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Review

Targeting N6-methyladenosine RNA modification combined with immune checkpoint Inhibitors: A new approach for cancer therapy

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ABSTRACT

Immune checkpoint inhibitors (ICIs) have revolutionized cancer immunotherapy by restoring the host antitumor immune response. Since 2011, various ICIs have been approved for the treatment of cancers, which has led to unprecedented prolongation of the survival time for some patients. Although ICIs have been successfully applied in the treatment of different cancers, the low effectiveness rate has dramatically restrained the clinical application of ICI treatment. N6-methyladenosine (m6A) modification is the most common RNA methylation. Recent studies have pointed out that m6A epigenetic modification could improve the efficacy of ICI blockade treatment. Here, we briefly summarize the relevant mechanisms of tumour immunity, the clinical application of ICIs, the resistance to ICI treatment in cancers, and the m6A epigenetic modification and how it regulates the response to ICI treatment. We attempted to provide a potential strategy for cancer therapy by targeting m6A modification combined with ICI blockade treatment.

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1. Background

Cancer is one of the most lethal chronic diseases in the world, with nearly 10 million deaths reported worldwide in 2020 [1]. Surgical resection, radiotherapy and chemotherapy are three major traditional cancer treatment methods. However, the accompanying limitations, including severe trauma, low targeting ability, high toxicity and strong drug resistance, markedly restrict their application in cancer therapy [2]. In recent years, cancer immunotherapies, especially immune checkpoint blockade (ICB) therapy, have achieved tremendous progress in the treatment of many malignant tumours [3,4].

ICIs have been a first-line therapy since they were discovered, which can alleviate the immunosuppressive tumour microenvironment [5]. In general, ICIs can elicit a powerful immune response by releasing the inhibitory braking of T cells, with the blockade of PD-1/PD-L1 and CTLA-4 being typical examples [6]. To date, the Food and Drug Administration (FDA) has authorized three kinds of ICIs, including antibodies against CTL4 (ipilimumab), PD-1 (pembrolizumab, cemiplimab and nivolumab), and PD-L1 (atezolizumab, durvalumab and avelumab). Most of these agents were initially approved for melanoma but have also been applied to other tumour types [7,8]. Although ICI therapy has been demonstrated to be successful in several cancers, the low effectiveness rate has significantly restrained the clinical application of ICI blockade treatment. Taking the therapeutic efficacy of pembrolizumab (anti-PD-1) as an example, the response rate among melanoma patients was only 33 %. Similarly, and in regard to lung cancer patients, only approximately 20–30 % of patients achieved the expected results with ICI blockade therapy [9].

Recent studies have indicated that epigenetic modification can not only promote cancer progression but also influence drug sensitivity [10]. Since epigenetic modifications are reversible by nature, strategies aiming to alleviate abnormal epigenetic modifications are probably effective combination treatments [11,12]. As a vital branch of epigenetic modification, m6A modification is the most commonly studied mRNA and ncRNA modification and can participate in various basic pathophysiological and metabolic processes of RNA, including splicing, nuclear export, translation, decay, folding and RNA-protein interactions [13–17]. Several studies have shown that aberrant expression of m6A regulators, including "writers" (methyltransferases), "readers" (binding proteins), and "erasers" (demethylases), might contribute to carcinogenesis, progression, and drug resistance in various cancers [18]. In addition, m6A modification has been demonstrated to be a potential target for cancer immunotherapy, which can function as a complement to immune checkpoint inhibitor therapy, thereby significantly improving the survival rate and enhancing the quality of life of cancer patients [7,19,20].

In this review, we briefly summarize the relevant mechanisms of tumour immunity, the principle and clinical applications of ICIs, and the role of m6A modification in cancer ICI treatment.

2. Mechanisms underlying tumour immunity

The human immune system is composed of immune defence, immunologic homeostasis and immune surveillance. First, immune defence can eliminate or inhibit viral infection and protect the host from virus-induced tumours. Second, immune homeostasis serves to remove pathogens and helps prevent establishment of the inflammatory environment facilitated by tumorigenesis. Third, immune surveillance can recognize and eliminate tumour cells according to their specific antigens or cell stress-induced molecules, and through these molecules, the immune system can discriminate cancer or precancerous cells from normal cells and eliminate them before they cause damage [21]. Even though the human body possesses a series of approaches for immune surveillance and immune clearance, tumour cells can still develop some strategies to weaken the immune system or evade the immune response, which leads to tumour immune escape [22].

Several potential mechanisms may underlie tumour immune escape.

(1) Low immunogenicity. Some tumours can escape recognition by the immune system because unlike normal cells, they do not have protein peptides that can be presented by MHC molecules. Other tumours might lose one or more MHC molecules or the expression of costimulatory proteins that are required for the activation and maturation of naive T cells.

(2) Lack of costimulatory molecules. The tumour antigens presented without the existence of costimulatory signals will lead to T cells' tolerance of these specific antigens.

(3) Antigen modulation. Initially, the immune system can recognize tumour antigens to attack tumour cells, whereas antibody-induced antigen internalization or the variation of antigens in tumours will lead to a decrease or even the disappearance of these antigens. The genetic instability of tumour cells is currently believed to contribute to the development of the antigen reduction
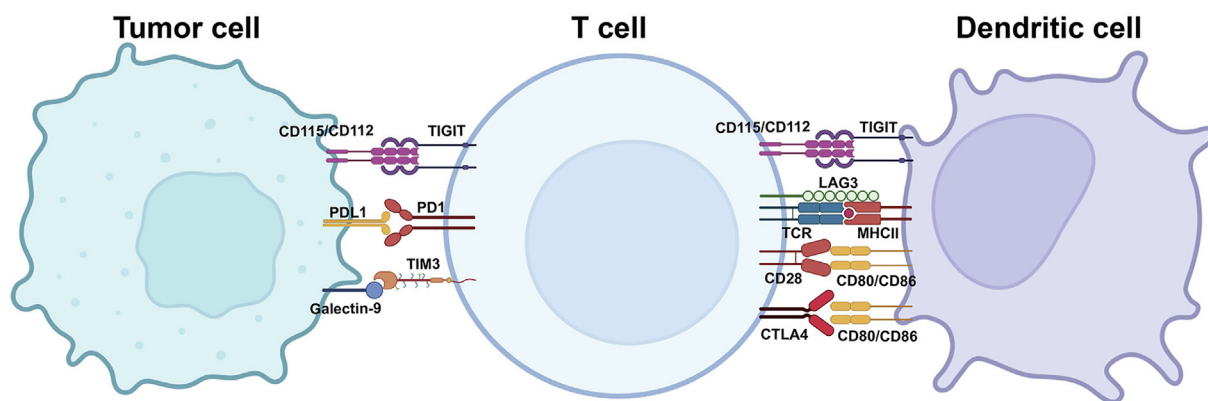
Fig. 1. Immune checkpoint signalling mechanisms.

equilibrium phase, which refers to slow or stagnant tumour cell proliferation caused by the immune system. Once the immune system fails in the fight against tumour cells, it will no longer destroy them, the result of which is robust proliferation of tumour cells. Moreover, tumour cells can escape an attack from lymphocytes by not expressing specific antigens and thus develop a selective advantage.

(4) Formation of an immune-privileged site. Tumour cells can secrete a variety of molecules, such as collagen, to form a physical barrier, which can prevent lymphocytes and antigen-presenting cells (APCs) from entering the tumour.

(5) Tumour-induced immunosuppression. Tumour cells can produce multiple immunosuppressive molecules, such as TGF-β, IL-10, IDO and PD-L1, to inhibit the immune response directly. They can also recruit regulatory T cells that secrete immunosuppressive cytokines [22,23].

3. Tumour immune checkpoint inhibitors and their clinical application

Next, we briefly outline the functional mechanisms of ICIs (Fig. 1) and their clinical applications based on the immune checkpoints they targeting.

PD-1 (CD279) is a type I transmembrane protein that is mainly expressed in activated T cells. PD-1 expression can also be detected in other cell types, including B cells and natural killer (NK) cells. PD-1 has been reported to eliminate the transmission of antigen recognition signals mediated by *T*-cell receptors [24]. Structurally, PD-1 contains a cytoplasmic tail and an extracellular domain similar to immunoglobulin (Ig). The cytoplasmic tail of PD-1 is composed of two immune receptor tyrosine-based structural motifs, the inhibitory motif (ITIM) and the switching motif (ITSM) [25]. Moreover, the inhibitory function of PD-1 is dependent on the phosphorylated tyrosine in ITSM [26]. PD-L1 and PD-L2 are two PD-1 ligands. PD-L1 is mainly expressed in tumour cells, and its expression is influenced by interferon-γ (IFN-γ) in the microenvironment. Once PD-L1 on tumour cells encounters PD-1 on T cells, their interaction will stop T cells from attacking tumour cells and cause immune escape [27,28]. Therefore, PD-1 on T cells plays a negative regulatory role in the immune system by acting as a brake on the immune system to prevent excessive immune activation. Tumour cells take advantage of this braking by overexpressing PD-L1 to escape attack from immune cells. Similarly, PD-1/PD-L1 inhibitors can block this signalling pathway to eliminate tumour cells by restoring the cytotoxicity of immune cells. To date, the FDA has approved three anti-PD-1 antibodies: nivolumab (IgG4 mAb), pembrolizumab (IgG4 mAb) and cemiplimab (IgG4 mAb). Pembrolizumab and cemiplimab have been demonstrated to work

well in the clinical treatment of melanoma and non-small cell lung cancer (NSCLC) patients [29]. In addition, nivolumab monotherapy is the first FDA-approved first-line immunotherapy for gastric cancer, which is also effective in the treatment of NSCLC, classical Hodgkin's lymphoma (CHL) and melanoma patients [30]. Since 2019, pembrolizumab has been approved and used as the first-line treatment for metastatic melanoma, some metastatic NSCLC and metastatic bladder cancer, refractory CHL and metastatic ESCC and as a second-line treatment for head and neck squamous cell carcinoma (HNSCC) [31–35]. Moreover, cemiplimab has been approved for the treatment of basal cell carcinoma, cutaneous squamous cell carcinoma (CSCC) and NSCLC. The FDA has also authorized three anti-PD-L1 antibodies, including atezolizumab (IgG4 mAb), avelumab (IgG1 mAb) and durvalumab (IgG1 mAb) [36]. Atezolizumab was the first FDA-authorized PD-L1 inhibitor for the treatment of patients with advanced or metastatic urothelial cancer in 2016 [37]. Atezolizumab has also been approved for patients with metastatic NSCLC that developed during chemotherapy or platinum-containing chemotherapy [38]. In 2017, avelumab was approved for the treatment of metastatic urothelial carcinoma and Merkel cell carcinoma (MCC) [39,40]. In addition, the FDA approved the combination of avelumab and the tyrosine kinase inhibitor axitinib for the first-line treatment of patients with advanced RCC in 2019[41]. In 2017, durvalumab was approved for the treatment of locally advanced or metastatic urothelial carcinoma for the first time [42]. Durvalumab, in combination with etoposide and carboplatin or cisplatin, has been approved as a first-line treatment for patients with advanced NSCLC [43].

CTLA-4 (CD152) is a type I transmembrane glycoprotein that is mainly expressed in T cells. It shares a pair of receptors with CD28—B7-1 (CD80) and B7-2 (CD86) —expressed on the surface of dendritic cells (DCs). In general, CD28 expression can be detected in both quiescent and activated T cells, while CTLA-4 is expressed only in activated T cells. The costimulatory checkpoint protein CD28 on T cells interacts with B7-1 and B7-2 on DCs to amplify the antigen recognition signal and thus successfully activate T cells [44]. To prevent excessive activation and proliferation of T cells, the inhibitory signals produced by the combination of CTLA-4 and B7-1/B7-2 are used to offset the signal activation through higher binding affinity [45–48]. As a vital immune balance modulator, CTLA-4 mainly functions by inhibiting the activation of effector T cells and promoting the proliferation of regulatory T cells (Tregs) in the tumour microenvironment to produce an immunosuppressive effect on tumour progression [49,50]. CTLA-4 inhibitors can target CTLA-4 to relieve Treg inhibition in the tumour microenvironment and induce the activation and proliferation of T cells through which they can attack tumour cells and achieve the goal of disease treatment. Ipilimumab (IgG1 mAb) is a mono-

clonal antibody against CTLA-4 and was the first ICI approved by the FDA in 2011 for patients with advanced melanoma [51]. The combination of ipilimumab with the PD-1 inhibitor nivolumab has been approved for the treatment of patients with metastatic colorectal cancer (CRC) with high microsatellite instability (H-MSI) or mismatch repair (MMR) [52]. Regardless of PD-L1 expression, the combination of ipilimumab with nivolumab has also been approved for patients with moderate- or low-risk renal cell carcinoma (RCC) [53]. In addition, ipilimumab combined with nabulizumab has also been used as a first-line treatment for NSCLC and malignant pleural mesothelioma (MPM) with tumour PD-L1 expression ≥ 1 % [54,55].

TIGIT (*T*-cell Ig and ITIM domain) is a member of the poliovirus receptor (PVR)/Nectin family that is predominantly expressed in T cells and NK cells [56,57]. TIGIT can bind to at least two Nectin family members, CD155 and CD112, and its affinity for CD155 is much higher than that for CD112 [58]. The interaction of TIGIT with CD155/CD112 dramatically weakens the cytotoxicity of targeting cells to achieve immunosuppression [59,60]. In addition, TIGIT can inhibit costimulation of DCs and result in reduced antigen presentation and immune activity of DCs. Therefore, the principle of TIGIT inhibitors in immunotherapy is to enhance the effect of T, NK and DC cells. By June 2020, 15 antibodies targeting the TIGIT-PVR pathway were under development, and tiragolumab has since entered the clinical trial phase. The combination of tiragolumab and atezolizumab targeting the TIGIT-PVR pathway is a promising first-line treatment for metastatic NSCLC patients with high PD-L1 expression and no EGFR or ALK mutation [61].

LAG-3 (CD223) is an inhibitory receptor of the type 1 Ig family. LAG-3 expression has been detected in a variety of immune cells, including activated T cells, Tregs and B cells [62,63]. LAG-3 can interact with various molecules and deliver inhibitory signals to regulate immune cell homeostasis, *T*-cell activation and proliferation, cytokine production, cytolytic activity, and other cellular functions [63]. In addition, persistent antigen stimulation, such as in cancer and chronic viral infection, can reflect LAG-3 expression and lead to *T*-cell failure and subsequent impairment of *T*-cell function [64]. Tumour cells are believed to use this strategy to escape immune surveillance during tumorigenesis and cancer progression. Opdualag is a fixed-dose combination of the LAG-3 blocking antibody relatlimab and the PD-1 blocking antibody nivolumab [65]. On 18 March 2022, Opdualag was approved by the FDA as a treatment option for adults and children older than 12 years with unresectable or metastatic melanoma.

TIM-3 (HAVCR2) is a type I membrane protein that is expressed in various immune cells, including Tregs, DCs, B cells, macrophages, NKs and mast cells [57]. It can mediate *T*-cell exhaustion and play a vital role in inhibiting antitumor immunity [66]. Aberrant STAT5 and p38 signalling was detected in Tim-3$^+$CD8$^+$ T cells, while blocking the Tim-3 pathway dramatically enhanced antitumor immunity and increased IFN-γ secretion in T cells [67]. A similar efficacy of Tim-3 was obtained in preclinical studies compared with that of PD-1 and LAG-3 inhibitors, and a synergistic effect of the three drugs was detected [68,69]. As a high-affinity humanized IgG4 (S228P) antibody targeting TIM-3, sabatolimab (MBG453) targeting TIM-3 on immune and bone marrow cells obtained fast certification from the FDA in 2021. Undoubtedly, ICIs represent a prominent class of drugs for human cancer therapy.

4. ICI resistance

Despite the advantages and robust development of ICIs in immunotherapy, their efficacy is usually short-term, and patients' responses are highly heterogeneous [70]. Even among melanoma patients with the highest response rate to ICIs, 60 % – 70 % had

no objective response to anti-PD-1 treatment [71]. Regarding lung cancer, only approximately 20–30 % of patients achieved the expected results when they received ICI blockade treatment [9]. ICI resistance is becoming a hot topic in tumour immunotherapy and can be divided into two categories: 1) primary resistance, which generally refers to patients who have no response at all from the very beginning and experience rapid tumour progression, and 2) acquired drug resistance, which refers to patients who initially respond to ICIs, but clinical and/or imaging progress ultimately occurs after treatment for a period.

Our understanding of the characteristics and mechanisms of primary and advanced ICI resistance is still limited. For primary drug resistance, the effectiveness rate of ICI treatment varies markedly among different cancers, from more than 80 % of patients with refractory Hodgkin's lymphoma to little or no response in mismatch repair-proficient colorectal cancer patients [72,73]. As the effectiveness rate of many tumours is between 20 % and 40 %, primary resistance or no response to ICIs remains a key issue. A recent study showed that only 12.5 % of the patients were estimated to benefit if they met the eligibility criteria for ICI treatment in 2018 [74]. Therefore, to increase the proportion of patients benefiting from ICI treatment, the factors that may lead to primary drug resistance must be thoroughly understood. The defects in antigenicity and adjuvanticity that shape tumour immunogenicity might be a probable explanation for the insensitivity of tumour cells to ICIs [75]. To address the challenge of primary drug resistance, extensive effort has been expended on combination treatment strategies, usually using empirical orthogonal therapies to expand the response population. In addition, potential biomarkers of the initial ICI response have been extensively studied, such as PD-L1 expression, the tumour mutational burden, tumour-infiltrating lymphocytes (TILs) and related gene expression characteristics.

In contrast to the primary drug resistance of ICIs, acquired drug resistance has not been thoroughly studied. Dysregulation of antigen presentation is suggested to be an effective mediator of acquired drug resistance. For example, interruption of MHCI presentation in lung cancer patients could decrease sensitivity to ICI treatment [84]. Moreover, in a patient with metastatic uterine leiomyosarcoma who responded well to anti-PD-1 treatment, one of the metastatic nodules was still insensitive to immunotherapy. Genomic and proteomic analyses of this nodule showed that the PTEN gene was mutated and that the expression of several neoantigens was decreased [85]. Although no evidence indicates that these features are related to drug resistance, the loss of neoantigen expression might also contribute to escape from cytotoxic *T*-cell attack.

5. Epigenetic modification of m6A

m6A modification is a dynamic and reversible process regulated by three types of enzymes: m6A methyltransferases, m6A demethylases and m6A binding proteins(Fig. 2). Their combined activities ensure the normal expression and translation of RNA [76].

M6A methyltransferases are also known as m6A writers. METTL3, METTL14, WTAP, RBM15, RBM15B, VIRMA and ZC3H13 are common methyltransferases [77,78]. METTL3, a protein with a molecular weight of 70 kDa, is the core catalytic component of the methyltransferase complex. The stable heterodimer formed by METTL3 and METTL14 at a ratio of 1:1 can induce m6A deposition in nuclear RNA transcripts [79]. WTAP is the regulatory component of the complex, which affects m6A deposition by binding to the METTL3/14 complex [80]. RBM15/15B interacts with METTL3 in a WTAP-dependent manner, which can help recruit the methyl-
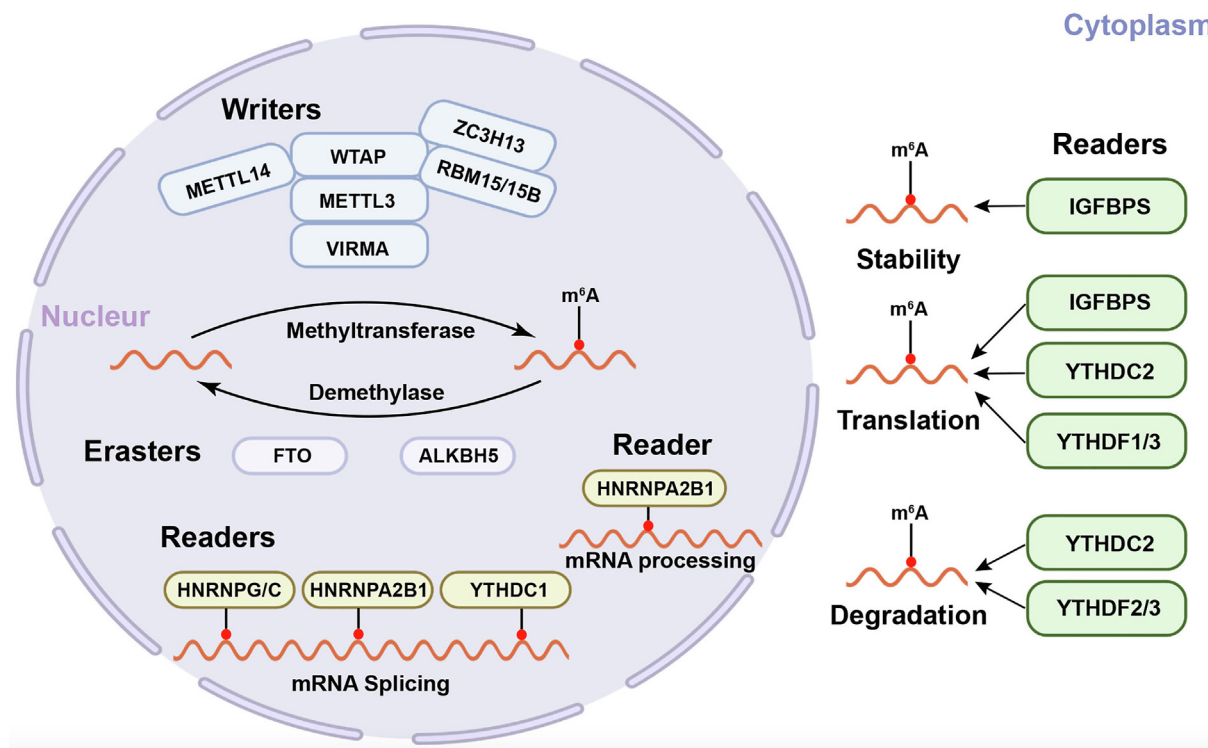
Fig. 2. The functional mechanism of m6A methylation and its machinery in RNA metabolism.

transferase complex to the U-rich region of mRNA [81]. VIRMA, also known as KIAA1429, is the largest scaffold component of the m6A methyltransferase complex, which plays a regulatory role in m6A methylation in the 3′-UTR and stop codon areas of genes [82,83]. ZC3H13 regulates nuclear m6A methylation by binding to other cofactors, such as WTAP and RBM15 [84]. These writer complexes are dramatically enriched in the RRACH (R = G or A; H = U, A or C) sequences of the stop codon, the 3′-UTR and long introns [85].

M6A demethylases are also known as m6A erasers. Fat and obesity-related protein (FTO) and AlkB homologue 5 (ALKBH5) are two common demethylases that contribute to the dynamic and reversible process of m6A modification [86,87]. FTO was the first protein discovered to catalyse m6A demethylation, which can affect the splicing and stability of mRNA by regulating m6A modification [88]. ALKBH5 is the second demethylase identified to reverse m6A modification, which can regulate mRNA output and metabolism through m6A methylation [87]. The biological effects of demethylases depend on the RNAs that they demethylate.

M6A-binding proteins are also known as readers of m6A modification. The YTH domain families (YTHDF1/2/3 and YTHDC1/2), heteronuclear ribonucleoproteins (HNRNPs; hnRNPC, hnRNPG and hnRNPA2B1) and insulin-like growth factor 2 mRNA binding proteins (IGF2BP1-3). These readers have been demonstrated to be involved in the regulation of RNA splicing, nuclear output, translation efficiency, RNA stability and RNA decay [89]. For example, the interaction of YTHDF1 with eIF3 can facilitate translation. YTHDF2 is the most widely studied m6A reader and can accelerate RNA decay modified by m6A methylation [90]. YTHDF3 affects the translation and decay of m6A-modified mRNAs through its synergistic effects with YTHDF1 or YTHDF2 [91]. Ribonucleoprotein HnRNPC/G is involved in RNA processing and maturation [92], while the RNA binding protein hnRNPA2B1 can bind to m6A-modified nuclear RNAs to participate in subsequent gene splicing

[92,93]. Readers from the IGF2BP family can recognize and bind to m6A modification sites, thereby increasing the stability and translation of target RNAs [94].

6. The involvement of m6A methylation in the responses of immune cells

6.1. T cells

T-cell development occurs in the thymus. Mature T cells can migrate to the surrounding organs to regulate the adaptive immune response and play an important role in the process of tumour immunity [95]. Some studies have shown that METTL3 deletion in CD4^{+} T cells can destroy the homeostasis and differentiation of T cells by downregulating activation of the IL-7/STAT5/SOCS pathway [96]. Interestingly, METTL3 deletion can enhance the stability of SOCS mRNA, thereby inhibiting the IL-2-STAT5 signalling pathway, which is crucial for the function of Tregs [97] T follicular helper (Tfh) cells are a special type of CD4^{+} T cells essential for humoral immunity [98]. In CD4^{+} T cells, METTL3 can stimulate the differentiation, proliferation and survival of Tfh cells by stabilizing Tcf7 transcripts, while conditional deletion of METTL3 can substantially impair these biological processes [98]. In addition, knockout of ALKBH5 could decrease the lactic acid content in the tumour microenvironment by downregulating the expression of the Mct4/Slc16a3 pathway, thus inhibiting the accumulation of Tregs and myeloid suppressor cells. Importantly, the absence of ALKBH5 can also enhance the efficacy of anti-PD-1 therapy [99].

6.2. Dendritic cells

DCs are important APCs. Immature DCs have a strong migration ability. After maturation, they can stimulate and activate T cells and function as a bridge between the innate immune response

and adaptive immune response [100]. Studies recently found that METTL3-mediated m6A modification could promote the activation and maturation of DCs. Specific deletion of METTL3 led to the impaired phenotype, functional maturation of DCs, decreased expression of costimulatory molecules, including CD40, CD80 and the cytokine IL-12, and a decreased response to *T*-cell stimulation. The mechanism underlying METTL3-mediated *T*-cell activation is that METTL3 can stimulate the translation efficiency of CD40, CD80 and Toll/interleukin-1 receptor (TIR) domain adaptor protein (TIRAP) [101]. YTHDF1 can enhance the translation of lysosomal protease-encoded mRNA, which can degrade tumour antigens in lysosomes. Deletion of YTHDF1 in DCs has been reported to inhibit the translation of lysosomal protease, which enhances the cross presentation of tumour antigens and promotes a more cytotoxic lymphocyte (CTL) response against tumours in DCs. In addition, the therapeutic effect of PD-L1 checkpoint blockade was enhanced in YTHDF1(-/-) mice [102]. YTHDF1 is suggested to be a new potential therapeutic target in anticancer immunotherapy.

6.3. Macrophages

Macrophages are phagocytes of the innate immune system, which mainly participate in the recognition, phagocytosis and degradation of pathogens and tumour cells, as well as the genesis and progression of tumours [103]. C1q + macrophages were found to express a variety of ligands that are immunoregulated by METTL14, and METTL14 regulates tumour-infiltrating CD8+ T cells through these ligands. In addition, specific knockout of METTL14 in macrophages drives the differentiation of CD8+ T cells towards dysfunction, thereby inhibiting the cytotoxicity of CD8+ T cells to tumour cells [104]. METTL3 depletion in macrophages reconstituted the tumour microenvironment by enhancing the infiltration of M1- and M2-like TAMs, as well as Tregs. M6A sequencing showed that METTL3 deletion damaged the YTHDF1-mediated translation of SPRED2, thus enhancing the activation of NFκB and STAT3 through the ERK pathway and resulting in increased tumour growth and metastasis. In addition, METTL3 consumption in macrophages also reduces the efficacy of PD-1 blockade therapy [105]. The above findings may provide new ideas for exploring the molecular mechanisms by which macrophages participate in cancer immunotherapy.

6.4. Natural killer cells

NK cells are innate lymphoid immune cells. As a core component of the innate immune system, NK cells play an important role in tumour monitoring [106]. Chen et al. found that METTL3 deletion in NK cells changed the homeostasis of NK cells and inhibited the function and infiltration of NK cells in the tumour microenvironment. The protein expression of m6A-modified SHP-2 is downregulated in METTL3-deficient NK cells. Decreased SHP-2 expression reduced the response of NK cells to IL-15, thus promoting tumour progression and metastasis [106]. Subsequently, Ma et al. found that YTHDF2 deletion in NK cells damages the antitumor and antiviral activities of NK cells in vivo. In terms of mechanism, YTHDF2 can sustain the homeostasis and terminal maturation of NK cells, which is related to the regulation of NK cell transport and Eomes, respectively. In addition, the formation of a STAT5-YTHDF2 positive feedback loop can also promote the effector function of NK cells and IL-15-mediated NK cell survival and proliferation [107]. These findings suggest that METTL3- and YTHDF2-mediated m6A methylation plays a regulatory role in antitumor immunity and NK cell homeostasis.

7. Association between m6A methylation and tumour immune checkpoint therapy

7.1. Nervous system tumours

Gliomas and glioblastomas (GBMs) are two common invasive brain tumours [108]. In recent years, a substantial number of studies have demonstrated that m6A modification plays an important role in their progression and anticancer effects [109]. The m6A scoring system established by Cai et al. showed that GBMs with high m6A scores had a better prognosis, while GBMs with low m6A scores had a worse prognosis. Moreover, the m6A score was significantly correlated with the expression of immune checkpoint genes, indicating that m6A modification may affect the efficacy of immunotherapy [110]. In contrast, some studies have demonstrated that immune checkpoint therapy is more effective for tumours with low m6A scores [111,112]. Zhao et al. confirmed that m6A modification of the regulatory factor HSPA7 can promote SPP1 expression and macrophage infiltration by regulating the expression of Yap1 and LOX in glioblastoma stem cells (GSCs) in vitro. This finding was also confirmed by a glioblastoma organ-like (GBO) model in which HSPA7 knockout enhanced the therapeutic effect of ICB treatment [113]. Yinyang 1 (YY1) is a zinc finger transcription factor that interacts with CDK9 to regulate transcriptional elongation in GSCs. Inhibition of METTL3 or YTHDF2 can stabilize interferon-related genes and activate interferon signals in other cell types. Targeting the YY1-CDK9 complex reduced the expression levels of METTL3 and YTHDF2, thereby inducing the interferon response, reducing regulatory *T*-cell infiltration, and enhancing the efficacy of immune checkpoint therapy in GBM [108]. Pan et al. also reported that the m6A-modified regulator ELAVL1 is an efficacy predictor for PD-L1 therapy [114].

7.2. Respiratory system tumours

Recent studies have also revealed the vital role of m6A modification in lung cancer [115]. CircIGF2BP3 is a circRNA derived from the back-splicing of IGF2BP3 between exons 4 and 13. The METTL3-mediated m6A modification of circIGF2BP3 and YTHDC1-related circularization helped circIGF2BP3 escape from the cytotoxicity of CD8+ T cells by stabilizing OTUB1 mRNA in a PKP3-dependent manner to reduce PD-L1 ubiquitination. Therefore, circIGF2BP3 is a potential therapeutic target to improve the efficacy of PD-1 antibodies [115]. YTHDF1 and YTHDF2 are also involved in PD-L1-mediated anticancer therapy in NSCLC. Overexpression of YTHDF1 and YTHDF2 was positively correlated with the prognosis of NSCLC patients, while silencing them could upregulate tumour PD-L1 expression and lead to a worse prognosis [116]. Patients with high-risk lung squamous cell carcinoma showed a more promising response to PD-1 treatment, and the expression of ALKBH5, METL3, HNRNPC and KIAA1429 was dramatically decreased compared with that in low-risk squamous cell carcinoma [116]. Moreover, multiple bioinformatics analyses also indicated the involvement of m6A regulatory factors in the prognosis and therapy of lung cancer by affecting immune checkpoints [117,118].

7.3. Urinary system tumours

7.3.1. Renal carcinoma
A recent study evaluated the m6A modification pattern and tumour immune landscape of 513 patients with clear cell renal cell carcinoma (CCRCC) to predict their responses to anti-PD-1 treatment. m6A scores were obtained using principal component analysis algorithms to accurately evaluate the m6A methylation

pattern in patients with CCRCC [119]. Another bioinformatics-based study showed that PD-L1 was overexpressed in the high-m6A score group in CCRCC, indicating that patients with high m6A scores may benefit from ICI treatment, which has been verified in 347 patients receiving ICI treatment [120]. LncRNAs have been demonstrated to be extensively modified by m6A, and their interaction might contribute to tumour progression, metastasis, drug resistance and the immune response [18] m6A modification can improve the stability of lncRNAs to promote their oncogenic functions mainly through the ceRNA network [121,122]. Regarding the mechanisms underlying the lncRNA-regulated m6A modification, a study demonstrated that lncRNA GATA3-AS could enhance the m6A reader protein KIAA1429-mediated m6A modification and promote the development of HCC [123] A prognostic risk model composed of seven m6A-related lncRNAs could be used to analyse the expression of immune checkpoint genes and immune cell infiltration in patients with different risks [124].

7.3.2. Bladder cancer

An m6A score model was constructed based on the transcriptome data and the adjusted clinical information of 716 bladder cancer samples from The Cancer Genome Atlas (TCGA) database. Immune response markers, such as PD1 and CTLA4, were found to be significantly correlated with the m6A score, indicating that the m6A score has predictive value for evaluating the effect of immunotherapy [125]. Ma et al. conducted a comprehensive RNA-seq analysis using data from the TCGA database and established nine m6A-related prognostic lncRNAs (m6A-RLPS) to verify a close correlation between tumour-infiltrating immune cells and the expression of immune checkpoint genes in bladder cancer (BLCA) [126]. However, these analyses came from bioinformatics tools only, and no further experiments were conducted to verify them. Therefore, whether changes in m6A modification readers can influence the effect of ICI treatment remains to be further studied in urinary system cancer.

7.4. Digestive system tumours

7.4.1. Gastric cancer

An analysis of 21 m6A regulators in 1938 gastric cancer (GC) samples indicated that m6A modification was significantly associated with the tumour immune microenvironment and tumour immunotherapy [127]. The high m6A score subtype showed deficient immune cell infiltration and a low survival rate, while the low m6A score subtype was associated with an increased neoantigen load and an increased response to anti-PD-1/L1 immunotherapy [127]. Mo et al. analysed 293 gastric adenocarcinoma samples from the TCGA database in a retrospective study and built an m6A risk scoring model, which was identified as an independent prognostic indicator for predicting the overall survival of patients with GC. A low risk score is associated with high expression of immune checkpoint genes, including PD-1, PD-L1 and CTLA-4, indicating that this score model can be used to evaluate the efficacy of immunotherapy for GC [128]. Another bioinformatics study evaluated the m6A modification in 407 GC clinical samples and constructed an m6A-related lncRNA pair signature (m6A LPS) to evaluate the status and prognosis of GC [129]. A close correlation was found between m6A-LPS and tumour-infiltrating cells. Higher expression of immune checkpoint genes and a stronger response to immunotherapy were detected in the low-risk group than in the high-risk group, suggesting that these m6A-related lncRNAs could remodel the tumour microenvironment and affect the anticancer ability of ICBs [129]. Although this hypothesis has not been clinically verified, it provides new insight into the prognosis of and therapeutic strategies for GC.

7.4.2. Oesophageal cancer

A recent study evaluated the differential expression of m6A regulatory factors in oesophageal cancer (ESCC) and normal tissues. Based on the expression of these regulatory factors, consensus clustering was adopted to identify PD-L1 expression, immune scores, immune cell infiltration and possible mechanisms in different ESCC clusters. As a result, PD-L1 was overexpressed in ESCC and was negatively correlated with the expression of YTHDF2, METL14 and KIAA1429. Moreover, immune scores, CD8^{+} T cells, resting mast cells and Tregs were significantly increased in Cluster 2, which suggested that m6A methylation regulators might mediate PD-L1 expression and immune cell infiltration and strongly affect the tumour immunological microenvironment of ESCC [130].

7.4.3. Colorectal cancer

According to the m6sig score extracted from the characteristic m6A-related genes, colorectal cancer (CC) patients could be divided into two subgroups with high and low m6sig scores. Patients with lower m6sig scores were found to have longer survival times and enhanced immune infiltration. Further analysis showed that accompanied by significantly mutated genes (SMGs), such as PIK3CA and Smad4, a lower m6sig score was also associated with a higher tumour mutation load, PD-L1 expression and a higher mutation rate [131]. In addition, patients with lower m6sig scores showed better immune responses and sustained clinical benefits in three independent immunotherapy cohorts [131]. m6A-related lncRNAs are also involved in immune infiltration and PD-L1 expression in CC [132]. As a demethylase, FTO can regulate PD-L1 expression in an IFN-γ-independent manner by regulating the methylation of PD-L1 mRNA [133]. Moreover, in mismatch repair-proficient (pMMR)/microsatellite instability-low (MSI-L) (pMMR-MSI-L) CC, deletion of METTK3 and METTL14 increased the infiltration of CD8^{+} T cells and the secretion of IFN-γ, CXCL9 and CXCL10 and enhanced the anti-PD-1 response [134]. Mechanistically, deletion of METTL3 and METTL14 could reduce the m6A modification of STAT1 and IRF1, as well as YTHDF2-mediated mRNA degradation, thereby increasing the expression of STAT1 and IRF1 in the IFN-γ-Stat1-Irf1 axis [134]. The above finding promotes a new understanding of RNA methylation in tumour immunotherapy.

7.4.4. Liver cancer

A recent study adopted five m6A-related genes, YTHDF1, HNRNPC, RBM15, METTL3 and YTHDF2A, in hepatocellular carcinoma (HCC) to conduct risk stratification based on their expression. The results showed that the expression levels of these genes had good predictive efficiency in predicting OS and DFS and was associated with the response to sorafenib treatment and anti-PD-1 immunotherapy [135]. In addition, m6A-related lncRNAs have also been reported to play an important role in the prognosis and ICI treatment of HCC, taking circRHBDD1, a new circular RNA highly expressed in HCC patients, as an example [136]. Studies have indicated that circRHBDD1 stimulates the recruitment of YTHDF1 to PIK3R1 mRNA and accelerates PIK3R1 translation in a m6A-dependent manner to affect metabolism. More importantly, targeting circRHBDD1 can improve the effect of anti-PD-1 therapy in mouse models [137]. The m6A regulator ZC3H13 was also closely correlated with tumour immune cell infiltration and the expression of immune cell biomarkers and immune checkpoint genes [138].

7.4.5. Pancreatic cancer

An m6A score model constructed based on the RNA-seq data of m6A regulatory factors in pancreatic ductal adenocarcinoma (PDAC) showed that the m6A score was associated with poor overall survival and increased tumour recurrence in PDAC patients. A

mechanistic study showed that PDAC with a high m6A score was characterized by decreased immune infiltration and *T*-cell exhaustion, while PDAC with a low m6A score was more sensitive to ICIs [139]. Hence, the m6A score model provides guiding significance for the prognosis of and therapeutic response to ICI treatment. In addition, Yao et al. established a prognostic risk model using five m6A methylation regulatory factors, ALKBH5, alkbh5, IGF2BP3, IRPPRC and KIAA1429, and based on these factors, PDAC patients were divided into a high-risk group and a low-risk group. The risk score was positively correlated with the tumour mutational burden (TMB). The high-risk group obtained a higher TMB value, while the low-risk group was associated with better efficacy of anti-PD-L1 immunotherapy [140]. However, the above conclusions are derived from bioinformatics analysis only, and prospective clinical studies are still needed.

7.5. Genital system tumours

7.5.1. Breast cancer

Twenty-four major m6A methylation regulatory factors were analysed using the RNA sequencing data of 775 breast cancer patients from TCGA. The consensus clustering algorithm was adopted to divide the patients into two subgroups based on the expression of the sem6A regulatory factors [141]. Compared with that in the hypomethylated subgroup, the infiltration of CD8+ T cells, helper T cells and activated NK cells was significantly increased in the hypermethylated subgroup, whereas the expression of PD-L1, PD-L2, TIM3 and C—C motif chemokine receptor 4 (CCR4) was lower in the hypermethylated subgroup than in the hypomethylated subgroup [141]. Consistently, a strong relationship between the expression of m6A regulatory factors and immune checkpoints has been reported in breast cancer [142]. These results suggest that the expression pattern of m6A regulatory factors might be a potential target and biomarker for immunotherapy for breast cancer. A recent study found that METTL3 directly interacted with PD-L1 to regulate the m6A modification of PD-L1, thereby affecting the stability of PD-L1 mRNA. IGF2BP3 could bind to PD-L1 mRNA in a METTL3/m6A-dependent manner, and IGF2BP3 knockdown could diminish the METTL3-enhanced stability of PD-L1 [20]. In addition, inhibition of METTL3 or IGF2BP3 could enhance antitumor immunity by influencing PD-L1-mediated *T*-cell activation, exhaustion and infiltration [20]. This finding will further promote our understanding of m6A methyltransferase in the anti-PD-1/PD-L1 treatment of breast cancer.

7.5.2. Ovarian cancer

Based on an expression analysis of 21 m6A RNA methylation regulators in the TCGA database, two different m6A patterns, m6A-cluster.A and m6Acluster.B, were obtained using the consensus clustering algorithm [143]. A total of 196 m6A modification-related genes were differentially expressed in the two clusters, and the underlying mechanism was also further studied. The principal component analysis algorithm was used in view of individual differences to calculate the m6A score of each sample to quantify the m6A pattern. Low m6A scores were associated with immune activation and an enhanced response to immune checkpoint inhibitors, while high m6A scores were related to tumour progression [143].

7.5.3. Prostate cancer

To identify an m6A regulatory pattern suitable for ICI treatment, an m6Ascore model was constructed to quantify the m6A modification based on the expression of m6A-related genes in individual prostate cancer (PC) patients. The response rate to immunotherapy in the low m6A score group with a poor prognosis was found to be higher than that in the high m6A score group. Hence, PC patients in the low m6A score group are more likely to benefit from ICI treatment [144].

7.6. Blood system cancer

7.6.1. Acute myelocytic leukaemia

A recent study investigated the association between factors regulating m6A modification and the antitumor immune response in acute myelocytic leukaemia (AML). High expression of immunomodulators, such as PD-L1, PD-L2, MRP1 and MRP2, was found to be associated with low m6A scores [145]. Deletion of FTO or its pharmacological inhibition could significantly reduce the self-renewal of leukaemic stem cells (LSCs)/initiated cells and reprogramme the immune response by inhibiting the expression of immune checkpoint genes, especially LILRB4. Moreover, silencing FTO could increase the sensitivity of leukaemic cells to the cytotoxicity of T cells and overcome the immune evasion induced by hypomethylating agents [146]. Recently, Cao et al. developed inhibitor-loaded glutathione (GSH)-bioimprinted nanocomposites (GNPIPP12MA) to target the FTO/m6A pathway in coordination with GSH depletion to enhance antileukaemogenesis [147]. GNPIPP12MA can increase the overall m6A modification in LSCs and enhance the response to PD-L1 blockade by increasing cytotoxic CD8+ *T*-cell infiltration [147]. In addition, it can also selectively target leukaemic mother cells and LSCs and induce ferroptosis by destroying intracellular redox homeostasis. Considering the existence of similar GSH-mediated signalling pathways in solid tumours, GNPIPP12MA may also have good potential in the treatment of other cancers.

7.7. Other cancers

7.7.1. Melanoma

Melanoma is one of the deadliest and most difficult cancers to treat, but breakthroughs in immunotherapy have markedly improved outcomes [148]. Recent studies based on bioinformatics analyses have demonstrated a close relationship between the expression of factors regulating m6A modification and immune checkpoints in melanoma [149]. As a demethylase, FTO has been demonstrated to be a stimulus for the development of melanoma. Deletion of FTO increased the m6A methylation of protumorigenic cell-intrinsic genes in primary melanoma, including PD-1, CXCR4 and SOX10, resulting in increased RNA attenuation by the m6A reader YTDHF2. FTO knockout can also increase the sensitivity of melanoma cells to IFN-γ, thus promoting the sensitivity of melanoma to anti-PD-1 therapy in mice [148]. Therefore, the combination of an FTO inhibitor and PD-1 blockade might reduce the resistance of melanoma to immunotherapy and improve the treatment response. In the anti-PD-1 treatment of melanoma, ALKBH5 deletion reduced the infiltration of Tregs and polymorphonuclear MDSCs by affecting m6A modification of the Mct4/Slc16a3 axis, thus enhancing sensitivity to anti-PD-1 treatment [99]. Hence, ALKBH5 might be a potential therapeutic target for cancer treatment alone or in combination with ICBs. Moreover, deletion of methyltransferases, including METTL3 and METTL14, inhibited m6A modification and enhanced the response of melanoma patients to PD-1 treatment. In addition, the lack of METTL3 and METTL14 in tumours leads to increased infiltration of cytotoxic CD8+ T cells and an altered tumour microenvironment [134].

7.7.2. Oral squamous cell carcinoma

Recent studies have found that METTL3 downregulation enhances the proliferation and metastasis of oral squamous cell carcinoma (OSCC) by reducing the m6A modification of PRMT5 and PD-L1 [150]. A similar role of METTL3 was also found in breast

Table 1

The common m6A modification regulators and their functional mechanisms.

Targets	Inhibitors	Function	References
FTO	MO-I-500	Inhibit the activity of FTO in m6A demethylation	[154]
	Fluorescein		[155]
	Meclofenamic acid		[156]
	Rhein	Competitively binds to the catalytic domain of FTO and inhibits it from binding to m6A-modified RNAs	[157]
	CHTB	Destroy the function of FTO and inhibit m6A demethylation	[158]
	N-CDPCB		[159]
	R-2HG	Confers anti-leukaemia and anti-glioma effects	[160]
	CS1/CS2	Inhibit the proliferation and self-renewal of cancer stem cells and enhance immune evasion	[146]
	DAC51	Inhibits the proliferation and self-renewal of cancer stem cells and enhances immune evasion	[161]
	Clausine E	Dose-dependently inhibits the demethylase activity of FTO	[163]
	Saikosaponin	Inhibits FTO to rescue m6A hypomethylation in MYC and RARA	[162]
	FB23/FB23-2	Inhibit the proliferation of human acute MLCs and promotes their differentiation/apoptosis	[165]
	MA/MA2	Inhibit the growth and self-renewal of GSCs	[164]
ALKBH5	2-{[1-hydroxy-2-oxo-2-phenylethyl] sulfanyl} acetic acid, 4-{[furan-2- yl] methyl} amino-1,2-diazinane-3,6- dione	Inhibits the proliferation of leukaemia cells including HL-60, CCRF-CEM and K562	[166]
	ALK-04	Inhibits the infiltration of Tregs and MDSCs and enhances the effect of anti-PD-1 therapy	[99]
	Curcumin	Inhibits ALKHB5 expression and induces the m6A modification of TRAF4	[167]
	Ena15/Ena21	Inhibit m6A demethylation in ALKBH5	[168]
METTL3/METTL14	STM2457	Inhibits the infiltration of Tregs and MDSCs and enhances the effects of anti-PD-1 therapy	[169]
	UZH1a	Inhibits the catalytic activity of METTL3	[170]
	Quercetin	Inhibit the proliferation of METTL3/METTL14	[171]
	Betaine		[172]
	SPI1		[173]
IGF2BP1	BTYNB	Reduces the stability of c-Myc, E2F1 and eEF2 mRNA and inhibits the proliferation and progression of ovarian cancer and melanoma	[174,175]

cancer [20], indicating the potential value of anticancer immunotherapy targeting METTL3.

7.7.3. Squamous cell carcinoma of the head and neck

Bioinformatics-based analysis demonstrated that lncRNAs related to m6A RNA methylation played an important role in the immune microenvironment of HNSCC [151,152]. Yi et al. further revealed the correlation of m6A methylation regulators with PD-L1 and immune infiltration [153]. These findings may provide a theoretical basis for m6A-related immunotherapy in HNSCC patients.

8. Therapeutic strategies targeting m6A regulators

The balance between m6A methylation and demethylation in specific RNA transcripts plays an important role in the progression of many tumours. Therefore, therapeutic strategies targeting these regulators may provide a new approach to cancer immunotherapy. In recent years, a variety of m6A inhibitors have been developed to promote traditional and regenerative medicine. FTO inhibitors, including MO-I-500, fluorescein, meclofenamic acid, rhein, CHTB, N-CDPCB, R-2HG, CS1/CS2, DAC51, clausine E, saikosaponin, 18077/18097, FB23/FB23-2 and MA/MA2, are representative m6A inhibitors and have shown significant antitumor effects both in vivo and in vitro (Table 1) [146,154–165]. Previous studies have mainly focused on FTO inhibitors; however, studies on inhibitors targeting other m6A proteins are still limited, although they might also be beneficial in m6A methylation-related cancers. With continuous breakthroughs in technology, a series of ALKBH5 inhibitors have also been developed, and 2-{[1-hydroxy-2-oxo-2-phenyl thyl]sulfanyl} acetic acid and 4-{[furan-2-yl]methyl}amino-1,2-dia zine-3,6-dione has been found to inhibit the proliferation of leukaemia cell lines [174] Alk-04, a specific ALKBH5 inhibitor, reduces the infiltration of Tregs and MDSCs and inhibits tumour growth by enhancing the efficacy of anti-PD-1 therapy [99]. Moreover, curcumin and Ena15/Ena21 function by inhibiting the expression and demethylation of ALKBH5, respectively [167,168]. Several

m6A methyltransferase inhibitors, such as STM2457, UZH1a, quercetin, betaine and SPI1, have also shown strong anticancer effects [169–173]. BTYNB screened out from a compound library was identified as a selective inhibitor of IGF2BP1 protein [174,175]. Considering the notable roles that m6A modification plays in tumour immunity, more selective and effective drugs targeting m6A-related factors must be developed and explored.

9. Conclusions

The growing successes in ICI therapy provide new hope to cancer patients. However, the low effectiveness rate has dramatically restrained its application. In this review, we explored the potential role of m6A methylation in ICI treatment. The m6A modification can affect tumour immunity by regulating multiple activities in various immune cells. The m6A regulatory factors are closely related to tumour immunity and immunotherapy. The aberrant expression of many m6A regulatory factors can affect anticancer immune function. Notably, m6A modification not only influences the expression pattern of immune checkpoint genes in a variety of cancers but also regulates the sensitivity to and effectiveness of ICI treatment in several preclinical animal models [20,99,99,102,115,134,136,137,148,176]. Therefore, the effective combination of m6A inhibitors and ICIs shows considerable therapeutic prospects. As studies on the relationship between m6A modification and tumour immunity are still at the initial stage, more intensive studies are needed to explore the underlying mechanism. In general, m6A modification is a rising star in the field of epigenetics and has strong therapeutic prospects for a wide range of cancers.

CRediT authorship contribution statement

Weiwei Liu: Conceptualization, Validation. **Chaoqun Liu:** Conceptualization, Validation. **Hui Wang:** Visualization, Data curation, Writing - review & editing. **Lijun Xu:**

Visualization, Data curation, Writing - review & editing. **Jueyu Zhou:** Visualization, Data curation, Writing - review & editing. **Sihua Li:** Visualization, Data curation, Writing - review & editing. **Yu Cheng:** Visualization, Data curation, Writing - review & editing. **Rui Zhou:** Visualization, Data curation, Writing - review & editing. **Liang Zhao:** Methodology, Resources, Supervision, Project administration. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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