Journal of Advanced Research 83 (2026) 1029–1045

Contents lists available at ScienceDirect

Journal of Advanced Research

journal homepage: www.elsevier.com/locate/jare

Review Article

Epigenetic orchestration of cancer-immune dynamics: mechanisms, technologies, and clinical advancements

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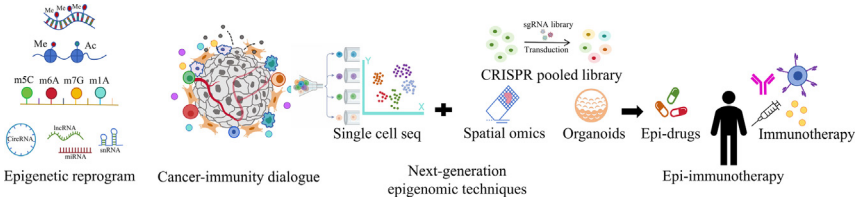
HIGHLIGHTS

- Epigenetic reprogramming as central axis of immune evasion that orchestrates immune evasion across all cancer-immunity cycle phases.
- Epi-drugs enhance immunotherapy by reversing suppression, boosting immunogenicity, and overcoming resistance.
- Cutting-edge technologies, including Single-cell epigenomics, CRISPR screens, spatial omics, and organoids reveal epigenetic vulnerabilities for precision immunotherapy.
- Mechanistic insights reveal novel therapeutic targets, including DNMTi-induced viral mimicry, EZH2-NK axis, and metabolic-epigenetic crosstalk in T cell exhaustion.
- Clinical trials explore epi-immunotherapy with epigenome editing and ctDNA monitoring for durable anti-tumour immunity.

GRAPHICAL ABSTRACT



Abbreviations: 5mC, 5-methylcytosine; AML, Acute myeloid leukemia; ALCL, Anaplastic large cell lymphoma; ADCC, Antibody-dependent cellular cytotoxicity; APC, Antigen-presenting cell; BCR, B cell receptor; BS-seq, bisulfite sequencing; BET, Bromodomain and extra-terminal motif; CHOP, C/EBP homologous protein; CI cycle, Cancer-immunity cycle; CTA, Cancer-testis antigen; CLL, Chronic lymphocytic leukemia; circRNA, Circular RNA; cHL, Classical Hodgkin lymphoma; CSF-1, Colony-stimulating factor 1; CRC, Colorectal cancer; ceRNA, Competing endogenous RNA; CR, Complete remission; CBP, CREB-binding protein; CTCL, Cutaneous T-cell lymphoma; CTL, Cytotoxic T lymphocyte; DcR3, Decoy receptor 3; DC, Dendritic cell; DBiT-seq, Deterministic Barcoding in Tissue for spatial omics sequencing; DLBCL, Diffuse large B-cell lymphoma; DNMT, DNA methyltransferase; DNMTi, DNA methyltransferase inhibitor; DOX, Doxorubicin; EEL FISH, Enhanced Electron Fluorescence in Situ Hybridization; Foxp3, Forkhead box protein P3; GM-CSF, Granulocyte-macrophage colony-stimulating factor; G-MDSC, Granulocytic MDSC; HCC, Hepatocellular carcinoma; HAT, Histone acetyltransferase; HDAC, Histone deacetylase; HDM, Histone demethylase; HLA, human leukocyte antigen; I-MDSC, Immature MDSC; ICBT, Immune checkpoint blockade therapy; ICI, Immune checkpoint inhibitor; iTreg, Induced Treg cell; LNP, Lipid nanoparticle; lncRNA, Long non-coding RNA; LSD1, Lysine-specific demethylase 1; MHC, Major histocompatibility complex; M-MDSC, Monocytic MDSC; MDSC, Myeloid-derived suppressor cell; NK cell, Natural killer cell; nTreg, Natural Treg cell; ncRNA, Non-coding RNA; NSCLC, Non-small cell lung cancer; pDC, Plasmacytoid dendritic cell; PM-MDSC, Polymorphonuclear/granulocytic MDSC; Treg, Regulatory T cell; SAM, S-adenosylmethionine; scCRISPR, Single-cell CRISPR screening; smFISH, Single-molecule fluorescence *in situ* hybridization; SOCS3, Suppressor of cytokine signalling 3; TCR, T cell receptor; Th, T helper; TET, Ten-eleven translocation; TF, Transcription factor; TNBC, Triple-negative breast cancer; TME, Tumour microenvironment; TNF, Tumour necrosis factor; TSG, Tumour suppressor gene; TAA, Tumour-associated antigen; TAM, Tumour-associated macrophages; TIL, Tumour-infiltrating lymphocytes; TSA, Tumour-specific antigen; IFN, Type I interferon; UCEC, Uterine corpus endometrial cancer; SAHA, Vorinostat; WASP, Wiskott-Aldrich syndrome protein.

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https://doi.org/10.1016/j.jare.2025.08.057

ARTICLE INFO

Article history:
Received 6 April 2025
Revised 26 August 2025
Accepted 27 August 2025
Available online 10 September 2025

Keywords:
Epigenetic immunotherapy
Cancer-immunity cycle
Immune evasion
Single-cell epigenomics
Personalized immunotherapy

ABSTRACT

Background: Epigenetic dysregulation plays a pivotal role in cancer immune evasion by orchestrating tumour antigen silencing, immune cell dysfunction, and the formation of an immunosuppressive microenvironment. By disrupting successive phases of the cancer–immunity cycle—from antigen presentation to T cell exhaustion—these aberrations facilitate immune escape and tumour progression, highlighting the need for targeted epigenetic intervention.

Aim of review: This review systematically dissects how epigenetic alterations impair anti-tumour immunity at each stage of the CI cycle. It not only integrates fragmented mechanistic evidence but also emphasizes underexplored crosstalk between specific epigenetic regulators and immune cell types. It further highlights emerging technologies—such as single-cell epigenomics, spatial multi-omics, and CRISPR-based screens—that are driving discovery of novel therapeutic targets and refining patient stratification.

Key scientific concepts of review.

We discuss how epigenetic interventions, alone or in combination with immunotherapies, can reinvigorate immune responses and overcome resistance to current treatments. A particular focus is given to how integrative high-resolution platforms are mapping immunoepigenetic landscapes, enabling mechanism-informed, precision immunotherapy strategies. By bridging epigenetic regulation with translational immuno-oncology, this review outlines a future where epigenetic reprogramming becomes central to overcoming immune evasion in cancer.

Contents

Introduction

Epigenetics, modifications are dynamic, reversible and heritable alterations in gene expression without altering the DNA sequence itself, including chromatin remodeling, histone modifications, DNA methylation, RNA modifications, and non-coding RNA (ncRNA) networks. These mechanisms orchestrate oncogenic and tumour-suppressive programs, driving cancer initiation and progression (Fig. 1) [1]. Beyond shaping tumour cell identity, emerging evidence highlights their pivotal role in the tumour microenvironment (TME), where epigenetic dysregulation disrupts immune function, silences tumour antigens, and fosters immunosuppressive niches [2,3]. This dual role makes epigenetic machinery both a driver of immune evasion and a therapeutic target.

Capitalizing on this duality, epigenetic-targeting agents (epidrugs) are reshaping the landscape of cancer immunotherapy. While immune checkpoint blockade (ICB) has revolutionized treatment, resistance remains a challenge. Tumours are often categorized as ‘cold’ or ‘hot’: ‘cold’ tumours exhibit low T-cell infiltration and weak immunogenicity, rendering them poorly

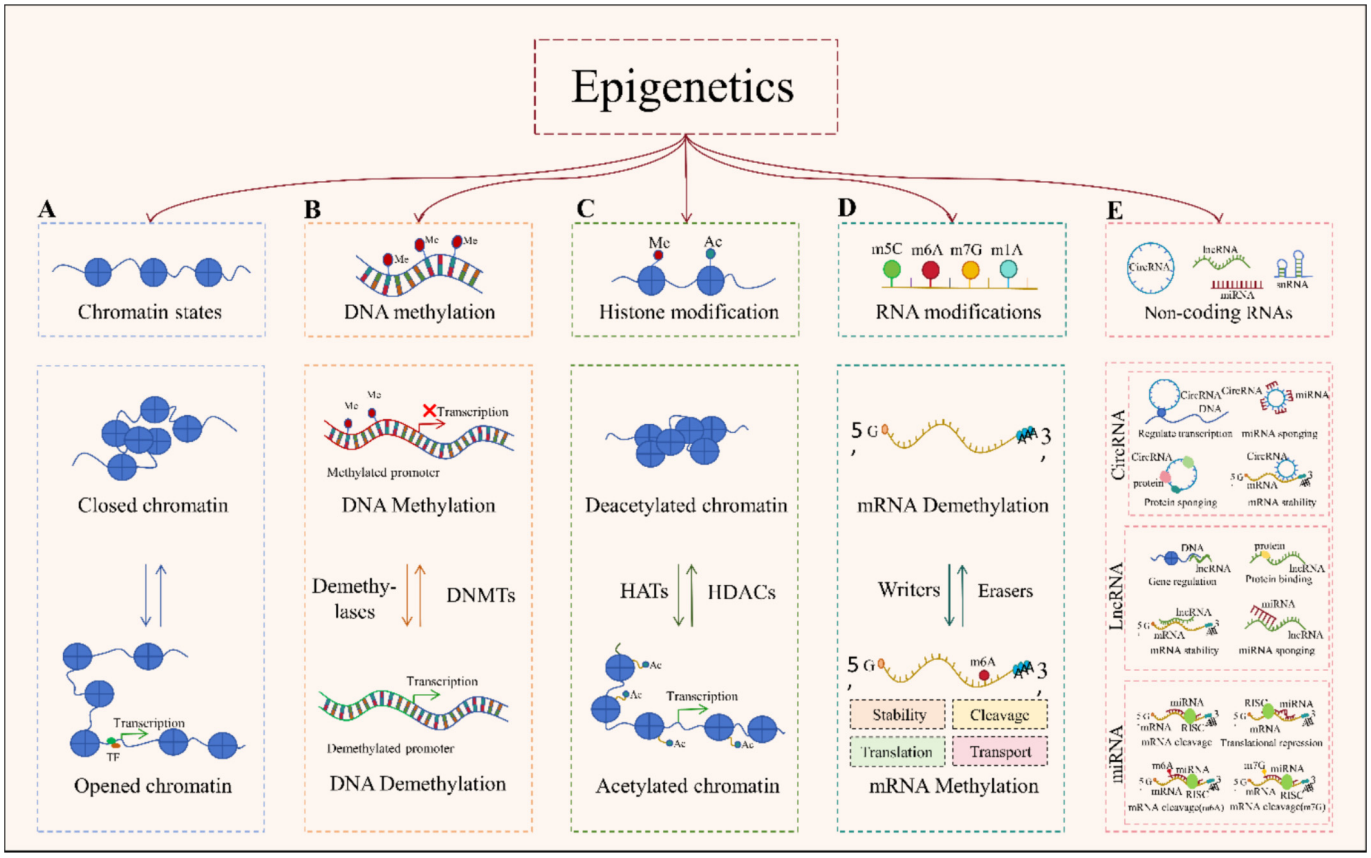

Fig. 1. Diagrammatical summary of epigenetic mechanisms. A. Chromatin remodelling. B. DNA methylation. C. Histone modifications. D. RNA modifications. E. Non-coding RNA.

responsive to ICB, whereas 'hot' tumours display high T-cell infiltration and robust immunogenicity, typically correlating with favourable ICB responses. Epi-drugs offer a solution—DNMT inhibitors and HDAC inhibitors can reactivate silenced tumour antigens, modulate immune checkpoints (PD-L1, CTLA-4), and restore T cell function. Combining DNMT inhibitors with anti-CTLA-4 therapy has tripled survival rates in refractory melanoma, while HDAC inhibitors (HDACis) enhance anti-PD-1 efficacy in non-small cell lung cancer [4,5]. These breakthroughs reposition epigenetic interventions as core strategies rather than adjunct therapies in cancer immunotherapy.

Advances in single-cell multi-omics and spatial epigenomics have further propelled this field. Technologies such as scATAC-seq and spatial CUT&Tag map epigenetic landscapes at single-cell resolution, revealing how chromatin accessibility, histone modifications, and RNA interactions shape immune cell trafficking, exhaustion, and plasticity in the TME. Meanwhile, CRISPR-based epigenomic screens have identified novel targets (e.g. EZH2 and BET), whose inhibition remodels myeloid suppression and enhances NK cell cytotoxicity. These tools decode cancer-immune crosstalk and lay the foundation for precision therapies tailored to tumour-specific epigenetic signatures.

This review explores the epigenetic landscapes of cancer-immune dynamics across three key frontiers: (a) How epigenetic drivers of immune evasion (e.g., T cell exhaustion, antigen silencing) are being rewired into therapeutic vulnerabilities. (b) From mechanistic insights to clinical breakthroughs, including combinations of epi-drugs with ICB, CAR-T, and cancer vaccines. (c) How cutting-edge epigenomic technologies are illuminating immune interactions within the TME, accelerating personalized immunotherapy. This review also discusses the current challenges in epigenetic-immune oncology from the perspectives of technology and translation. By bridging molecular insights with translational innovations, this review charts a roadmap for epigenetically empowered cancer immunotherapy.

Epigenetic reprogramming of the cancer-immunity dialogue: From immune evasion to therapeutic vulnerability

The concept of the CI cycle describes the sequential steps by which the immune system detects and eliminates tumour cells [6]. These include tumour antigen release, antigen capture and presentation by APCs, T cell priming and activation, effector T cell trafficking, infiltration into the tumour microenvironment (TME), and cytotoxic T cell-mediated killing. Successful completion of this cycle results in the elimination of nascent tumours; however, cancers arise when tumours exploit immune evasion mechanisms at one or more of these steps. Notably, Epigenetic modifications are now recognized as key regulators of each phase of the CI cycle, influencing both immune evasion and therapeutic reprogramming. This section explores their dual role—how epigenetic dysregulation enables immune escape and how targeting these mechanisms unveils new therapeutic vulnerabilities.

Suppression of tumour antigenicity

Tumour antigens are classified into tumour-specific antigens (TSAs) and tumour-associated antigens (TAAs), depending on whether their expression is restricted to tumour cells or also found in non-malignant tissues. TSAs arise from somatic mutations that are absent in germline DNA, classifying them as "non-self" antigens. Neoantigens are well-characterized TSAs. TAAs are self-antigens encoded in germline DNA that exhibit high and preferen-

tial expression on tumour cells. Epigenetic modifications can regulate the expression of both types. For example, certain neoantigen-encoding genes become silenced by DNA methylation, allowing cancer cells to diminish the display of "non-self" neoantigens and thereby escape immune detection [7]. Likewise, cancer-testis antigens (CTAs) are typically restricted to germline cells but are aberrantly overexpressed in various tumours. It is well-established that families of CTA genes (e.g., MAGE, GAGE, XAGE, BAGE, PAGE) are frequently downregulated in tumours via promoter DNA hypermethylation or through targeting by non-coding RNAs [8–10]. This epigenetic silencing of CTAs and other TAAs diminishes the repertoire of antigens presented to T cells, contributing to immune evasion.

In addition to antigen abundance, effective T-cell recognition requires proper antigen processing and presentation machinery within tumour cells and APCs. The major histocompatibility complex (MHC) molecules (called human leukocyte antigens, HLA, in humans) present peptide antigens to T cells. Tumours often show loss or downregulation of MHC class I (required for $CD8^+$ T cell recognition) or key antigen-processing components, thereby avoiding cytotoxic T cell killing. For example, epigenetic silencing of MHC-I genes via PRC2/EZH2-mediated H3K27me3 and antigen processing machinery (e.g., proteasome subunits) impair antigen presentation, permitting tumour cells to "hide" from T cell surveillance [11]. Thus, reversing epigenetic repression on tumour antigens may re-prime tumours and boost their immunogenicity.

Dysregulation of antigen-presenting cells

Professional antigen-presenting cells (APCs)—dendritic cells (DCs), macrophages, and B cells—play a crucial role in T cell activation and anti-tumour immunity, but tumours exploit epigenetic reprogramming to impair their function. DCs are essential for priming T cells with tumour antigens, however, DC differentiation and function can be disturbed by tumour-derived epigenetic signals. For instance, in breast cancer, tumour-derived miR-155 suppresses CCR7, a key receptor for DC maturation and migration, leading to dysfunctional DCs with impaired T cell activation [12]. Similarly, N6-methyladenosine (m6A) methylation by YTHDF1 inhibits tumour antigen cross-presentation in DCs, weakening $CD8^+$ T cell priming [13]. Tumours also epigenetically modulate macrophages, shifting tumour-associated macrophages (TAMs) toward an immunosuppressive phenotype. For example, Decoy Receptor 3 upregulation in cancers recruits histone deacetylases (HDACs) to silence CIITA, downregulating MHC-II antigen presentation in macrophages [14]. Exosomal miR-27a-3p further enhances immune evasion by increasing PD-L1 expression on macrophages [15], whereas miR-149 counteracts pro-tumoural macrophage activity by inhibiting CSF1, reducing macrophage accumulation in triple-negative breast cancer (TNBC) [16].Though primarily involved in antibody production, B cells also function as APCs. Epigenetic defects, such as Wiskott-Aldrich Syndrome Protein (WASP) deficiency, alter chromatin architecture, biasing B cell antigen presentation toward Th2, Th17, and Treg responses instead of cytotoxic Th1 activation, thereby promoting tumour tolerance [17]. These findings underscore how tumours exploit epigenetic mechanisms to disrupt APC function, suppress immune activation, and sustain immune evasion. Targeting those dysregulated epigenetic regulators such as EZH2 and HDACs have been reported to enhance antigen presentation [18].

T cell dysfunction and exhaustion

T cell activation, differentiation, trafficking, and effector functions are tightly regulated by epigenetic modifications, which influence their ability to mount effective anti-tumour responses [19]. $CD8^+$ T cells, primed by MHC-I molecules, undergo clonal expansion and differentiation into cytotoxic T lymphocytes (CTLs) and memory T cells. Tumour-reactive $CD8^+$ T cells exhibit specific DNA methylation patterns; for instance, in colorectal cancer (CRC), CD39 and CD103 demethylation distinguishes tumour-reactive T cells from bystander TILs [20]. This suggests that DNA methylation status helps fix the identity and persistence of tumour-specific T cells. Moreover, T cell activation under the harsh metabolic conditions of the TME can be bolstered by epigenetic regulators – one report showed that miR-155 sustained $CD8^+$ T cell activation and glucose metabolism under nutrient stress, enhancing their function in the TME [21]. Unlike $CD8^+$ T cells, $CD4^+$ T cells are primed by MHC-II molecules and undergo proliferation and differentiation into various subtypes, including helper T cells (e.g. Th1, Th2, Th17, nTreg, and iTreg), Th9 cells, and Tr1 cells. Many studies have demonstrated that epigenetics play a definite role in directing the differentiation of CD4 + T cells and in preserving the function of memory T cells, ensuring a well-coordinated and sustained immune response. In lung cancer, miR-7 deficiency promotes Th1 polarization in adoptive T cell therapy [22], while hypermethylation of IFNG in colon tumours restricts Th1 differentiation, compromising immune responses [23].

After activation, T cells traffic to and infiltrate tumours, a process regulated by chemokines, adhesion molecules, and receptor expression. Tumours often silence chemokine genes (e.g., CCL5, CXCL9/10) via DNA methylation or polycomb-mediated histone modifications (e.g., EZH2) to prevent T cell infiltration [24,25]. Tumour-derived circRNF216 further regulates chemokine expression, influencing T cell migration [26]. Conversely, integrins and homing receptors on T cells, required for tumour infiltration, are also epigenetically modulated, with a permissive chromatin state essential for effective T cell trafficking. Tumours that impose repressive epigenetic marks at these loci create physical barriers to T cell entry, contributing to immune evasion. Once at the tumour site, effector T cells ($CD8^+$ CTLs and $CD4^+$ Th cells) execute cytotoxic functions, which are epigenetically regulated. $CD8^+$ T cells rely on perforin, granzymes, and IFN-γ for cytotoxicity, with activating histone marks regulating gene expression. For instance, loss of lysine demethylase 6B (KDM6B) impairs granzyme B production, reducing $CD8^+$ T cell cytotoxicity [27]. This indicates that certain activating histone marks removed by KDM6B are necessary for expression of cytotoxic genes. Interestingly, metabolites in the TME can exert epigenetic effects on T cells – a metabolite indole-3-lactic acid modifies chromatin accessibility, enhancing cholesterol metabolism and $CD8^+$ T cell function [28]. Such findings tie the metabolic environment to epigenetic reprogramming of T cell effector activity. $CD4^+$ T cells, critical for supporting $CD8^+$ responses, are also epigenetically shaped. Persistent STAT5 activation remodels chromatin, increasing Th cell expansion, tumour infiltration, and boosting $CD8^+$ responses [29].

A major obstacle is T cell exhaustion, a dysfunctional state induced by chronic antigen exposure and immunosuppressive TME signals [30]. Exhausted T cells upregulate inhibitory receptors (PD-1, TIM-3, Galectin-9) and develop a stable exhaustion-associated chromatin landscape, locking them in a non-functional state. Tumour-derived miRNAs can be transferred via exosomes, promoting exhaustion by enhancing inhibitory pathways [31]. Likewise, chronic cytokine and metabolic stress in the TME (e.g. glucose deprivation) can enforce epigenetic changes in T cells. glucose deprivation in ovarian cancer sustains high miR-101 and miR-26a, which suppress EZH2, impairing T cell memory and differentiation [32]. These heritable epigenetic alterations stabilize T cell exhaustion, preventing effective tumour rejection. However, this also presents a therapeutic opportunity: epigenetic reprogramming of exhausted T cells, using demethylating agents or inhibitors targeting exhaustion-associated chromatin regulators, could

restore their functionality. Thus, epigenetic modulation of T cell activation, differentiation, trafficking, and exhaustion is a critical determinant of anti-tumour immunity, shaping whether T cells will successfully eliminate tumours or succumb to immune evasion.

Amplification of immunosuppressive cells

Tumours exploit epigenetic reprogramming to shape an immunosuppressive microenvironment, recruiting regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), and tumour-associated macrophages (TAMs) to dampen anti-tumour immunity. These cells rely on epigenetic modifications for lineage specification and suppressive function, making them key players in immune evasion.

Tregs, specialized in immune suppression, are maintained by FOXP3 demethylation, ensuring stable expression and suppressive function. Tumours reinforce this epigenetic state, promoting Treg differentiation via miR-192-5p-mediated NF-κB modulation in gastric cancer and miR-34a suppression in hepatocellular carcinoma, which upregulates CCL22, attracting Tregs to the tumour [33–35]. The presence of epigenetically reinforced Tregs in the TME is a major barrier to effective immunity, as they produce immunosuppressive cytokines (IL-10, TGF-β) and consume IL-2, starving effector T cells. Targeting Treg-specific epigenetic regulators like EZH2 is being explored to destabilize Tregs and relieve immune suppression.

MDSCs, potent T cell suppressors, arise from myeloid progenitors under tumour-driven epigenetic alterations. Tumours modulate C/EBPβ, SOCS3, and IRF8 expression via non-coding RNAs, skewing differentiation towards immunosuppressive MDSCs [36]. Overexpression of NNMT reduces H3K27me3 on the CSF2 promoter, increasing GM-CSF production and expanding MDSCs [37]. Additionally, RNA methyltransferase METTL1 enhances chemokine expression, promoting MDSC infiltration [38], while CCRK-EZH2 signalling upregulates IL-6, reinforcing MDSC expansion and T cell suppression [39]. MDSCs further support Treg development, creating a multi-layered immunosuppressive network [40]. Disrupting MDSC-related epigenetic pathways, such as EZH2 inhibition or miRNA circuit targeting, could help dismantle this axis of immune evasion.

TAMs, derived from monocytes, shift between pro-inflammatory M1 and immunosuppressive M2 phenotypes based on epigenetic cues. DNA methylation and m6A RNA modifications regulate this polarization, with tumour-derived signals reinforcing the M2/TAM state [41,42]. Tumours secrete exosomal non-coding RNAs (e.g., miR-548 t-5p, circSMARCC1, SNHG12) to modulate IL-33, CCL5, IL6R, promoting TAM-mediated immune suppression [43–45]. TAMs not only inhibit T cells but also remodel the extracellular matrix and facilitate metastasis. They, in turn, release exosomal miRNAs that influence PD-L1 expression and T cell differentiation [46]. This indicates a complex cross-talk where epigenetic messages are exchanged between TAMs and T cells to amplify immune suppression. Strategies such as HDAC and DNA methylation inhibitors aim to reprogram TAMs from M2 to M1, restoring their tumouricidal activity and ability to activate T cells.

In summary, epigenetic modifications serve as a common thread in the generation of an immunosuppressive microenviron-

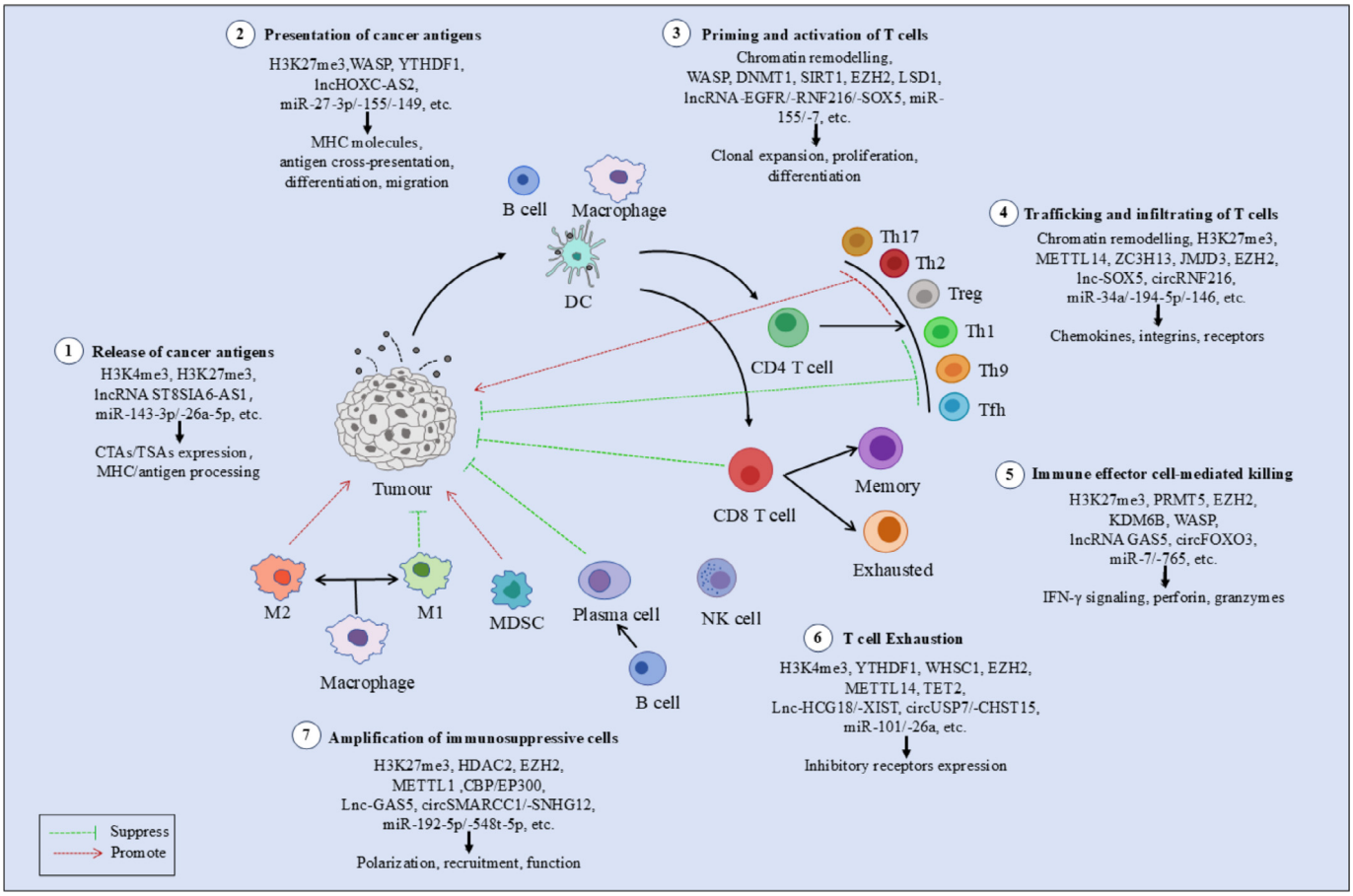

Fig. 2. Epigenetic regulations in cancer-immunity cycle. Epigenetic machinery participates in all aspects of anti-cancer immunity. Dysregulated epigenetic regulators can regulate the behaviour and function of each cellular player in the CI cycle via targeting essential genes, indicating the universality and flexibility of epigenetic modification in anti-cancer immunity.

ment (Fig. 2). Recognizing these epigenetic mechanisms presents opportunities for therapies that selectively disrupt the suppressive programming of these cells, potentially reactivating anti-tumour immunity.

Harnessing epigenetic drugs to reboot anti-tumour immunity: Synergy and beyond

Strategies to overcome epigenetically driven immune evasion

Given the multiple ways epigenetic alterations enable tumours to evade immunity, an emerging concept is to exploit these alterations as therapeutic targets. Key approaches include: (a) Restoring tumour antigen expression. Epigenetic silencing of tumour antigens (neoantigens, CTAs, etc.) and antigen-processing genes can be counteracted by drugs that inhibit DNA methylation or histone deacetylation. DNA hypomethylating agents (e.g., azacitidine, decitabine) demethylate gene promoters, re-expressing tumour-specific antigens (TSAs/TAAs) and antigen-presenting machinery. This increases the pool of antigens available for immune detection and can turn an "invisible" tumour into a visible one. Similarly, HDAC inhibitors relax chromatin and reactivate MHC molecules and co-stimulatory ligands, exposing tumours to immune surveillance. (b) Reactivating silenced immune pathways (viral mimicry and cytokines). Tumours epigenetically suppress interferon signalling and endogenous retroviruses (ERVs), reducing immune activation. DNMT inhibitors can induce "viral mimicry", causing ERV transcription and triggering type I interferon responses, recruiting immune cells to the tumour microenvironment (TME) [47]. This essentially fools the cancer cells into sounding an antiviral alarm, which can recruit and activate immune cells in the TME. Additionally, epigenetic drugs can be used to upregulate key chemokines and cytokines that were suppressed. For example, demethylation can increase expression of T cell-attracting chemokines like CXCL9/CXCL10, leading to improved T cell trafficking to tumours [48]. Such epigenetic reprogramming of the tumour's cytokine/chemokine profile can convert a "cold" tumour (non-inflamed, poorly infiltrated) into a "hot" tumour (inflamed, T-cell rich) that is more susceptible to immune attack. (c) Reprogramming immunosuppressive cells. Tregs, MDSCs, and TAMs rely on epigenetic regulation for their suppressive function. EZH2 inhibitors prevent $CD4^+$ T cells from converting into Tregs and reduce MDSC activity [49]. BET inhibitors downregulate PD-L1 expression on tumour and immune cells, reducing inhibitory signals to T cells [50]. HDAC inhibitors repolarize M2-like TAMs toward an M1 phenotype and disrupt MDSC differentiation [51]. Targeting tumour-secreted ncRNAs via anti-miR oligonucleotides can further block Treg/MDSC recruitment. Selective delivery of epigenetic drugs to these suppressive cells (e.g., via nanoparticles) may effectively lift tumour-induced immune suppression. (d) Preventing T cell dysfunction and exhaustion. Preclinically, several epigenetic therapies (e.g., azacytidine and entinostat) have been shown to improve the efficacy of ICB via preventing or partially reversing T cell exhaustion [48]. LSD1 inhibitors improve T cell activation and memory formation, boosting anti-PD-1 responses. By maintaining T cells in a stem-like or effector state, epigenetic interventions preserve anti-tumour immunity under persistent stimulation. Overall, these strategies epigenetically "reprogram" tumours and immune cells, rebooting the cancer–immunity dialogue to restore immune attack against.

Epi-drugs in cancer immunotherapy

Translating the above strategies into the clinic relies on the increasing number of Epi-drugs that can safely be used in patients. Over the past two decades, several epigenetic therapies have gained approval in oncology, and many others are in development; these agents are now being integrated with immunotherapy approaches. Key classes of Epi-drugs relevant to immunotherapy include as following. An overview for their actions and clinical implications are listed in Table1.

DNMT inhibitors

Inhibitors of DNMTs, such as 5-aza-2′-deoxycytidine and 5-azacytidine, inhibit DNA methyltransferases and cause passive demethylation of DNA during replication. They are FDA-approved for certain leukaemia and myelodysplastic syndrome, and in solid

Table 1
Comparative overview of major epigenetic drug classes in cancer immunotherapy.

Drug class	Mechanism	Approved/Clinical use	Immunotherapy synergy	Major toxicity
DNMT Inhibitors	Inhibit DNA methyltransferases → demethylation, reactivation of silenced genes	FDA-approved: azacitidine & decitabine for AML, MDS;in trials for solid tumours (NSCLC, CRC, melanoma) with ICIs	Restore MHC-I and antigen presentation; upregulate IFN response genes; sensitize "cold" tumours to ICB	Myelosuppression, neutropenia, anemia, gastrointestinal (GI) symptoms
HDAC Inhibitors	Block histone deacetylases → ↑ histone acetylation, open chromatin, enhanced gene transcription	FDA-approved: vorinostat (CTCL), romidepsin (CTCL/PTCL), belinostat (PTCL), panobinostat (MM); in trials with anti–PD-1/PD-L1	Increase tumour antigenicity; modulate Treg and MDSC suppressive function; enhance effector T/NK cytotoxicity	Fatigue, thrombocytopenia, QT prolongation, cytopenias
HMT/HDM Inhibitors	Inhibit histone methyltransferases (e.g., EZH2, PRMT5) or demethylases (e.g., LSD1, KDMs) → alter chromatin marks	FDA-approved: EZH2 inhibitor (tazemetostat) for EZH2-mutant follicular lymphoma & epithelioid sarcoma;others (LSD1, BET, PRMT5) in early-phase trials	Promote T cell infiltration; reduce Treg stability (EZH2i) ; suppress MDSCs; convert "cold" tumours to "hot"	Fatigue, GI symptoms, hematologic toxicity, risk of infections, occasional secondary malignancies
ncRNA-targeting & Other Agents	Target oncogenic ncRNAs (miRNA mimics/antagonists), or DNA damage sensing pathways (e.g., PARP inhibitors)	FDA-approved: PARPi (olaparib, rucaparib, niraparib, talazoparib) for BRCA-mut ovarian, breast, pancreatic, prostate cancers; ncRNA-targeting agents (e.g., MRX34) tested but halted due to safety	PARPi-induced DNA damage promotes cGAS-STING signaling, enhancing ICB; ncRNA agents can regulate immune checkpoint gene expression	irAEs (MRX34: cytokine release, hepatotoxicity); PARPi: fatigue, hematologic toxicity, GI symptoms, MDS

tumours they are known to induce the expression of silenced genes, including tumour antigens and immune-regulatory genes. These inhibitors can upregulate MHC molecules, cancer-testis antigens, and co-stimulatory genes in cancer cells, such as MHC I, B2M, CALR, CD58, PSMB8, and PSMB9, facilitating T-cell recognition of tumours [52]. Furthermore, they also provoke the viral mimicry response that draws the attention of innate immune cells, in this way, creating a more immunogenic tumour profile. For example, the DNMT1 inhibitor 5-azacytidine has been shown to enrich immunomodulatory pathways, including interferon signalling and cytokine/chemokine production, which are crucial for recruiting and activating immune cells like CD4 + T cells, CD8 + T cells, and NK cells [47]. Decitabine has been found to upregulate NKG2D- and DNAM-1-activating ligands on acute myeloid leukaemia (AML) cells, enhancing their susceptibility to NK cell-mediated lysis [53]. These insights have led to multiple clinical trials testing DNMT inhibitors in combination with checkpoint inhibitors (anti-PD-1/PD-L1 or anti-CTLA-4) in solid tumours. The rationale is that a DNMTi may prime tumours to be attacked by the immune system or to overcome resistance in patients who initially did not respond to immunotherapy.

HDAC inhibitors

HDACi, such as vorinostat, romidepsin, panobinostat, entinostat, prevent the removal of acetyl groups from histones, generally leading to a more open chromatin state and increased gene transcription. Several HDACis are approved for T-cell lymphomas and multiple myeloma [54]. In the context of immunotherapy, HDACis have shown the ability to modulate tumour immunogenicity and activities of immune cells. For example, Trichostatin A, a specific inhibitor of Class I/II HDACs, has been shown to enhance tumour immunogenicity, such as MHC I, MHC II, TAP-1, TAP-2, LMP-2, LMP7, and Tapasin [55]. Panobinostat, a non-selective HDAC inhibitor, induces type I interferon (IFN) production in plasmacytoid dendritic cells (pDCs) through the transcriptional activation of IFN genes, accompanied by increased H3K27 acetylation. This epigenetic modification is critical for the therapeutic efficacy of HDAC inhibitors in AML [56]. HDACis including vorinostat, entinostat, and ricolinostat (HDAC6 inhibitor) have been reported to promote T cell activation and exhibit antitumour activity in various cancers [57,58]. Currently, etinostat is in clinical trials combined with PD-1 or CTLA-4 inhibitors, particularly after a phase II trial in metastatic breast cancer suggested that entinostat plus anti-PD-1 could improve outcomes in certain patients (NCT02697630). The ongoing studies will clarify the role of HDACis in immunotherapy combinations, including whether they primarily act on tumour cells, immune cells, or both.

Histone methyltransferase and demethylase inhibitors

Histone methyltransferases (HMT) are responsible to add methyl groups to histones. Targeting HMTs such as EZH2 and LSD1 is a new approach to modulate the epigenome. EZH2 inhibitors (tazemetostat being the first FDA-approved in epithelioid sarcoma and follicular lymphoma) block the enzyme that deposits repressive H3K27me3 marks [59]. In immune context, Inhibition of EZH2 using 3-Deazaneplanocin A in prostate cancer models could trigger a robust double-stranded RNA-STING-ISG stress response, leading to active anti-tumour effects [60]. It was also reported that inhibition of EZH2 could promote NK cell activation and increase NKG2D expression, which participate in the recognition and eradication of tumour cells [61].

There is considerable interest in using EZH2 inhibitors to make "cold" tumours more immune-reactive, given EZH2's role in silencing genes (including some chemokines and antigen-presenting genes) in tumours. LSD1 (KDM1A) inhibitors are another class in trials; LSD1 regulates gene expression in both tumour cells and immune cells. LSD1 inhibition remarkably increased tumour immunogenicity and infiltrated T cells in poorly immunogenic tumours, thereby enhancing anti-PD1 therapeutic effects in checkpoint blockade refractory mouse melanoma [62]. Other histone-modifying enzymes being targeted include PRMT5 (an arginine methyltransferase) and BET proteins (bromodomain inhibitors like JQ1 or ODM-207) – while BET inhibitors are not classical epigenetic drugs in terms of modifying chromatin, they prevent reading of acetylated histones and have been shown to enhance T cell persistence and suppress certain immunosuppressive cells [63,64].

Targeting ncRNA and other epigenetic modulations

Non-coding RNAs are flexible epigenetic regulators that buffer the disturbance in cellular process and transmit signals among cellular components in TME. Increasing evidence has showed their potency in regulating tumour immunity that merit much attention. Injection of lncRNA NKILA knockdown CTLS effectively suppressed the growth of breast cancer patient-derived xenograft mice by increasing CTL infiltration [65]. The miRNA targeting drug, MRX34, a lipid nanoparticle (LNP) formulation combined with miR-34a mimics, has been shown to enhance TILs and reduce CD8 + PD1 + cells in vivo. Moreover, the combination of MRX34 with radiotherapy results in a more significant increase in CD8 + T cell numbers compared to either treatment alone. Some drugs overlap epigenetic and immunogenic effects, such as PARP inhibitors (PARPi, which primarily target DNA repair but can cause accumulation of cytosolic DNA fragments that activate the cGAS-STING pathway, leading to interferon production). While PARPi are not Epi-drug, their combination with DNMTis could heighten the viral mimicry and inflammation in tumours [66]. Additionally, HAT (histone acetyltransferase) inhibitors and chromatin remodelling inhibitors (like AT-rich interaction domain inhibitors) are in earlier stages, and their impacts on anti-tumour immunity are under exploration [67,68].

Combination approaches in immunotherapy

Epigenetic priming plus ICB therapy

The ICB therapy such as anti-PD-1/PD-L1 and anti-CTLA-4 antibodies have been approved for clinical treatment of many cancers. However, the objective response rate (ORR) of ICB monotherapy is only 15 ~ 20 %, a majority of patients do not respond or eventually develop resistance [69]. Emerging evidence have found that hyperactivated epigenetic regulators critically modulate tumour immune evasion and ICB sensitivity or resistance by altering antigen presentation, IFN-γ signalling and TME remodelling. It was found that higher HDAC1/2/3 activity exhibited deficient IFN-γ signalling and worse response to ICB therapy in HCC [70]. Therefore, targeting these regulators with epigenetic drugs (epi-drugs), such as DNA methyltransferase or HDAC inhibitors, may synergistically enhance ICB efficacy by restoring antigenicity and reversing immunosuppressive circuits. For example, adding azacitidine to anti-CTLA-4 therapy in an ovarian cancer model restored chemokine production by NK and T cells, increased TILs, and resulted in better tumour control than checkpoint inhibitor alone [71]. Decitabine treatment has been shown to induce epigenetically suppressed MHC and cytokine genes in a colon cancer model, leading to greater $CD8^+$ T cell accumulation and enhanced efficacy of anti-PD-1 therapy [72]. These findings provide a rationale for ongoing trials (in lung cancer, melanoma, breast cancer, etc.) where patients receive a hypomethylating agent or HDACis together with a PD-1 or CTLA-4 blocker. In some studies, patients who had progressed on ICB therapy achieved renewed responses when an epigenetic agent was added, suggesting the combination can reverse certain resistance mechanisms. It's important in these trials to monitor immune biomarkers – for instance, checking if

PD-L1 levels, T cell infiltration, or IFN-γ-responsive gene signatures increase after epigenetic priming. That would confirm the immunological potentiation by epigenetic therapy. Combination regimens will also need to optimize scheduling (e.g., giving the epigenetic drug in cycles that align with T cell priming phases) to maximize benefits while minimizing any potential lymphocyte suppression.

Targeting multiple checkpoints and pathways

Immune modulators including checkpoint inhibitors, cytokines and co-stimulatory agonists are cornerstone therapies in cancer immunotherapy, as they reinvigorate anti-tumour immunity by enhancing T cell activation, promoting antigen presentation and disrupting immunosuppressive TME. However, their efficacy is often constrained by epigenetic dysregulation of immune pathways, such as DNA hypermethylation of IFN-γ signalling genes or histone deacetylation-mediated silencing of cytokine receptors. In this way, combining epigenetic therapy with multiple immunomodulators may be another approach in cancer immunotherapy. For example, entinostat plus NHS-IL12 combination not only restored antigenicity but also amplified type I IFN responses, driving the innate and adaptive immune milieu to an inflamed landscape in mouse colon carcinomas and poorly immunogenic breast tumours [73], thereby biasing the cytokine support towards effector cells. Similarly, epigenetic drugs also function as adjuvants which are crucial in vaccine. They can effectively boost vaccine-targeted antigen expression or presentation and reshape the TME to boost immunity [74,75]. For example, decitabine could restore NY-ESO-1, a highly immunogenic TSA, and led to improved immune response when combined with a protein vaccine [76]. The HDACi AR-42 combined with a DNA vaccine targeting HPV protein E7 could enhance anti-cancer effect of $CD8^+$T cell in lung cancer [77]. Adoptive cell transfer therapies like CAR T-cells or TIL therapy may also benefit from epigenetic conditioning of the tumour. It has been reported that several factors impeding CAR T cell therapy in solid tumours, including antigen heterogeneity, immunosuppressive TME, poor persistence of *ex vivo* expansion, and impaired infiltration and trafficking of CAR T cells [78]. The epigenetic reprogram in these processes derive approaches to pre-treated ep-drugs briefly before infusion of CAR T cell or combine epi-drugs with CAR T therapy to render the tumour cells more susceptible [79]. A fascinating concept is giving epigenetic modulators that act on the T cells *ex vivo* during their expansion – for example, using a EZH2 inhibitor or DNMT inhibitor in the TIL expansion culture to prevent differentiation into an exhausted state, yielding a more potent product for infusion [80]. While these combination strategies are complex, they underscore the versatility of epigenetic interventions as an adjunct to various forms of immunotherapy.

Harnessing metabolic-epigenetic crosstalk to overcome therapeutic resistance

Tumours can adapt under the pressure of immunotherapy, often through epigenetic changes (since those are more readily acquired than genetic mutations). Key mechanisms involve the epigenetic silencing of interferon-stimulated genes or antigen presentation machinery and upregulation of immunosuppressive ligands and enzymes after prolonged exposure to immunotherapy. For instance, tumours recurring after PD-1 therapy might show transcriptional silencing of genes in the antigen presentation pathway or upregulation of alternative immune checkpoints [81]. Additionally, in immune effector cells themselves, chronic antigen exposure imprints a stable exhaustion program characterized by persistent expression of inhibitory receptors and loss of effector function [82]. Combining epigenetic drugs can counteract this adaptive resistance by restoring these epigenetic imbalances. A clinical trial in refractory Hodgkin lymphoma showed that combining decitabine with camrelizumab resulted in a twofold improvement in efficacy compared to camrelizumab monotherapy and improved ORR and CR in resistant patients, mechanistically associated with epigenetic enhancement of T cell activation, as evidenced by increased IFN-γ and HLA-DR expression [83].

In addition to epigenetic reprogramming, tumour resistance can be shaped by metabolic rewiring which ties into epigenetics via metabolites. Metabolites serve as either substrates or cofactors for catalyzing epigenetic modifications, thereby coupling tumour metabolism with immune dysfunction. Recent studies have reported that lactate-driven histone lactylation (e.g., H3K18la) promoted M2 polarization of TAMs and resistance to PD-1 blockade [84]; α-ketoglutarate (α-KG) supported histone demethylases that remodel B-cell chromatin and suppressed T-cell responses [85], while accumulation of its oncometabolite 2-hydroxyglutarate (2-HG) blocked T cells in an exhausted state [86]. Similarly, tumour glycolysis elevated Acetyl-CoA, fueling histone acetylation (H3K27ac) and PD-L1 upregulation [87]. These examples highlight that metabolic–epigenetic crosstalk is a causal driver of immune resistance.

Therapeutically, this axis can be targeted by combining epi-drugs with metabolic interventions. For example, vitamin C supplementation enhances TET demethylase activity and synergizes with DNMT inhibitors [88]; mannose supplementation preserves T-cell stemness through epigenetic maintenance [89]; and ACAT2 inhibition blocks lactate–cholesterol metabolism to restore PD-1 sensitivity in preclinical pancreatic cancer [84]. Early-phase clinical trials of epi-drug plus ICI combinations (e.g., DNMTi or HDACi with PD-1 blockade) have shown modest but encouraging activity, though results remain heterogeneous. Moving forward, rational integration of metabolic modulators, epigenetic agents, and ICIs—guided by biomarkers such as histone lactylation signatures or metabolite ratios—may offer a promising route to overcome therapeutic resistance.

As illustrated in recent studies and summarized in Fig. 3, epigenetic therapies and ICIs operate through complementary mechanisms: epigenetic agents can alleviate immune suppression and enhance tumor immunogenicity, thereby priming the tumor microenvironment for a more effective immune response, while immunotherapy mobilizes effector cells to target the now-recognizable cancer cells. For instance, the Phase II clinical trial NCT02697630 evaluating entinostat in combination with Pembrolizumab in metastatic uveal melanoma (UM) reported an objective response rate (ORR) of 14 %, which compares favorably to the historical ~ 5 % response rate observed with anti–PD-1 monotherapy. Additionally, the median overall survival (OS) reached 13.4 months versus the typical benchmark of 10 months, and the treatment was well-tolerated, with only 34 % of patients experiencing grade ≥ 3 immune-related adverse events (irAEs) and no treatment-related deaths. Notably, this trial also suggested that patients with BAP1 wild-type tumors might derive more benefit, indicating a potential predictive biomarker for treatment stratification. In contrast, the Phase II trial NCT02260440 investigating azacitidine plus Pembrolizumab in microsatellite-stable (MSS) metastatic colorectal cancer (CRC) did not yield meaningful clinical benefit, despite a favorable safety profile. One hypothesized reason for the limited efficacy is the use of a non-standard, low dose of azacitidine, which may have been insufficient to elicit the necessary immunomodulatory effects. These examples underscore the complexity of translating epi-immunotherapy combinations into clinical success. Key factors such as epigenetic context, biomarker selection, dosing regimens, pharmacokinetics, and tumor subtype-specific biology all play critical roles in shaping treatment outcomes. While the results thus far are encouraging, they also highlight the need for careful optimization and patient stratification.

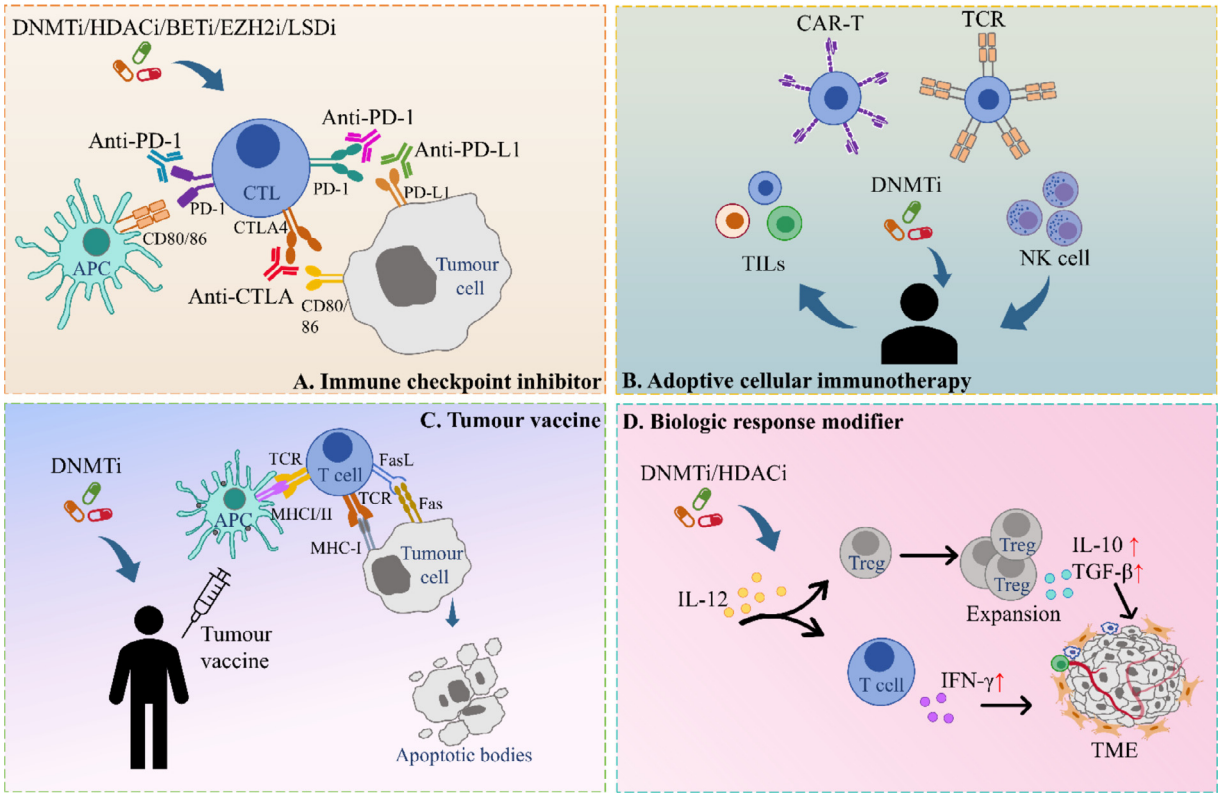

Fig. 3. Epigenetic interventions in cancer immunotherapy. A. ICB combined with Epi-drugs. B. Adoptive cellular immunotherapy combined with DNMTis (e.g., 5-azacytidine, decitabine) enhance adoptive immunotherapy in blood cancers. C. Tumour vaccines combined with DNMTis have been trialed with vaccines for recurrent ovarian cancer. D. Biologic response modifier combined with HDACis show promise in lymphoma and solid tumours.

Continued clinical efforts, as summarized in Table 2, are expected to provide further insights into how to effectively integrate epigenetic therapy with immunotherapy across different cancer settings.

Next-generation epigenomic technologies: Decoding complexity for precision immunotherapy

Recent technological advancements are transforming epigenetic research in cancer immunotherapy, providing remarkably high resolution and new strategies to target immune evasion. Innovations such as single-cell omics, CRISPR-based functional screens, spatial omics, and patient-derived organoid cultures now allow researchers to dissect the tumour microenvironment and its epigenetic landscape in greater detail than ever before (Fig. 4). These tools enable the identification of epigenetic regulators of immune escape and the testing of interventions to restore anti-tumour immunity, ultimately assisting to develop more effective cancer immunotherapies.

Decoding tumour heterogeneity with single-cell sequencing

Nowadays, single-cell sequencing has been rapidly developed and recolonizing our understanding of tumour heterogeneity, unveiling distinct cellular subpopulations and their functional dynamics. Techniques integrating epigenomics with single-cell profiling, termed single-cell epigenomics, such as scATAC-seq (chromatin accessibility), scCUT&RUN (histone modifications), scMNase-seq (nucleosome positioning), and scBS-seq (DNA methylation), etc. have mapped the epigenetic landscape at high resolution. These approaches uncover how epigenetic diversity drives phenotypic plasticity and therapeutic outcomes. For instance, a landmark study employing scATAC-seq and scRNA-seq to profile tumor-infiltrating lymphocytes (TILs) in triple-negative breast cancer (TNBC) following paclitaxel monotherapy or its combination with anti-PD-L1 treatment revealed that CXCL13 + CD8 + T cells represented the dominant subset driving therapeutic efficacy. These cells exhibited enhanced chromatin accessibility in genes associated with effector/memory differentiation (e.g., CD44, CXCR4, KLRG1 [activation marker], and ICOS [co-stimulatory signaling]) and diminished accessibility in exhaustion-associated transcriptional regulators (CREM, MYO7A), suggesting that epigenetic reprogramming in CXCL13 + T cells orchestrates their phenotypic shift toward superior cytotoxic and memory potential [90]. In addition, a recent work in B-cell non-Hodgkin's lymphoma uncovered chemotherapy-induced epigenetic and transcriptomic reprogramming in T cells that compromises their suitability for CART-T therapy. This paradigm underscores the need for epigenetic quality control in T-cell therapy through patient stratification, HDAC/DNMT pre-therapy modulation, and allogeneic donor screening to optimize T-cell therapies [91]. Taken together, single-cell epigenomics is not merely a diagnostic tool but a blueprint for therapeutic engineering, empowering personalized T-cell therapies.

CRISPR screening for identifying epigenetic targets

The CRISPR-based system is a highly efficient tool for genomic editing through actions of Cas proteins. Currently, various whole-genome or customized CRISPR pooled libraries applied in different models enable researchers to identify the key epigenetic regulators in tumor progression or treatment. For example, CRISPR-Cas9 pooled library screens in tumour cells co-cultured with cytotoxic T lymphocytes have shown that loss of SWI/SNF chromatin remodeler subunits sensitizes tumours to T cell killing, indicating these

Table 2
The combined use of epigenetic drugs and immunotherapy in selected clinical trials.

Immunotherapy	Epi-immuno combo	Intervention / Treatment	Cancer type	Phase	Efficacy outcomes	NCT no.
Immune checkpoint inhibitor	anti-PD-1/PD-L1 + DNMT inhibitor	Drug: Azacitidine Biological: Pembrolizumab	Chemo-refractory Metastatic CRC	II	ORR 3 % (1/30; 95 % CI 0.1–17 %), mPFS 1.9 mo, mOS 6.3 mo; manageable safety	NCT02260440
		Drug: Decitabine Biological: Camrelizumab	Relapsed/Refractory Classical Hodgkin Lymphoma	II/III	Results not yet available	NCT04510610
			Relapsed or Refractory AML.	III	Results not yet available	NCT04722952
		Drug: Azacytidine Drug: Homoharringtonine Drug: Cytarabine Biological: Anti-PD-1 mAb				
		Drug: Azacitidine Biological: Avelumab	Refractory/Relapsed AML	Ib/II	CR 10.5 % (with thrombocytopenia); mOS 4.8 mo; manageable safety	NCT02953561
		Drug: Guadecitabine Biological: Atezolizumab	Urothelial Cancer	II	Results not yet available	NCT03179943
		Drug: Guadecitabine Biological: Durvalumab	Advanced HCC, Pancreatic Adenocarcinoma, and Cholangiocarcinoma/Gallbladder Cancer, Advanced Kidney Cancer	Ib, Ib/II	Results not yet available	NCT03257761, NCT03308396
Immune checkpoint inhibitor	Anti-PD-1/PD-L1 + HDAC inhibitor	Drug: Entinostat Biological: Nivolumab	Previously Treated Unresectable or Metastatic Cholangiocarcinoma and Pancreatic Adenocarcinoma	II	ORR 11 % (3 PRs); DoR 10.2 mo; Grade ≥ 3 TRAEs 63 %	NCT03250273
		Drug: Entinostat Biological: Pembrolizumab	Non-Inflamed MM	III/IV	Results not yet available	NCT03765229
		Drug: HBI-8000 Drug: Placebo Biological: Nivolumab	Unresectable or Metastatic Melanoma Not Previously Treated With PD-1 or PD-L1 Inhibitors	II/III	ORR 65.8 % (including CR 15/8% and PR 50 %), CBR 87 %, mPFS 36.9 mo in Phase II; Phase III ongoing	NCT04674683
		Drug: Domatinostat Biological: Avelumab	Advanced Unresectable/Metastatic Merkel Cell Carcinoma Progressing on Anti-PD-(L) 1 Antibody Therapy	II	Results not yet available	NCT04393753
		Drug: Tazemetostat Biological: Durvalumab	Beat Cancer	II	Results not yet available	NCT04705818
		Drug: Belinostat Biological: Durvalumab	ARID1A Mutated Cancers with Focus on Urothelial Carcinoma	I	Results not yet available	NCT05154994
Immune checkpoint inhibitor	Anti-PD-1/PD-L1 + BET inhibitor	Drug: BET Bromodomain Inhibitor ZEN-3694 Drug: Nab-paclitaxel Biological: Pembrolizumab	Metastatic TNBC	Ib	Results not yet available	NCT05422794
		Drug: BMS-986158 Biological: Nivolumab	Selected Advanced Solid Tumours or Hematologic Malignancies	I/IIa	PR in 2 patients (Schedule A); SD in 26–38 %; clinical benefit ~ 30 %	NCT02419417
	anti-PD-1/PD-L1 + EZH2 inhibitor	Drug: Tazemetostat Biological: Pembrolizumab	Pembrolizumab- or Nivolumab-Resistant, Recurrent or Metastatic HNSCC, Advanced Urothelial Carcinoma	I/II	Results not yet available	NCT04624113, NCT03854474
	anti-PD-1/PD-L1	Drug: INCB059872	Advanced Malignancies (Including AML/MDS, SCLC, Myelofibrosis, Ewing sarcoma,	I/II	Results not yet available	NCT02712905

Table 2 (*continued*)

Immunotherapy	Epi-immuno combo	Intervention / Treatment	Cancer type	Phase	Efficacy outcomes	NCT no.
	+ LSD1 inhibitor	Drug: All-trans retinoic acid Drug: Azacitidine Biological: Nivolumab	and poorly Differentiated Neuroendocrine Tumours)			
		Drug: Bomedemstat Biological: Atezolizumab	Newly Diagnosed Extensive Stage Small Cell Lung Cancer	I/II	Results not yet available	NCT05191797
	anti-CTLA4 + DNMT inhibitor	Drug: SGI-110 Biological: Ipilimumab	Unresectable or Metastatic Melanoma	Ib	Results not yet available	NCT02608437
Immune checkpoint inhibitor	anti-CTLA4 + HDAC inhibitor	Drug: Panobinostat Biological: Ipilimumab	Unresectable Stage III or Stage IV Melanoma	I	ORR 12 % (2 PRs); SD 35 %; one patient maintained response > 24 mo	NCT02032810
	anti-CTLA4 + EZH2 inhibitor	Drug: CPI-1205 Biological: Ipilimumab	Advanced Solid Tumours (Unresectable or Metastatic Melanoma, Metastatic NSCLC, Advanced or Metastatic RCC, Unresectable or Metastatic Urothelial Carcinoma (Urethra, Bladder, Ureters, or Renal Pelvis))	I	Results not yet available	NCT03525795
Tumour vaccine	CDX-1401 Vaccine + Anti-PD-L1 + DNMT inhibitor	Drug: Guadecitabine Drug: Poly ICLC Biological: Atezolizumab Biological: DEC-205/NY-ESO-1 Fusion Protein CDX-1401	Recurrent Ovarian Cancer	II	Results not yet available	NCT03206047
Adoptive cellular immunotherapy	NKR-2 CAR-T + DNMT inhibitor	Drug:5-azacytidine (AZA) Biological: NKR-2 CAR-T Cells	naïve AML or MDS	I	Results not yet available	NCT03612739
Adoptive cellular immunotherapy	Decitabine-primed Tandem CAR19/20 engineered T + DNMT inhibitor + HDAC inhibitor	Drug: Chidamide Drug: Decitabine Biological: Decitabine-primed Tandem CAR19/20 engineered T cells	Aggressive Relapsed and/or Refractory Non-Hodgkin's Lymphoma	I/II	Results not yet available	NCT04553393
Biological response modifier	NHS-IL12 + anti-PD-1 + HDAC inhibitor	Drug: Entinostat Drug: NHS-IL12 Biological: Bintrafusp Alfa (Targeting TGF-β and PD-L1)	Advanced Cancers (Including HPV-Associated Malignancies, Small Bowel, and Colon Cancers)	I/II	Results not yet available	NCT04708470
	QBM076 + anti-PD-1 + HDAC inhibitor	Drug: LCL161 Drug: Everolimus Drug: Panobinostat Drug: QBM076 Drug: HDM201 Biological: PDR001 (Spartalizumab)	CRC, NSCLC, TNBC, RCC	Ib	Results not yet available	NCT02890069

Abbreviation: PTCL: peripheral T-cell Lymphoma; CTCL: cutaneous T-cell Lymphoma; MDS: myelodysplastic syndromes.

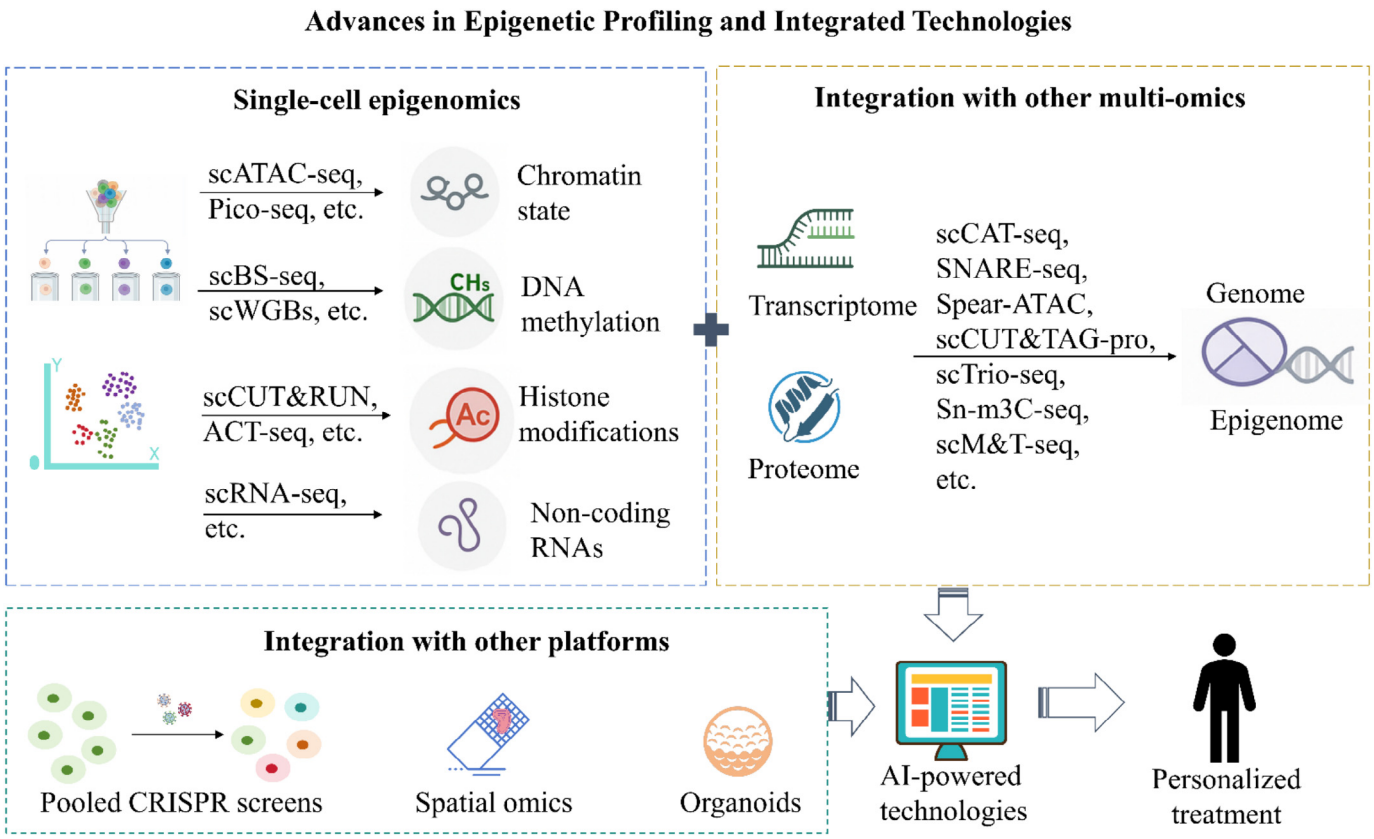

Fig. 4. Advancement of epigenomic sequencing. A. Single-Cell Epigenomics. B. Multi-Omics Integration. C. Advanced Epigenomics: integrated with CRISPR screens, spatial omics, and organoid models to enhance functional and spatial analysis.

factors normally protect tumours from immune attack [92]. A major innovation is integrating CRISPR perturbations with epigenomic profiling. Perturb-ATAC, for instance, combines CRISPR interference with ATAC-seq, revealing how gene silencing reshapes chromatin landscapes [93]. Applied in immuno-oncology, this method can identify gene knockouts that alter chromatin at immune-relevant loci, such as PD-L1, cytokine genes, or antigen presentation machinery, thus linking epigenetic regulation to immune evasion [94]. An even more high-throughput variant, Spear-ATAC (Single-cell perturbation ATAC-seq), profiles thousands of CRISPR perturbations at single-cell resolution, simultaneously mapping how 414 different gene knockouts impact chromatin accessibility across 104,592 cells [95]. By mapping these chromatin-based immune escape mechanisms, CRISPR approaches provide a list of actionable epigenetic targets, whose inhibition could enhance tumour immunogenicity and restore immune surveillance. Apart from the high-throughput CRISPR screening platforms, it has been developed the locus-specific CRISPR system by using catalytically inactivated Cas9 variant (dCas9) or Cas12a variant (dCas12a), in which these orthologue Cas9/12a variants retain DNA targeting but lack nuclease activity. The engineered CRISPR-dCas9/dCas12a system can be fused to epigenetic modifiers (DNMTs, TET demethylases, HATs, etc.) and also artificial transcription factors (e.g., p65-HSF1 and multiple copies of VP16 motif) to manipulate the epigenome or gene transactivation/repression [96]. For example, a recent study developed a CRISPR/dCas9 DNA methylation editing system to uncover the cancer-specific DNA methylation patterns in hematological malignancies [97]. Using CRISPR/dCas9 SunTag-directed DNMT3A methylation system on targeted DNA locus unraveled the epigenetic regulatory mechanisms of GBP4 and its effects on T cell exhaustion and antitumor immunology in pancreatic cancer [98].

Spatial omics and tumour microenvironment mapping

Spatial omics technologies integrating epigenomic, transcriptomic, and proteomic profiling with spatial resolution, bridge the gap between single-cell sequencing and tissue architecture, and preserving the spatial context of gene expression and epigenetic modifications in tumours. For example, spatial-ATAC-seq identified closed chromatin regions at CXCL13 loci in hypoxic tumor cores, correlating with reduced cytotoxic T cell infiltration, while spatial CUT&Tag detects H3K27me3-rich zones in stromal compartments that suppress dendritic cell activation [99]. Such insights highlight how localized epigenetic barriers dictate immunotherapy resistance. Advanced platforms like DBiT-seq and Spatial Glue enable multi-omics integration (epigenome-transcriptome-proteome) within the same tissue slice. For instance, DBiT-seq revealed co-localized H3K9me3 (repressive mark) and PD-L1 overexpression in glioblastoma-associated macrophages, directly linking epigenetic silencing to immune exclusion [100]. Similarly, SpatialGlue resolved spatial domains in breast cancer, distinguishing exhausted T cell clusters (low CD28 accessibility) from functional ones near tertiary lymphoid structures [101].

Beyond static profiling, spatial omics decodes dynamic cell–cell communication. Spatial transcriptomics localized PD-L1 + cancer-associated fibroblasts (CAFs) to tumour-stroma interfaces, adjacent to TIM-3 + exhausted T cells, forming immunosuppressive hubs. Integrating this with epigenetic data (e.g., PD-L1 enhancer activity in CAFs) identifies actionable targets for epigenetic drugs [102]. Based on these findings, emerging applications are focusing on spatially guided interventions. For example, mapping CXCL13 + C D8 + T cell niches in NSCLC guided CAR-T dosing to tumour margins, improving efficacy [103]. By mapping immune escape mechanisms at the tissue level with spatial resolution and multi-omics

depth, spatial omics may redefine precision immunotherapy—targeting epigenetic vulnerabilities in suppressive microenvironments while ensuring therapies reach the right cells and spare healthy tissues.

Organoid models for immunotherapy research

Three-dimensional (3D) tumour organoids, derived from patient tumours, offer physiologically relevant models that preserve cellular heterogeneity and stem-like subpopulations more effectively than traditional 2D cultures. Integrating organoids with single-cell epigenomic sequencing enable mechanism exploration of how epigenetic programs regulate tumour biology in context. For instance, scATAC-seq on glioblastoma organoids identified closed chromatin regions at CXCL13 loci, mirroring primary tumour but lacking regulatory elements critical for T cell recruitment, highlighting the role of the TME in immune evasion [104]. In hepatobiliary tumour organoids, subclone-specific chromatin accessibility patterns (e.g., LEF/TCF4 silencing) directly correlated with multidrug resistance, demonstrating how epigenetic heterogeneity drives therapeutic failure [105]. These findings underscore the potential of epigenetic drugs (e.g., HDAC inhibitors) to reprogram resistant subclones by restoring immune checkpoint expression or reversing chromatin silencing.

Beyond intrinsic tumour properties, organoids, organoid-immune co-culture systems enable ex vivo dissection of tumour-immune dynamics [106]. Co-culturing patient-derived organoids (PDO) with autologous T cells or CAR-T cells allows real-time assessment of immunotherapy efficacy. For instance, NSCLC organoids with endogenous TILs revealed PD-L1 enhancer hypermethylation in resistant clones, which was reversible using DNMT inhibitors, restoring T cell cytotoxicity [107] Paired with single-cell sequencing, these platforms distinguish epigenetic differences between immunotherapy-responsive and resistant cells, guiding precision strategies. Organoids integrated with epigenomic and immune components represent a paradigm shift for dissecting cancer–immune crosstalk and bridging preclinical discoveries to clinical applications.

Future perspectives and challenges

Epigenetic modifications have emerged as master regulators at almost every phase of the CI cycle, orchestrating immune evasion and therapeutic resistance. For instance, DNA methylation and histone deacetylation can epigenetically repress genes encoding antigen presentation machinery (e.g. MHC-I components) [11], T cell–recruiting chemokines (e.g. CXCL9/10) [108], and interferon signalling pathways (e.g. IFN-γ–responsive genes) [109], thereby impairing tumour antigen visibility, DC–mediated T cell priming, T cell infiltration, and effective cytotoxicity. In this way, epigenetic reprogramming by epi-drugs (e.g. DNMTis, HDACis, EZH2 inhibitor, etc.) offers a transformative strategy to revive anti-tumour immunity. These advances underscore the rationale for combining epi-drugs with ICIs and other immunotherapies.

Nevertheless, the translation of epigenetic insights to immunotherapy still meets great challenges. The regulatory networks are extraordinarily complex and remain only partially understood. In particular, metabolic–epigenetic feedback loops—where oncogenic metabolites impose histone modifications that stabilize immune dysfunction—are emerging as a critical determinant of drug resistance. Lactate-induced histone lactylation, α-KG/2-HG–driven methylation dynamics, and acetyl-CoA–mediated histone acetylation exemplify how metabolic rewiring locks T cells into exhaustion programs or promotes macrophages toward an M2 phenotype, thereby sustaining therapeutic resistance. Future research should therefore integrate metabolic and epigenetic profiling to identify predictive biomarkers such as histone lactylation signatures, α-KG/2-HG ratios, or acetylation landscapes that stratify patients for epi-immunotherapy. Rational sequencing of metabolic modulators (e.g., ACAT2 inhibitors, vitamin C, mannose) with epi-drugs and ICIs may achieve durable reprogramming of the TME, though careful optimization of dose, timing, and patient selection will be essential to maximize efficacy and minimize toxicity.

The absence of validated epigenetic biomarkers/signatures limits the identification of patients that are responsive to epi-immunotherapy combinations, as demonstrated in the phase II AML trial (NCT02775903) where the survival outcomes did not show significantly improved despite epigenetic modulation [110]. Furthermore, current epi-drugs often exhibit suboptimal specificity and lead to off-target effects [111]. This may disrupt immune homeostasis in unintended ways and underscores the need for isoform-selective modulators. Indeed, combining epi-drugs with immunotherapies have shown promising synergistic potential as they can heighten tumour immunogenicity and foster a more permissive TME for immune attack. However, heightened immune activation from dual therapy can also exacerbate immune-related adverse events (irAEs) such as autoimmunity. Besides, differences in epi-drug dosage, duration, and inter-patient epigenomic heterogeneity can lead to inconsistent efficacy with some patients deriving little added benefit or even developing resistance to the combination. Thus, while epigenetic adjuvants clearly hold promise to amplify anti-tumour immunity, careful optimization is required to mitigate the risks.

Identifying epigenetic signatures that pinpoint which patients are most likely to benefit from a given combination would enable more personalized and safer deployment of these powerful epi-immunotherapy approaches. Emerging technologies in multi-omics and artificial intelligence (AI) are poised to accelerate progress on these fronts. Advanced single-cell and spatial sequencing techniques now permit high-resolution mapping of epigenetic states within tumours, offering new insight into local immune evasion mechanisms. Such multi-omics profiling with applications in high-throughput functional genomic screens (e.g., pooled CRISPR screens) and physiologically relevant models (e.g. patient-derived organoids) have already uncovered novel epigenetic regulators of anti-tumour immunity and potential therapeutic targets. At the same time, integrated data analysis powered AI technologies, particularly machine learning (ML) and deep learning, are being used to discover complex biomarker signatures that predict immunotherapy outcomes with remarkable efficiency and precision [112]. ML algorithms such as support vector machine, gradient boosting machines and convolutional neural networks have been using to leverage epigenomic data (DNA methylation, histone marks) integrated with multi-omics features to predict therapeutic response, stratify patients, and uncover immune evasion mechanisms within the TME [113]. The application of these cutting-edge tools is expected to refine patient selection and guide precision epigenetic therapy: by matching the right epigenetic drug to the right patient at the right time, based on multi-omics biomarkers, clinicians could avoid futile treatments and focus on responders.

However, translating these advances into routine clinical practice will require overcoming significant technical and translational barriers. These include: (i) the risk of off-target effects in pooled CRISPR-based functional screens, which may confound interpretation and raise safety concerns for clinical translation [114]; (ii) the high costs, technical complexity, and lack of standardized protocols that limit the integration of single-cell and spatial epigenomics into clinical workflows. (iii) the poor compatibility of these technologies with formalin-fixed, paraffin-embedded (FFPE) clinical

specimens [115], which poses a major challenge for large-scale clinical implementation; (iv) the scarcity of validated epigenetic biomarkers across diverse patient cohorts, which hampers translation of correlative findings into robust, predictive tools. Large-scale, multi-centre studies will be required to assess the reproducibility, specificity, and clinical utility of candidate biomarkers. In parallel, regulatory considerations surrounding epigenetic drugs—with their potential for broad and pleiotropic effects—underscore the need for long-term safety monitoring, especially in combination regimens. Overcoming these challenges will require close interdisciplinary collaboration among researchers, clinicians, computational biologists, and regulatory bodies. By continuing to unravel the complex epigenetic underpinnings of tumour–immune interactions and rigorously testing new combination strategies and enabling technologies, the field can make meaningful progress toward clinical translation.

In summary, epigenetic therapy has firmly established itself as a compelling partner for immunotherapy, capable of reshaping the immune landscape of tumours. The coming years will focus on meeting the outstanding challenges – refining specificity, identifying biomarker-defined patient subsets, managing toxicity, and reducing practical obstacles – to fully realize the potential of epigenetic immunomodulation. With sustained research efforts and carefully designed clinical trials, it is anticipated that epigenetic adjuncts will become an integral component of next-generation immuno-oncology treatment paradigms, ultimately improving responses and outcomes for cancer patients.

CRediT author statement

Ning Wang conceived the overall idea and finalized the manuscript. Yuanjun Lu drafted the whole manuscript and prepared figures and tables. Shuoshuo Ma collected the data and organized tables. Yau-Tuen Chan, Ya Wu, and Yibin Feng revised the manuscript. All authors approved the final manuscript.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Ethics approval and consent to participate

Not applicable.

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.

Acknowledgements

This work was supported by Sichuan Science and Technology Program (Project code: 2024YFHZ0366), and Outstanding youth fund of Sichuan Cancer Hospital (Project code: YB2024011).

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