the production of cytotoxic substances, such as like perforin and granzyme [19]. However, without the stimulation

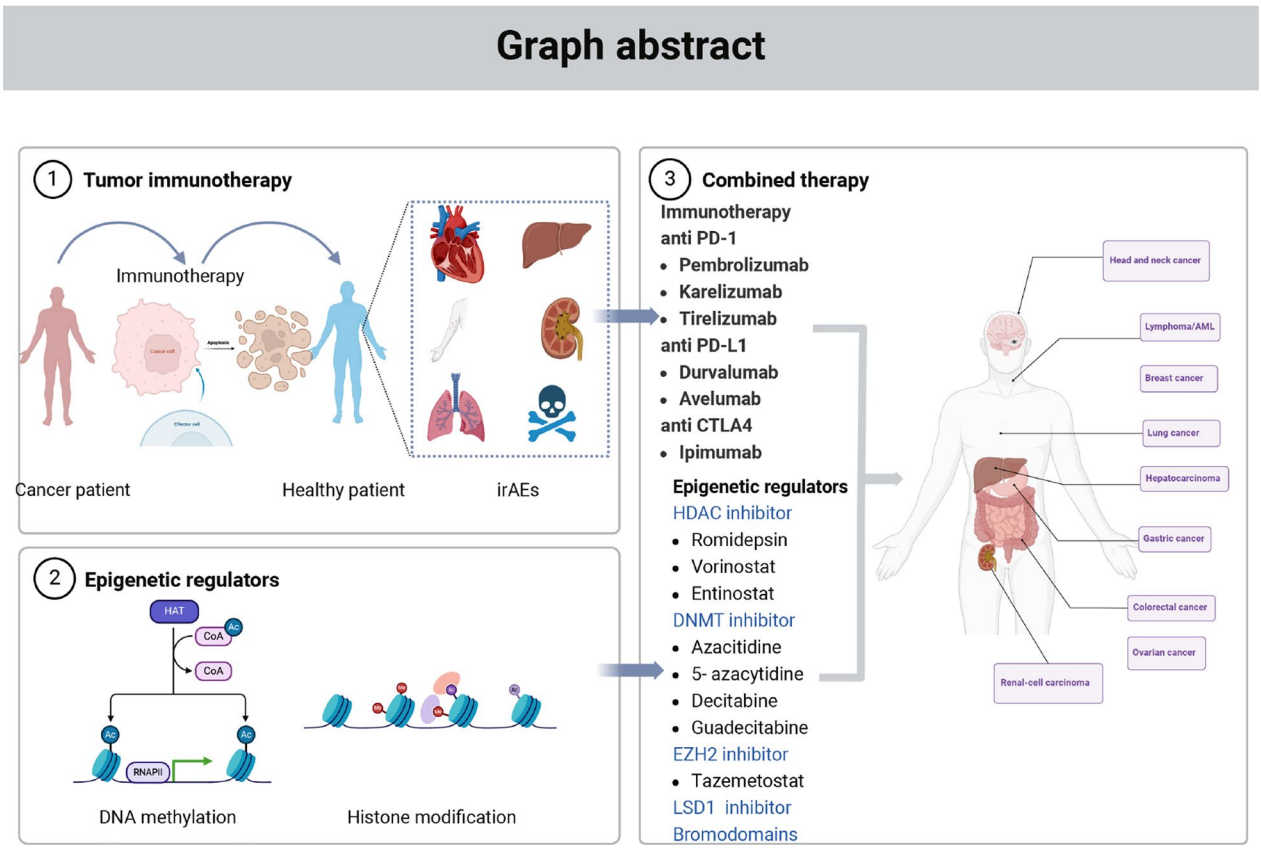

Fig. 1 Advantages of cancer treatment strategies employing epigenetic regulators in conjunction with tumour immunotherapy. Tumour immunotherapy has shown strong therapeutic advantages and therapeutic potential in tumour treatment, and the treatment of tumour patients is developing well, but there are still some adverse events related to immunotherapy, such as myocarditis, liver function damage, rash, renal function damage, lung injury, and even life-threatening in severe cases. Epigenetic regulatory factors show therapeutic potential in tumours and other diseases. The purpose of this review is to discuss the research effect, clinical application potential and problems of tumour immunotherapy, such as immune checkpoint inhibitor drugs such as pabolizumab, karelizumab, duvalizumab and epigenetic regulators such as romidixin and decitabine, in the treatment of various types of tumours

of $CD8^+$ T cell proliferation or in the immunosuppressive TME [20], NK cell activation is restricted, which promotes tumour progression [21]. NK cells impair the growth capacity of MYCN-amplified cancer cells and prevent TGF-β1-mediated immunosuppression [20]. In ovarian cancer, NK cells play a comparable role. TNF-α and interferon (IFN)-γ mediate immune escape in NK cells [21].

Macrophages

Tumour-associated macrophages (TAMs), which are the predominant components of the TME, influence tumour malignancy and immune evasion. Histologically, TAMs are divided into M1-TAMs and M2-TAMs [22]. During the early stage of cancer, TAMs exhibit a proinflammatory M1 phenotype and exert an antitumour role. However, under the influence of TME, they eventually transform into an immunosuppressive and angiogenic M2 phenotype, which can enhance T cell depletion, inhibit cytotoxic T cell viability and reduce ICIs activity [23]. Tumour cells induce the polarisation of M2-TAMs and increase PD-L1 expression in M2 macrophages, while enhancing PD-L1 expression in cancer cells to promote migration and immune evasion [24, 25].

DCs

DCs are the most potent antigen-presenting cells in the body. They efficiently uptake and process antigens, presenting them to the immune system through major histocompatibility complexes I and II, thus serving a pivotal role in initiating, regulating and sustaining the immune response [26]. HLA-G facilitates tumour immune evasion. Tumours induce the expression of HLA-G in DC-10 cells, which results in T cells dysfunction [27, 28].

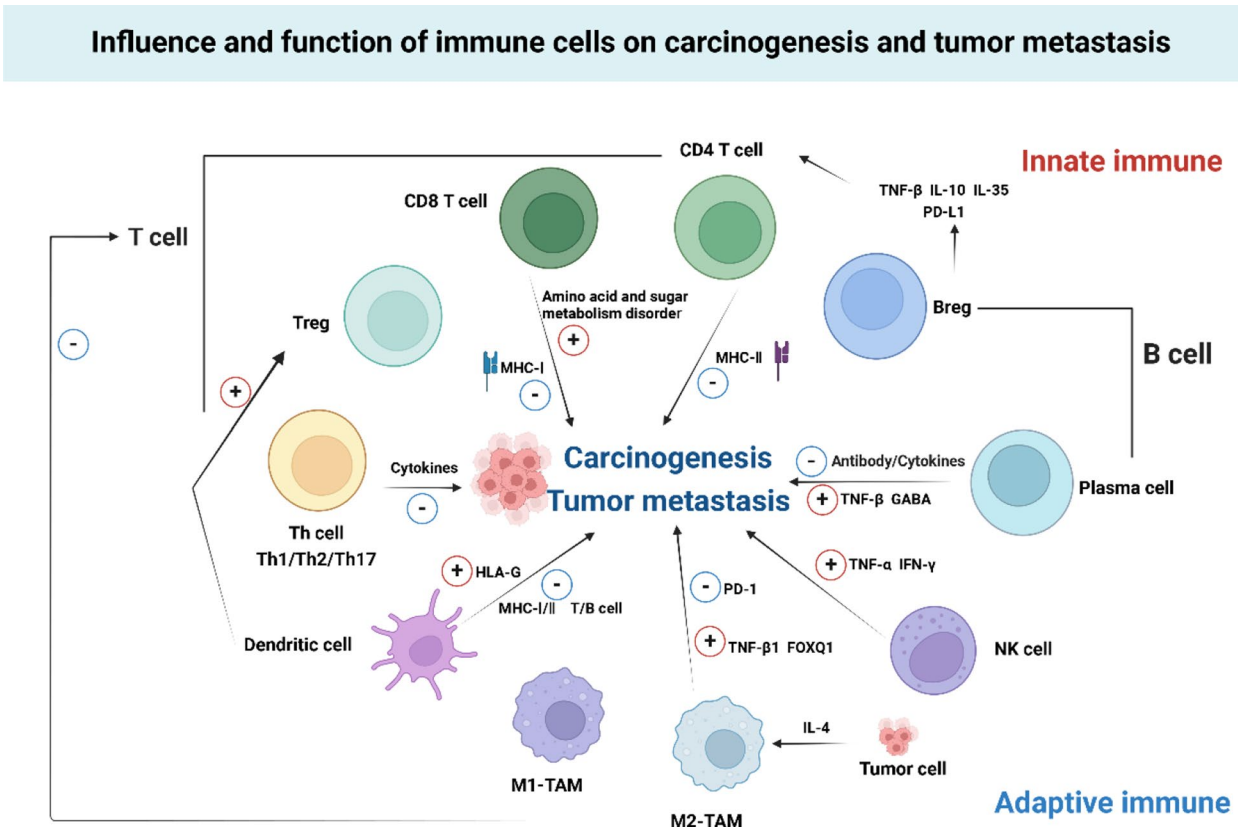

Fig. 2 Influence and function of immune cells on carcinogenesis and tumour metastasis. Immune cells in the body participate in innate and adaptive immune responses in the process of tumour occurrence, respectively. In different types of immune responses, immune cells interact with each other through secretion factors and other mechanisms. These effects have both positive and negative effects on tumour occurrence, development and tumour metastasis

Current situation of tumour immunotherapy

Since its introduction, tumour immunotherapy has garnered significant interest. Currently, the most commonly used and well-characterised tumour immunotherapy modalities include cytokine therapy, immune vaccine therapy, ICI therapy and adoptive cell transplantation (ACT) therapy (Fig. 3). The immunotherapy drugs currently approved by the US Food and Drug Administration (FDA) are listed in Table 1, and several have come to the forefront of cancer treatment.

Cytokine therapy

Cytokines are among the first immunotherapeutic drugs approved by the FDA at the end of the twentieth century [29]. They serve as messengers through precise autocrine and paracrine signalling and methodically regulate innate and adaptive immunity [30]. Certain key anti-tumour effector functions require cytokine release or cytokine-mediated activation of anti-tumour immunity. Only IFN-α and IL-2 have been approved by the FDA for treating cancer.

IFN-α and IFN-γ

Various IFN-α preparations have been approved for the treatment of various intractable diseases, including haematological diseases, such as polycythaemia vera, primary thrombocytosis, myeloid leukaemia [31, 32], metastatic melanoma [33], metastatic renal cell carcinoma, advanced mycosis fungoides [34] and high-risk uveal melanoma [35]. IFN-γ has shown good initial results in a phase II trial [36]. It caused changes to the TME, particularly T cell infiltration and MHC-I expression on the tumour surface. The combination of IFN-γ and anti-PD1 therapy may have some potential benefits for the treatment of sarcoma [37].

ILs

IL-2 has been used to treat metastatic melanoma and renal cell carcinoma, demonstrating satisfactory therapeutic efficacy [38].The potential role of IL-2 and NK cell activation in metastatic osteosarcoma has also been examined [39]. Other cytokines were found to enhance antigen activation and promote the infiltration of effector

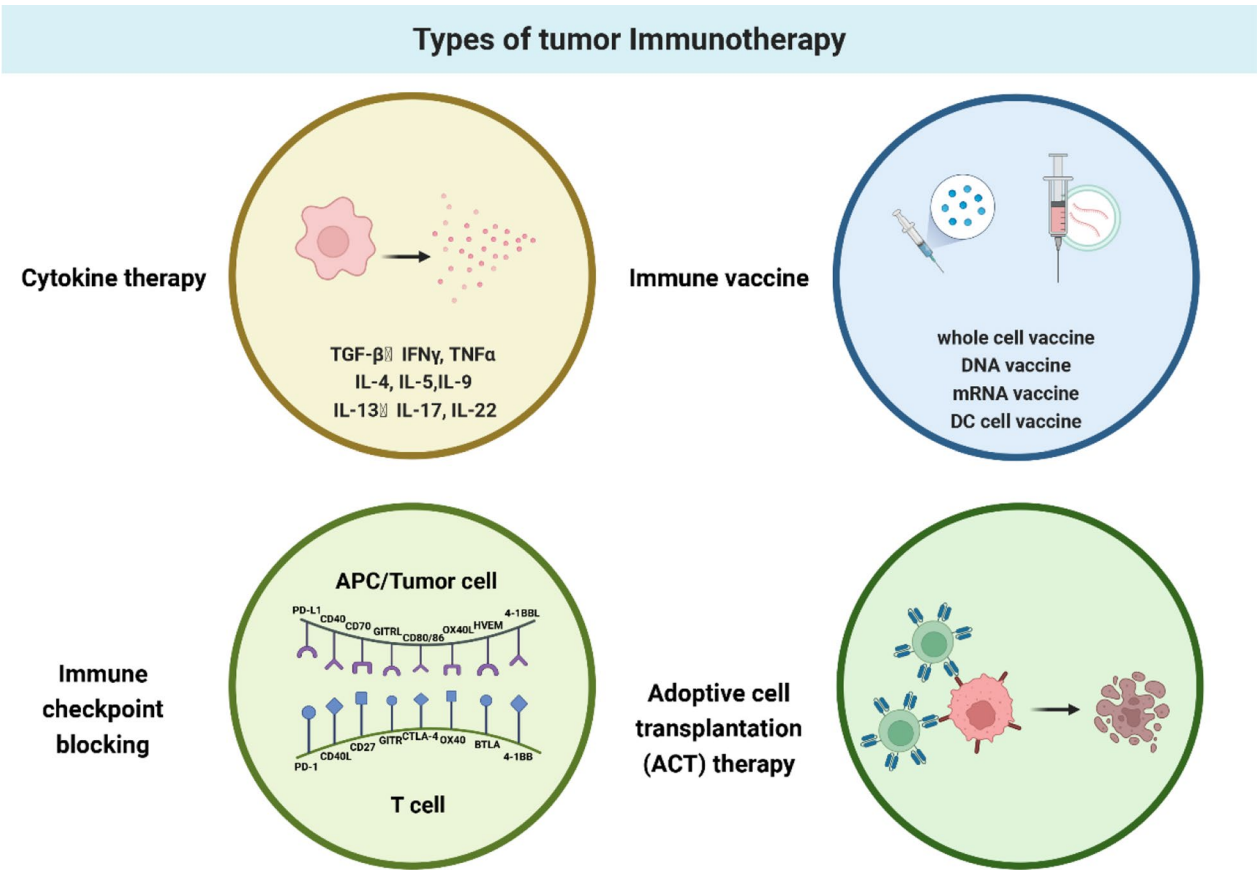

Fig. 3 Types of tumour immunotherapy. Tumour immunotherapy can be divided into cytokine therapy, immune vaccine, immune checkpoint blocking and adoptive cell transplantation. Cytokines mainly include TGF-β, IFN γ, TNF-α, IL-4, IL-5, IL-9 and so on. Immune vaccines are divided into whole-cell vaccine, DNA vaccine, mRNA vaccine and DC cell vaccine. The commonly used inhibitors of ICB are PD-1, PD-L1 and CTLA-4 inhibitors. CAR-T therapy is often used in adoptive cell transplantation therapy

Table 1 Immunotherapy drugs approved by the FDA

Approach	Content	Target	Diseases
Cytokine	IL-2	IL-2R	Metastatic melanoma and metastatic renal cell carcinoma
	INF-α	INF-αR	Metastatic renal cell carcinoma, Kaposi's sarcoma, follicular lymphoma, chronic myeloid leukaemia, cervical intraabdominal tumour and high-risk melanoma
Vaccine	Peptide-based vaccine	MHC class I molecule MHC class II molecule	Melanoma, lung cancer, esophageal cancer, pancreatic cancer
	mRNA vaccine	APC	Colorectal cancer, melanoma and NSCLC
Immune checkpoint inhibitor	Nivolumab Pembrolizumab Cemiplimab	PD-1	Melanoma, NSCLC, renal cell carcinoma, HL, head and neck cancer, urothelial carcinoma, colorectal cancer, hepatocellular carcinoma, and SCLC, gastric cancer, cervical cancer, and Merkel cell carcinoma, Metastatic cutaneous squamous cell carcinoma
	Atezolizumab Avelumab Durvalumab	PD-L1	Bladder cancer, NSCLC, triple-negative breast cancer, small cell lung cancer, Merkel cell carcinoma, urothelial carcinoma
	Ipilimumab	CTLA-4	Melanoma, renal cell carcinoma, and colorectal cancer
Adoptive cell transplantation therapy	CAR T cell therapy	CD19	Large B cell lymphoma, AML, and large B cell lymphoma

cells to the tumour site, demonstrating anti-tumour activity, with IL-12, IL-15 and IL-21 as the most prominent examples. The anti-tumour effect of IL-15 was evaluated in preclinical studies and clinical trials [40], and its effect on NK cells was evaluated in various paediatric tumours, including rhabdomyosarcoma [41].

Limitations of the therapy

Cytokine therapy also has some drawbacks. Because of the short half-life of cytokines, large doses are often required for treatment. However, rapid injection can cause blood vessel leakage and cytokine release syndrome (CRS) [42]. Additionally, cytokine therapy increases the survival of regulatory T cells, induces T cell death and ultimately leads to an immune attack on normal tissues [42, 43]. For example, IL-18 exerted its anti-tumour effects after being activated through enzymatic conversion. Administering high doses of IL-18 intravenously to achieve effective concentrations poses adverse risks and limited therapeutic efficacy. Treatment with IL-18 alone does not elicit a tumour-specific immune response [44].

Cancer vaccines

The development of tumour vaccines is fundamentally similar to that of infectious disease vaccines, They include whole-cell vaccines, DNA and messenger RNA (mRNA) vaccines, antigen vaccines and DC vaccines [45]. Cancer vaccines specifically activate the immune system and target tumour cells [45, 46] and often result in a specific and durable immune response to TAs. There are two types of cancer vaccination strategies: preventive strategies and therapeutic strategies.

Preventive vaccines and therapeutic vaccines

Antigens derived from pathogenic viruses are the most common tumour-specific antigens selected for the development of preventive vaccines. Vaccines targeting the Epstein–Barr virus associated with Hodgkin's lymphoma and nasopharyngeal carcinoma, as well as the hepatitis B and C viruses associated with hepatocellular carcinoma (HCC), have been developed as tumour preventive vaccines [47]. The objective of therapeutic vaccines is to induce tumour regression, eliminate minimal residual disease, establish durable anti-tumour memory and prevent non-specific or adverse reactions. Adenovirus vaccines have been shown to induce strong anti-tumour immunity [48]. Various peptide-based cancer vaccines have been used to treat various cancers, such as melanoma, lung cancer, oesophageal cancer and pancreatic cancer [49]. Experimental results on DNA cancer vaccines are scarce, whereas mRNA vaccines have been used utilized to treat colorectal cancer, melanoma and non-small-cell lung cancer (NSCLC) [50].

Limitations of therapy

Although cancer vaccines have provided benefits to patients, multiple clinical trials with larger data samples are needed to confirm their efficacy [42]. Similar to other immunotherapeutic modalities, tumour vaccine therapy faces challenges involving tolerance and immunosuppression [51, 52]. Tumour vaccines have the disadvantages of low immunogenicity, slightly poor stability, and high production costs, which require the use of adjuvants. Moreover, RNA vaccines are susceptible to extracellular degradation by enzymes, whereas DNA vaccines pose a risk of integration into the host genome [53].

Immune checkpoint blocking (ICB)

The discovery of ICIs has fundamentally transformed the systematic methods of cancer treatment and improved the clinical outcomes for many cancer types [54]. Currently, the primary immune checkpoint molecules that have garnered the most attention are PD-1, PD-L1 and CTLA-4 [55], for which numerous well-established drugs are widely used for treatment. However, several new checkpoint inhibitory inhibitors, including B and T lymphocyte attenuators, have been identified and evaluated for their efficacy in anti-cancer immunotherapy [56].

CTLA-4

CTLA4 is an immune negative regulatory factor that aggregates onto the plasma membrane after T cell activation and binds with DCs and other antigen-presenting cells to inhibit T cell activation and expansion [57, 58]. Interestingly, when CTLA4 is blocked, the function of Treg cells is also affected, which further contributes to the suppression of T cell responses [59, 60]. The application of anti-CTLA4 is based on relieving the inhibition of the original anti-cancer T cell response and stimulating new treatment responses. Preclinical studies in mouse models demonstrated significant potential, prompting Pfizer and BMS/Medarex to advance two distinct anti-CTLA4 antibodies into clinical trials. The Phase II trial failed to meet the anticipated target of tumour regression; however, BMS/Medarex remained confident in their long-term benefits. Consequently, a comprehensive randomised phase III trial was launched for patients with relapsed and refractory metastatic melanoma, with overall survival as an endpoint. Both antibody groups exhibited a two-fold survival benefit lasting over 2.5 years [61, 62]. Additionally, preclinical studies were also found that using a fucosylated derivative of ipilimumab (BMS-986218) was enhanced the depletion of T cells by anti-CTLA4 [60, 63].

PD-1/PD-L1

PD-1 interacts with its ligand PD-L1 to inhibit T cell receptor-mediated cytotoxicity and the proliferation of $CD8^+$ T cells, thereby weakening immune surveillance and promoting tumour cell immune evasion [64, 65]. Antibody-based PD-1/PD-L1 inhibitors have demonstrated remarkable effectiveness in treating various advanced cancers. The FDA has approved six monoclonal antibodies targeting either PD-1 or PD-L1 for the treatment of haematological and solid tumours. These antibodies enhance T cell function by blocking PD-1, thereby significantly increasing overall survival within a tolerable range [66–68]. The combination of anti-PD-1 and anti-PD-L1 drugs (such as atezolizumab and duvalizumab) was considered to be effective for metastatic triple-negative breast cancer (TNBC). A robust immune microenvironment and extensive TA exposure in early breast cancer increased the response to checkpoint inhibitors [69, 70]. The combination of paclitaxel and atezolizumab significantly enhanced PD-L1 metastasis and overall survival in patients with advanced TNBC [71].

The existing anti-PD1/PD-L1 therapies block the binding between PD-1 and PD-L1, effectively activating depleted immune cells and triggering the anti-tumour immune response [72]. Recently, peptide-based or non-peptide-targeted PD-1/PD-L1 small molecule inhibitors have been developed [73, 74]. The peptide-based PD-1/PD-L1 inhibitor AUNP-12 is a branched peptide consisting of 29 amino acids, which effectively suppresses the growth and metastasis of tumour cells [74]. In addition to AUNP-12, non-peptide PD-1/PD-L1 inhibitors have also been developed. For example, derivatives of sulfamethoxazole and sulfamethoxazole antibiotics exhibit low cytotoxicity and block the PD-1 by inhibiting the binding of mPD-1 and mPD-L2 [75]. Compared with monoclonal antibodies, small molecule inhibitors have several advantages, including excellent stability, superior membrane permeability, non-immunogenicity and, most importantly, oral administration [76]. However, these small molecule immune checkpoint modulators remain in the early stages of development, which highlights the need for safer and more effective treatment options.

B and T lymphocyte attenuators (BTLA)

BTLA interacts with HVEM in the TNF receptor superfamily. Abnormal cells evade immune system surveillance through immune checkpoints, such as BTLA in cancer [77]. Blocking BTLA enhances the efficacy of cancer treatment by inhibiting IL6/IL-10-induced $CD19^{high}$ B lymphocytes [78]. The overexpression of BTLA/HVEM is associated with reduced anti-tumour immunity and poor prognosis. In preclinical studies, targeting BTLA demonstrated superior therapeutic effects and enhanced anti-tumour immune capabilities [77].

Limitations of therapy

ICIs have achieved success as immunotherapeutics. However, most patients still fail to achieve the desired clinical response. ICIs have a low reaction rate and often exhibit drug resistance. For example, the objective response rate for TNBC against PD-L1 treatment is below 10% [79, 80]. Interactions among components of the TME may occur, such as M2-TAMs and cytotoxic T cells. During the early stages of cancer progression, the TME promotes the generation of M2-TAMs, which in turn, suppress the activity of cytotoxic T cells and enhance T cell elimination. This results in tumour immune evasion and immune suppression [81]. Additionally, adverse events caused by ICIs have serious side effects on multiple organs, such as the skin, gastrointestinal tract, liver and heart. Following ICI treatment, autoimmune disease and other adverse events have been observed, including fatigue, diarrhoea, colitis, myalgia, hepatitis, myocarditis and pericarditis [79, 80, 82]. Additionally, ICIs have other limitations, such as high production costs, low tissue permeability and poor oral bioavailability [83].

ACT therapy

ACT therapy leverages tumour-reactive immune cells to target tumour cells expressing specific surface markers for eradication [84]. ACTs are classified into allogeneic and transgenic T cell therapies, such as CAR-T and TCR-T cell therapies. This requires the genetic modification of T cells to express chimeric antigen receptors (CARs) or T cell receptors (TCRs) [85].

CAR-T

T cell populations are the primary agents used in this therapy. The development of CAR-T cell therapy is based on laboratory research and clinical studies of activated killer cells and tumour-infiltrating lymphocytes (TILs) for cancer treatment [86]. The first ACT therapy was performed using autologous TILs from patients with metastatic melanoma [87]. This approach is currently used to treat relapsed or refractory large B cell lymphoma, with early successes observed [88]. ACT shows therapeutic potential for treating refractory epithelial cancer with autologous TILs [89]. CAR-T cells are also effective for treating B cell malignancies, such as Hodgkin's lymphoma [90]. Furthermore, post-allogeneic haematopoietic stem cell transplantation treated CAR-T cells proliferate and persist in B-ALL patients, exhibiting anti-leukaemia activity [91].

TCR-T

TCR-T therapy holds great promise as a new treatment option for patients with various solid tumours. After decades of research, T cells have been successfully modified to express TCRs. These receptors can specifically bind to antigens on tumour cells, thereby activating the T cells to destroy abnormal cells [92, 93]. Recently, TCR-T therapy was evaluated in phase I clinical trials for patients with relapsed or refractory acute myeloid leukaemia (AML) and myelodysplastic syndrome (MDS) [94, 95]. TCR-T therapy has remarkable potential for delivering benefits to patients with lymphoma and myeloid leukaemia, without inducing significant toxicity associated with TCR-T cells [96].

Limitations of the therapy

The effectiveness of CAR-T or TCR-T therapy is remarkable, and it will remain one of the most effective modalities for tumour treatment in the future. The adverse reactions and severity of CAR-T therapy after treatment vary greatly among individuals, prompting studies into the causes of these side effects. CRS and immune effector cell-associated neurotoxic syndrome are the most prevalent adverse reactions Additionally, as with other immunotherapies, low response rates and drug resistance are also major limitation of CAR-T or TCR-T therapy, which result in shorter recurrence and remission times [97, 98].

Dual role of tumour immunotherapy

Despite the remarkable efficacy of immunotherapy, safety concerns may arise. As an innovative treatment, immunotherapy exhibits outstanding efficacy, yet it also has limitations and challenges. The adverse effects of immunotherapy can be complex. Therefore, how should we rationally view these side effects? Tumour resistance to immunotherapy result from multiple factors within an unfavourable TME, which hinders the infiltration of cytotoxic anti-tumour cells, rendering these cells unsuited for survival and normal function post-invasion. In this section, we discuss the primary challenges of tumour immunotherapy, the dual role of immune cells within the TME (Fig. 4), and the pros and cons of tumour immunotherapy.

Enigmas in tumour immunotherapy

Immune evasion

In addition to drug resistance and systemic side effects, immune evasion remains a significant unsolved challenge in tumour immunotherapy. The composition of the immune system undergoes a profound change to counteract cancer progression following immunotherapy. However, tumour cells often employ immune evasion to mitigate the adverse effects of the immune response. Upregulation of PD-L1 induces immune evasion. Another key factor in immune evasion is MHC-I, which is internalised and degraded by CEMIP to facilitate the immune evasion of tumour cells [99]. Moreover, chromosomal deletions contribute to immune evasion. A homozygous deletion at chromosome 9q21.3 results in the loss of CDKN2A/B function and subsequently reduces the IFN gene cluster, and leading to a weakened surveillance function of $CD8^+$ T cells during tumour cell immune evasion [100].

Epigenetic characteristics of the TME

Epigenetic regulation of spatiotemporal gene expression, such as the CpGI methylation silencing of tumour suppressor genes, is essential for embryonic development, cell differentiation and carcinogenesis. Epigenetic regulation is considered a transcription regulatory factor within the immune system. It can influence the surveillance of tumours and the maintenance of microenvironmental homeostasis in immune cells [101]. Therefore, a comprehensive analysis of epigenetics may provide insight into immune surveillance and homeostasis development. For example, the extent of methylation at differentially methylated sites is markedly different between innate and adaptive immune cells and defines their epigenetic and gene expression profiles [102]. Epigenetic regulation ensures that immune cells exhibit appropriate gene expression patterns in different tissue microenvironments to establish their proper differentiation and function. Epigenetic alterations in the TME orchestrate tissue hypoxia and potentially influence cellular responses to hypoxia and cancer cell metabolism. Epigenetic modifications stabilise binding between hypoxia-inducible transcription factor (HIF) and its transcriptional targets, which in turn, affects histone demethylase activity following direct HIF transactivation and promotes tumour growth [103].

Tumour heterogeneity

Tumour heterogeneity studies have focused on the driving effect of gene variations (such as gene mutations, single nucleotide polymorphisms and copy number variations) and polymeric gene expression, and genomic intratumoural heterogeneity is prevalent in many cancers [104, 105]. Proteins encoded by the aberrant expression of splice variants drive the unique characteristics of cancer cell growth, differentiation and other biological functions. These processes are intimately associated with the response of tumour-specific or microenvironmental factors [106]. Recent studies have revealed a role for epigenetic regulation in tumour heterogeneity caused by alternative splicing [107]. Enzymes proteins and their cofactors associated with DNA methylation and histone

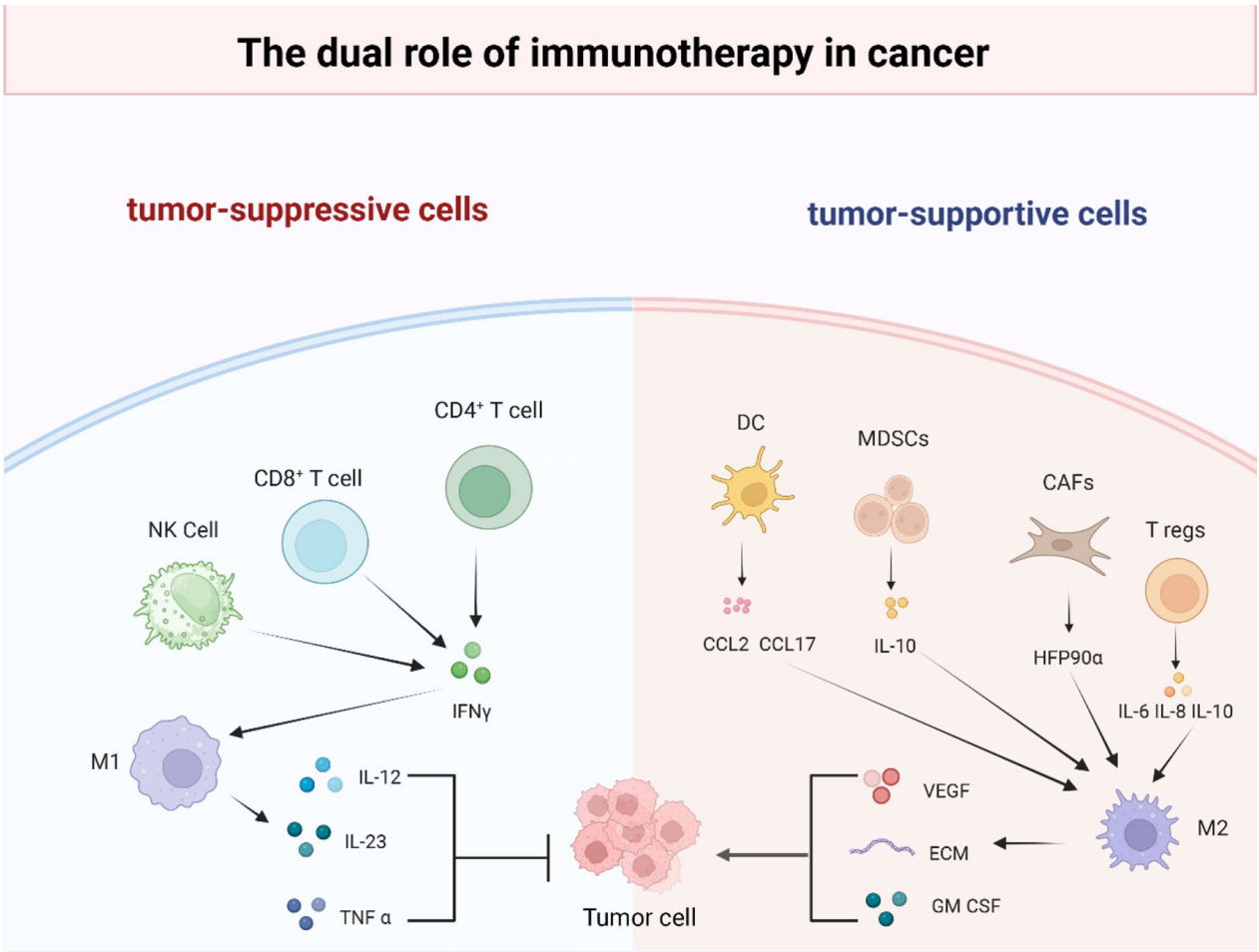

Fig. 4 The dual role of immunotherapy. Tumour immunotherapy plays an anti-tumour role mainly through the immune function of immune cells in the body. Immune cells in the body can be roughly divided into two categories, namely, fall-inhibitory cells and fall-supportive cells, and each immune cell plays a corresponding role mainly by secreting different factors

modification interact with the structure of chromatin regions to form a complex regulatory network that not only determines the initiation of gene transcription, but also affects alternative splicing of mRNA [107, 108]. Therefore, more studies are needed to understand the relationship between tumour heterogeneity, the immune background and its association with epigenetic regulation.

Dual functions of immune cells in carcinogenesis

Myeloid-derived suppressor cells (MDSCs)

MDSCs induce immunosuppression and promote cancer progression through adaptive and innate immunity [109]. In the TME, MDSC differentiation and immunosuppressive properties are modulated through signal regulatory protein α (SIRPα)/CD47 signal transduction. The binding of CD47 and SIRRP α decreases the phagocytosis of MDSCs, promotes the expression of immune checkpoint and inhibits the proliferation of T cells.

Cancer-associated fibroblasts (CAFs)

CAFs are integral to the TME. They mediate the immunosuppression of the TME by secreting the extracellular matrix (ECM) and cytokines [110] that regulate growth [111, 112]. CAF-derived cardiotropic factor-like cytokine 1 (CLCF1) increases tumour cell secretion of chemokine (C-X-C motif) ligand 6 (CXCL6) and TGF-β, which activates the ERK1/2 pathway and generates more CLCF1 through a positive feedback loop, thus accelerating the carcinogenesis of HCC [113].

Tumour-associated endothelial cells (TECs)

TECs are also important components of the TME. TECs promote tumour angiogenesis and modulate cytotoxic T cells in the TME to induce carcinogenesis [114]. TECs

secrete IL-10 and TGF-β to suppress the proliferation of $CD8^+$ T cells, decrease the secretion of cytokines, such as IL-2, and facilitate the immune evasion of tumour cells [115]. Silencing GPNMB expression in TECs resulted in accelerated tumour progression and increased $CD8^+$ T cell depletion, following TEC injection in a mouse model [116].

Tumour-associated macrophages

Based on their immune-related functions, TAMs may be categorised into two distinct subtypes: M1-TAMs, which enhance immune surveillance, and M2-TAMs, which promote immune evasion. M1-TAMs express high levels of TNF, which mediates cytotoxic effects against tumour cells and exerts anti-tumour functions. M2-TAMs may be further divided into M2a, M2b and M2c phenotypes based on the level of cytokines expressed. M2-TAMs promote T cell depletion, repress cytotoxic T cell activity and ultimately induce tumour immune evasion [117].

Tumour immunotherapy as a double-edged sword

In recent years, tumour immunotherapy has emerged as one of the most effective strategies to cure cancer. It has attracted widespread interest among researchers and clinicians. However, studies have revealed that immunotherapy is a double-edged sword. For example, patients with advanced melanoma were randomly assigned into two groups, in which one group was administered the ICI ipilimumab and the other received TILs. Approximately half of the patients in both groups showed improved outcomes, with favourable median progression-free survival and overall survival and disease objective response rates of approximately 21% and 50%, respectively. Nevertheless, 57% of the patients undergoing TIL therapy, as well as those on ipilimumab, experienced treatment-related adverse events, with a notable incidence of graft-versus-host disease [118]. Patients with NSCLC were randomly assigned to receive nivolumab plus ipilimumab therapy and chemotherapy. The immunotherapy-based combination resulted in longer overall survival compared with the chemotherapy, with similar adverse event rates of 32.8% and 36.0%, respectively [119]. The aforementioned immunotherapy has shown significant efficacy in the treatment of various types of cancer, however, no single immunotherapeutic drug can cure the majority of patients, and most high-level treatment-related adverse events and related deaths occur during immunotherapy. These remain obstacles to the future application of immunotherapy. There is evidence that tumourigenesis immune evasion and resistance are regulated by epigenetic mechanisms. The epigenetic upregulation of MHCs and associated TAs, such as CD80 and CD86, promotes TA presentation, thereby modulating the TME or enhancing the antigenicity of tumour cells [120]. The FDA has currently approved the DNMTis 5-azitidine and 5′-2′-deoxycytidine for the treatment of MDS, which may reverse immune evasion during tumour therapy and progression [121, 122]. In conclusion, the combined application of epigenetic regulation and immunotherapy may yield favourable outcomes in cancer treatment.

Epigenetic regulation of immune cells

Epigenetic regulators in tumour therapy

The epigenetic characteristics of cancer cells are markedly distinct from those of normal cells. Consequently, epigenetic modification has emerged as a promising target for tumour therapy. Epigenetic regulators agents are primarily classified into DNMTis, HDACis, EZH2is, LSD1is and bromodomain and extra-terminal (BET) inhibitors (BETis) [75, 123].

DNMTis

DNMTs are important epigenetic enzymes that are involved in the carcinogenesis [124]. Besides conspicuously modifying and regulating tumours through methylation, DNMTs also induce tumour immune signal transduction, which through the transcriptional activation of transposable elements, results in the formation of dsRNA, subsequently inducing the expression of type I IFNs and cytokines [125]. Interestingly, DNMTis participate in the synergistic action of poly (ADP-ribose) polymerase inhibitors, an enzyme involved in the DNA damage response pathway, to increase anti-tumour efficacy. Among the many DNMTis, 5-azacytidine (5-Aza) and decitabine (DAC) are the early applications, 5-Aza and DAC are currently approved by the FDA for the treatment of AML and MDS [126].

HDACis

HDACs primarily regulate gene expression, including PD-L1, by removing acetyl groups from histones [127]. Numerous HDACs have been developed which promote $CD8^+$ T cell activity and enhance anti-tumour immune responses by suppressing MDSCs [128]. Among HDACis, vorinostat, romidepsin and heliostat are approved for the treatment of HL, T cell lymphoma, and other tumour types [129–131]. The HDAC2i valeric acid showed a positive therapeutic effect in the treatment of HCC by inducing PD-L1 expression and tumour immune evasion through IFN-γ regulation [132]. Furthermore, HDAC exhibited synergistic anti-tumour effects in combination with chemotherapy and radiotherapy [133, 134].

EZH2is

EZH2 is an important H3K27 histone methyltransferase. Numerous EZH2is have been developed, including the

well-known tazemetostat [135]. However, EZH2is in immunology exhibit a bidirectional effect. On the one hand, they positively affect the immune response against cancer. EZH2is were shown to inhibit the transcriptional upregulation of USP22 expression, which in turn, stabilised PD-L1. Inhibition of EZH2 enhanced the stability of PD-L1 and decreased K48-mediated ubiquitination [136]. Subtoxic doses of EZH2 prevented cancer immune resistance during immunotherapy [137]. On the other hand, EZH2is is only effective for a limited number of tumour types, which may be due to their effect on the TME. For example, EZH2i GSK126 treatment reactivated Th1 chemokines, facilitating the transport of effector T cells into the TME. GSK126 inhibited EZH2 and simultaneously promoted the development of MDSCs [138, 139]. Although EZH2 appears to act as a double-edged sword in immunotherapy, combining EZH2 targeting with cancer immunotherapy remains a promising strategy.

LSD1is

LSD1 is a histone demethylase that regulates the H3K4me and H3K9me. It has emerged as an important anti-tumour target [140]. LSD1 deficiency reverses its immunosuppressive function by decreasing the amount of PD-L1 and preventing its transport to other cancer cells via exosomes, thereby restoring the cytotoxic function of T cells within the TME [141, 142]. Several irreversible and reversible LSD1is are undergoing clinical trials, which encompass both irreversible inhibitors (such as TCP, ORY-1001, GSK2879552, INCB059872, ORY-2001, IMG-7289, TAK-418) and reversible inhibitors (such as CC-90011 and SP2577) [143–145]. A novel quinazoline-based LSD1i was shown to promote the response of tumour cells to T cell killing by reducing PD-L1 expression and disrupting the PD-1/PD-L1 interaction [123]. Moreover, numerous natural products act as LSD1is and exhibit positive anti-tumour effects. These include berberine alkaloids, flavonoids and polyphenols, which are used for in the treatment of acute leukaemia [146]. Although there are currently no marketed drugs targeting LSD1, it is important to develop new LSD1is.

BETis

The inhibition of BET is considered another potential strategy for epigenetic-driven cancer therapy [147]. The BET protein BRD4 directly binds to acetylated lysine on histone tails, thereby facilitating gene transcription by RNA polymerase II [148]. BETis, such as JQ1 and RVX, showed their ability to inhibit PD-L1 binding targets during treatment [149]. BET inhibition by JQ1 significantly reduces the expression of PD-L1 on tumour cells, tumour-associated DCs and macrophages and exhibits increased anti-tumour cytotoxic T cell activity [150]. Clinical trials for haematological malignancies have demonstrated anti-tumour efficacy and manageable toxicity profiles for BETis [151]. However, despite extensive research on BETis, no drug has been approved to date.

Immune cell-related epigenetic mechanisms

Immune cells, particularly T, B and NK cells, play an important role in tumour immunotherapy. Throughout the development and differentiation of immune cells, they undergo various epigenetic modifications, including histone modifications, such as methylation and acetylation, as well as changes in DNA methylation [152].

Innate immune cells

NK cells play a vital role in suppressing tumour cell immune evasion and preserving cellular homeostasis [153]. The maturation, development and function of NK cells are tightly controlled by epigenetic modifications. They can also be exploited for cancer progression and immune evasion. Unlike genetic determinants, epigenetic factors are induced by a variety of triggers, such as increased activity of NK cells after exercise [154]. Recent studies indicate that while chronic antigens trigger the proliferation of CD8 memory T cells via whole-genome epigenetic mediation, NK cells demonstrate robust activation when treated with agonist antibodies in conjunction with IL-15, thus highlighting the specific patterns of epigenetic reprogramming associated with NK cells. [155].

DCs initiate adaptive immune responses against infections and diseases they rapidly integrate signals from their tissue microenvironment and respond accordingly. This dynamic process relies on various enzymes that facilitate epigenetic modifications, which are pivotal for the presentation of immune antigens. However, contrary to conventional understanding, DNA demethylation in infected DCs follows gene transcription. This suggests that DNA demethylation may play a more limited role in the regulation of DCs [156].

Cytokines play an important role in regulating the specific transcriptional programmes of innate immune cells. IL-4 influences STAT6 expression and promotes DNA demethylation, thereby leading to DC differentiation [157]. Additionally, within specific immune microenvironments, numerous chemokines, including CXCL8, CXCL1 and CXCL12, are influenced by DNA methylation. Their regulation in both the inflammatory microenvironment and the TME is closely linked to epigenetic mechanisms [158, 159].

$CD8^+$ T cells

During the immune function of $CD8^+$ T cells, homologous antigen signals control the initiation, expansion and

effector/memory T cell generation. Upon secondary antigen stimulation, memory $CD8^+$ T cells engage with the TCR and integrate IL-2 signalling during expansion to synergistically enhance the accessibility of whole-genome chromatin. Memory T cells exhibit functional differences from naive T cells, which arise from epigenetic modifications that regulate gene transcription. The regulation of epigenetic genes is related to the stability and extensibility of T cell memory, which has the potential to selectively modify T cell memory in diseases through DNA methylation and histone modification [160]. Consequently, the epigenetic programming and function of certain memory $CD8^+$ T cells are influenced by antigen epitope selection [161]. The inhibition of EZH2 and LSD1 expression results in reduced suppression of $CD8^+$ T cells, an increased frequency of $CD8^+$ T cells producing IFN-γ and decreased expression of tumour-infiltrating T cell inhibitory markers. This results in positive changes to the anti-tumour immune response [142, 162].

B lymphocytes

B lymphocytes are important mediators of humoral immunity. Antibody-secreting cells (ASCs) or plasma cells secrete antibodies, which are essential for maintaining robust humoral immunity within the body. The process by which B cells develop and differentiate into ASCs or plasma cells, stimulated by antigens and other signals, requires epigenetic regulation and transcriptional reprogramming. During cell division, this process involves the interaction between transcription factors that initiate gene expression programmes and epigenetic mechanisms. Epigenetic markers at various stages can regulate the differentiation of B cells [163]. However, the development, differentiation and distribution of B lymphocytes must be maintained in a state of relative balance. Under normal circumstances, B cells within the tumour maintain a balance between the extrafollicular and germinal centre responses. Once this programme is disrupted, epigenetic-metabolic crosstalk is associated with glutamine-derived metabolites, which promote T cell-driven immunosuppressive programmes [164].

Epigenetic regulators combined with immunotherapy: a novel frontier for cancer therapy

Numerous small molecule inhibitors targeting epigenetic regulatory enzymes have been developed. The immune cycle has been reported to be modulated through epigenetic therapy to enhance antigen presentation and T cell activation and infiltration as well as counteract immunosuppression. Epigenetic therapy and immunotherapy have shown a synergistic effect. Epigenetic regulators upregulate the expression of the MHC, co-stimulatory molecules and tumour-associated antigens, whereas immune checkpoints promote cytokine secretion (Fig. 5) and tumour elimination are restored, leading to an increased clinical remission rate by the combination of epigenetic therapy with ICIs [165, 166]. Thus, integrating epigenetic regulation with immunotherapy represents a promising strategy for tumour treatment and warrants further exploration.

Epigenetic regulators combined with immunotherapeutic strategies

Epigenetic drugs target various aspects of histone modification and DNA methylation involved in the epigenetic regulation, including DNMTis, HDACis, EZH2is, LSD1is and BETis [167]. These drugs modify the epigenetic landscape of cancer cells, thereby regulating gene expression and inhibiting cancer cell growth [168]. There are currently numerous clinical trials involving the combination therapy of epigenetic regulators and ICIs.

Combination therapy of DNMTis with ICIs

DNMTis activate immune cell activity, thereby enhancing the chemotherapeutic drug sensitivity in patients. Consequently, numerous combinations of DNMTis and ICIs are in clinical trials. For example, 5-azacytidine combined with pembrolizumab is being evaluated in metastatic colon cancer (NCT02512172) [169], whereas DAC is being evaluated in combination with tirelizumab for AML, and has shown promising results in terms of objective response and progression-free survival (NCT04541277) [170]. Moreover, various combinations of DAC and ICIs are also being tested in both liquid and solid tumours (Table 2).

Combination therapy of HDACis with ICIs

Most clinical data on successful combination therapy using ICIs and HDACis revolve around pembrolizumab, such as vorinostat in combination with pembrolizumab for oestrogen receptor (ER)-positive breast cancer (NCT02395627) [171], non-Hodgkin's lymphoma (NCT03150329) [129], squamous cell carcinoma of the head and neck (HN) and salivary gland carcinoma (SGC) (NCT02538510) [172]. Triple therapy consisting of tamoxifen, vorinostat and pembrolizumab for the treatment of hormone-resistant breast cancer, resulting in prolonged survival for patients (NCT02395627) [171] (Table 2). Despite some clinical data supporting its use, an understanding of the efficacy of combining HDACis with immunotherapy remains limited. To develop more effective treatments, further studies are necessary.

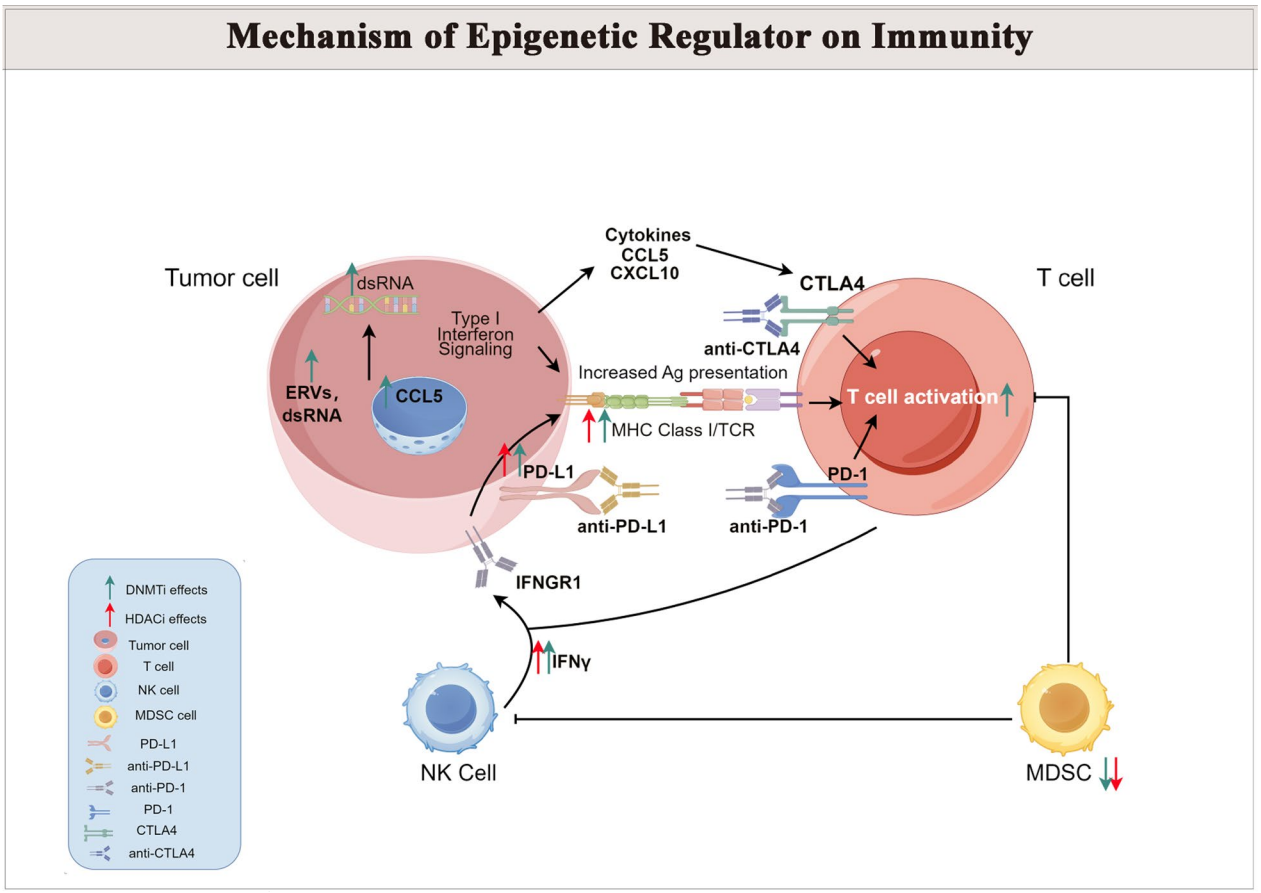

Fig. 5 Mechanism of epigenetic regulator on immunity. Epigenetic therapy and immunotherapy have a synergistic effect, which is mainly mediated by the de-repression of the promoter encoded by ERVs. Epigenetic regulators upregulate MHC, co-stimulatory molecules and tumour-associated antigens, and immune checkpoints CTLA4, PD1 and PD-L1 induce the increase of cytokine secretion and inhibit MDSCs, remodelling tumour immune microenvironment

Combination therapy of EZH2is with ICIs

Tazemetostat is the most extensively studied EZH2i in clinical settings. In January 2020, the drug received accelerated approval in the United States as first-line treatment for specific cases of epithelioid sarcoma [173]. One clinical study reported the results of tazemetostat in combination with pembrolizumab for the treatment of urothelial carcinoma [174] (Table 2). However, further studies on the potential synergistic effects of EZH2is and ICIs are required before formulating a final combination therapy strategy.

Other potential combination therapies

BETis represent a promising therapeutic approach to overcome immune resistance and enhance anti-tumour efficacy by inhibiting the PD-1/PD-L1 axis. Several clinical trials evaluated the combination of BETis and ICIs for the treatment of various types of cancer (NCT03059147, NCT03292172 and NCT02419417) (Table 2) [75, 175]. Despite the absence of clinical trials reports on LSD1is, they have been shown to enhance the response to PD-1 inhibition, resulting in increased $CD8^+$ T cell infiltration and significant tumour growth inhibition in vivo [176].

Impact of epigenetic regulators on the host immune response and tumour immunotherapy

The synergistic effect of epigenetic therapy and immunotherapy is mediated by the suppression of the promoters encoded by endogenous retroviral elements (ERVs). The results of immunopeptidomics indicate that epigenetic therapy regulates T cell responses and subsequently modulates tumour immune effects, thereby effectively controlling the progression of cancer cells [177]. On the one hand, epigenetic therapy increases the immunogenicity of cancer cells or remodels the immunosuppressive TME by upregulating the expression of the MHC, co-stimulatory molecules and tumour-associated antigens, followed by enhanced antigen presentation. On the other hand, it also induces increased cytokine secretion by upregulating the expression of immune checkpoints

Table 2 Clinical study on the combination of epigenetic regulating drugs and tumour immunotherapy

Identifier	Cancer types	Epigenetic drug	Immunotherapy drug	Trial phase	Number of subjects	Effect
NCT03150329	Non-Hodgkin lymphoma	Vorinostat	Pembrolizumab	I	52	Objective remission rate and progression-free-survival are better
NCT02395627	Estrogen receptor (ER) positive breast cancer	Vorinostat	Pembrolizumab	II	34	Objective remission rate and progression-free-survival are better
NCT02512172	Colorectal cancer	5-azacytidine and romidepsin	Pembrolizumab	I	27	Lack of further analysis of pharmacokinetics (PK) and pharmacodynamics (PD)
NCT02437136	NSCLC	Entestat	Pembrolizumab	II	76	Objective remission rate and progression-free-survival are better
NCT03250962	Classical Hodgkin lymphoma	DAC	Karelizumab	II	61	Objective remission rate and progression-free-survival are better
NCT02608437	Non-Hodgkin lymphoma	Guadecitabine	Ipilimumab	Ib	19	Objective remission rate and progression-free-survival are better
NCT04541277	AML	DAC	Tirelizumab	II	27	Objective remission rate and progression-free-survival are better
NCT02959437	NSCLC	Epacadostat	Pembrolizumab	I/II	70	Patients with advanced solid tumours who have received immunotherapy before have no clinical response
NCT03308396	Clear cell renal cell carcinoma	Guadecitabine	Duvalizumab	Ib/II	57	Did not reach the expected PFS goal
NCT02775903	AML	5-azacytidine	Durvalumab	II	129	Clinical efficacy has not improved
NCT02775903	MDS	DAC	Duvalizumab	II	84	The toxicity is greater, and the clinical outcome has not been significantly improved
NCT02953561	AML	DAC	Avelumab	Ib/II	19	Good tolerance, but limited clinical activity
NCT02996474	AML	DAC	Pembrolizumab	I	10	Prolong survival time
NCT02961101 NCT03250962	HL	DAC	Karelizumab	II	61	Prolong survival time
NCT02538510	Squamous cell carcinoma of head and neck (HN) and salivary adenocarcinoma (SGC)	Vorinostat	Pembrolizumab	II	50	Prolong survival time
NCT03820596	Relapsed or refractory extranodal natural killer T cell lymphoma	Chidamide	Sintilimab	Ib/II	38	↑DOR /PFS
NCT02220842	Recurrent/refractory (R/R) diffuse large B cell lymphoma (DLBCL)	Tazemetostat	Atezolizumab	Ib	43	Safe and tolerant, moderate anti-tumour activity

CTLA4, PD1 and PD-L1 [120]. The mechanism by which epigenetic drugs affect tumour immunity can be broadly categorised as outlined below.

Enhancing the role of innate immunity

Epigenetic therapy upregulates the expression of IFN-stimulated genes, including PD-L1, activates pattern recognition receptors (PRR) and downstream IFN pathways [178], induces the expression of endogenous retroviruses

and triggers a viral mimicry response. This increases the level of double-stranded RNA (dsRNA) and activates the innate immune signalling pathway of TNBC to exert anti-tumour effects [179].

Enhancing the role of immune cells (NK cells, T cells and macrophages)

Enhancing the cytotoxic activity of NK cells Inhibition of HDAC3 facilitates NK cell infiltration. HDAC3 orchestrates the deacetylation of ATF3 to enhance its transcriptional repression capabilities. Consequently, suppressing HDAC3 promotes CXCL12 secretion, which in turn, stimulates NK cell recruitment and activation, thus enhancing NK cell infiltration [180]. DNMTis and HDAC6is also induce the expression of cytokines, chemokines and the MHC-I antigen-presenting complex in cancer cells by enhancing the type I IFN response. Increased tumour killer cells NK and NK T cells were detected following treatment with DNMTis and HDAC6is in a mouse model of ovarian cancer. This indicates that epigenetic drugs enhance the anti-tumour effect of NK cells [181].

Targeting adaptive immune T cells to augment their immune response In tumour cells that highly express CXCL9, 10 and 11 chemokines, HDAC3 binds to their promoter regions to inhibit expression. The HDAC3is, entinostat and panobinostat, not only inhibit tumour growth by recruiting $CXCR3^+$ T cells, but also act through additional mechanisms [182]. HDAC6is, including QAPHA and zabadinostat (CXD101), induce the expression of the tumour immune-related major histocompatibility complex II (MHC-II) on tumour cells, thereby enhancing the recruitment and activation of $CD4^+$ and $CD8^+$ T lymphocytes [183–185]. The subtype-selective HDACi tucidinostat enhances the activity of chemokine ligand 5 (CCL5) and the expression of co-stimulatory molecules on monocytes, while also promoting the migration of $CD8^+$ T cells into tumours by attenuating the NF-κB signalling pathway [186]. The synergistic use of HDACis and anti-PD-1 antibody increases the expression of CCL5 and CXCL9, subsequently suppressing tumour growth and the infiltration of $CD3^+$ and $CD8^+$ T cells and controlling tumour progression [187]. HDAC1is elevate the histone acetylation of the PD-L1 gene by activating the transcription factor STAT1, thereby enhancing PD-L1 expression. This process not only attenuates the infiltration of $CD8^+$ T cells, but also decreases the number of MDSCs within the TME. The concomitant administration of thioamide and anti-PD-1 antibody cause a significant facilitated a reduction in tumour volume and promotes survival [188].

Enhancing the function of the TAM The DNMTi 5-azacytidine induces the activation of M1-like macrophages, which is characterised by increased p65 phosphorylation and IL-6 expression. Subsequently, 5-azacytidine-activated T cells in adipose tissue exert their immune and anti-tumour effects [189]. The administration of DNMTi DAC in vivo promotes IL-8 release and macrophage M2-like differentiation, with increased expression of CD206 and ALOX15, to enhance the anti-tumour effect. However, DAC also induces a reduction in the levels of the inflammatory cytokines IL-1β, TNF-α and IFN-γ and polarises M2, which may cause immune tolerance during immunotherapy [190].

Additional immunomodulatory effects of the epigenetic drugs

Enhancement of the role of antigen presentation DNMT restores tumour immunogenicity, thereby promoting DC maturation. It also enhances the activity of antigen-presenting cells and the phagocytosis of tumour cells by antigen-presenting cells. For example, zebularine stimulates the cGAS-STING-NF-κB/IFNβ pathway to augment the immunogenicity of tumour cells and increases the processing and presentation of antigens, thereby inducing tumour cell destruction mediated by $CD4^+$ and $CD8\ T^+$ cells [191].

Suppression of immunosuppressive cells HDAC directly acts on immune cells and exerts its function by attenuating the function of naive T cells, boosting $CD8^+$ T cells and NK cells to regulate immunity, while targeting Tregs to regulate tumour immune suppression and alter the TME [192]. The pan-histone deacetylase inhibitor suberanilohydroxamic acid (SAHA) inhibits the binding of c-Myc to the CCL1 promoter, subsequently suppressing the transcription of CCL1. Furthermore, it blocks the interaction between CCL1 and CCR8, which also downregulates the activity of Tregs and alleviates the immunosuppressive effects of the tumour [193].

Enhancement of CAR-T cell activity and function The HDACi M344 and thioamide enhance the acetylation of TCF4, LEF1 and CTNNB1 by suppressing the expression of HDAC1 and activating the canonical Wnt/β-catenin pathway, thereby inducing sustained anti-tumour effects in CAR-T cells [194].

Advances in and challenges of combined immunotherapy in clinical settings

In recent years, the use of epigenetic regulatory factors alongside tumour immunotherapy has resulted in notable progress in cancer treatment. There has been an increase in the number of combination therapies. In addition to the integration of epigenetic regulators and ICIs, there is increased interest in the synergistic use of epigenetic therapies, such as CAR-T, ACT and tumour vaccines. Apart from their use in conjunction with

immunotherapy, epigenetic modifiers are also used in combination with other tumour treatment modalities, such as radiotherapy or chemotherapy. Studies of tumour combination therapy have progressed from cell culture and animal models to clinical trials, with its application expanding from initial blood cancers to various solid tumours [195, 196]. Furthermore, in the majority of preclinical and clinical research, combination therapy has demonstrated favourable therapeutic outcomes, as evidenced by alterations in disease remission rates, extensions in both disease-free and overall survival durations and a minor reduction in adverse events. Despite significant advancements in the integration of epigenetic modifiers with tumour immunotherapy, numerous barriers and shortcomings persist in the pathway to their broad clinical adoption and dissemination.

The application of combination therapy is subject to certain requirements and limitations, with only a small proportion of patients benefitting from it. For example, the epigenetic regulators azacytidine and bevacizumab may not exert their expected anti-tumour effects in patients with solid tumour who have undergone related immunotherapy in the past [197]. Although combination therapy is viable for certain tumours, it does not enhance clinical efficacy. Compared with azacitidine alone, first-line combination therapy with duvalizumab and azacitidine did not benefit older patients with AML [198]. In addition to issues regarding clinical efficacy and target beneficiaries, combination therapy may increase drug toxicity. In patients with high-risk MDS, azacitidine combined with the ICI duvalizumab was more toxic compared with azacitidine alone, without any improvement in clinical efficacy [199]. The same toxicity issues were observed in the combined treatment of small-cell lung cancer (SCLC). Administering atezolizumab and antazoline alongside carboplatin and etoposide was unsafe, because it resulted in severe neutropenia and thrombocytopenia. In summary, the use of epigenetic modifiers and tumour immunotherapy faces challenges, including efficacy and toxicity. It will be necessary to focus on the epigenetic regulation mechanisms of tumour immunity to address the deficiencies in their clinical application.

Conclusion

This review explored the application of immunotherapy and epigenetic regulators in tumour treatment, highlighted recent advances in the integration of tumour immunotherapy with epigenetic therapy and summarised the application of various combination approaches in cancer treatment. Most clinical trials have demonstrated the benefits of tumour immunotherapy, based on improved overall and disease-free survival rates in patients with cancer. However, clinical use still faces limitations, such as a restricted patient base and an increased incidence of adverse reactions. Epigenetic regulators play a significant role in immunomodulation-mediated anti-tumour effects and in counteracting immune evasion, particularly within the context of immunotherapy. We expect further studies to conclusively validate these findings, eagerly anticipating the early and effective application of immunotherapy and epigenetic regulation in patients with cancer. Nevertheless, continued exploration into their underlying mechanisms is essential, based on the clinical benefits observed in most combined application trials. This will lead to their rapid and widespread clinical adoption, offering more effective and safer treatment options for patients with cancer. Generally, the integration of tumour immunotherapy and epigenetic regulation therapy results in positive anti-tumour effects and will likely contribute to cancer treatment in the future.

Abbreviations

5-Aza	5-Azitidine
ACT	Adoptive cell transplantation therapy
AID	Acquired Immune Deficiency Syndrome
AML	Acute myeloid leukemia
APCs	Antigen-presenting cells
ASCs	Antibody-secreting cells
Breg cells	B regulatory cells
CAFs	Cancer-associated fibroblasts
CAR T cell therapy	Chimeric antigen receptor T cell therapy
CCL5	Chemokine ligand 5
CLCF1	Cardiotrophic factor-like cytokine 1
CRS	Cytokine release syndrome
CTLA-4	Cytotoxic T lymphocyte-associated protein 4
CXCL6	Chemokine ligand 6
DAC	Decitabine
DCs	Dendritic cells
DNMTis	DNA methyltransferase inhibitors
DNMTs	DNA methyltransferase
dsRNA	Double-stranded RNA
ECM	Extracellular matrix
ERVs	Endogenous retroviral elements
FDA	Food and Drug Administration
GM-CSF	Granulocyte-macrophage colony stimulating factor
HDAC	Histone deacetylase
HDACis	Histone deacetylase inhibitors
HGF	Hepatocyte growth factor
HIF	Hypoxia-inducible transcription factor
HL	Hodgkin's lymphoma
HR-MDS	High-risk myelodysplastic syndrome
HSCT	Hematopoietic stem cell transplantation
ICANS	Immune effector cell-associated neurotoxic syndrome
ICB	Immune checkpoint blockade
ICIs	Immune checkpoint inhibitors
IFN	Interferon
IFI16	IFN inducible protein 16
IFN-α	Interferon-α
IL-2	Interleukin-2
IL-10	Interleukin-10
ILGF1/2	Insulin-like growth factor 1/2
ISG	IFN-stimulated gene
iNOS	Inducible nitric oxide synthase
MDS	Myelodysplastic syndrome
MDSC	Myeloid suppressor cells
MHC-I	Major histocompatibility complex I
MHC-II	Major histocompatibility complex II
mRNA	Messenger RNA

NSCLC	Non-small cell lung cancer
PRR	Pattern recognition receptors
SAHA	Suberanilohydroxamic acid
SCLC	Small cell lung cancer
SIRPα	Signal regulatory protein α
Stau1	Staufen1
TAMs	Tumour-associated macrophages
TCR	T cell receptor
TECs	Tumour-associated endothelial cells
TFHL	T-follicular helper cell lymphomas
TGF-β	Transforming growth factor β
TGM2	Transglutaminase 2
TILs	Tumour-infiltrating lymphocytes
TME	Tumour microenvironment
TNBC	Triple negative breast cancer
TNF	Tumour necrosis factor
Tregs	Regulatory T cells

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Author contributions
Huan Zhang contributed to the data curation; formal analysis; methodology; writing—original draft; preparing Figures 1–5. Yutong Pang and Ling Yi were involved in the data curation; formal analysis; and investigation. Xiaojue Wang performed the formal analysis and investigation. Panjian Wei curated the data. Haichao Wang contributed to the data curation; formal analysis; investigation; methodology; and software. Shuye Lin contributed to the data curation; formal analysis; funding acquisition; methodology; project administration; resources; supervision; visualization; writing—original draft; and writing—review and editing.

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Availability of data and materials
No datasets were generated or analysed during the current study.

Declarations

Consent for publication
The authors declare no competing interests.

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