

EPIGENETIC REGULATION OF ANTITUMOR IMMUNITY: NEW FRONTIERS IN CANCER IMMUNOTHERAPY

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Abstract

While oncology has vastly changed with the arrival of cancer immunotherapy, the issue of resistance obstacles (primary and acquired) to durable responses is still omnipresent. Recently, epigenetic mechanisms such as DNA methylation, histone modifications, chromatin remodeling, and non-coding RNAs have come to play important roles in the regulation of tumor antigenicity, antigen presentation, interferon signaling, expression of immune checkpoints, and functional status of infiltrating immune cells. Both malignant cells and immune cells show epigenetic dysregulation that leads to immune evasion and formation of immunosuppressive tumor microenvironment, including T-cell exhaustion, macrophage polarization to M2 type and generation of myeloid-derived suppressor cells. Of note, epigenetic modifications can be reversed, which is not true for genetic changes, and therefore may provide a novel therapeutic opportunity. Epigenetic-targeting agents such as DNA methyltransferase inhibitors, histone deacetylase inhibitors, EZH2 inhibitors and BET inhibitors can induce the re-expression of silenced tumor antigens and re-establish antigen presentation, alter immune checkpoints, and re-educate other (immunosuppressive) myeloid cells, transforming immunologically "cold" tumors into "hot" tumors that are responsive to immune checkpoint blockade. However, trials of epigenetic treatment in conjunction with immunotherapy have produced promising but inconsistent outcomes, and there are a number of obstacles to wide-scale use, such as varying efficacy on different types of disease context, off-target effects and the requirement for predictive biomarkers. The development of emerging single-cell and spatial epigenomic technologies, multi-omic integration, and precision epigenetic editing holds the promise of

achieving cell, locus, and context specific interventions. In this review, the molecular mechanisms by which epigenetic modifications are connected to antitumor immune responses are summarized, existing therapeutic approaches and clinical findings are discussed, and the future path for precision epigenetic tumor immunotherapies that boost the success of the treatment while limiting its toxicity is outlined.

1. Introduction

Traditionally, the interplay between the immune system and cancer cells has been studied in terms of genetic changes: neoantigens, genetic instability that leads to immune recognition and genetic changes that lead to immune evasion. But changes in genes are not enough to explain the reversible dynamic of immune evasion in the course of tumor development and in response to treatment. Epigenetic mechanisms offer an additional level of regulation which allows quick response to immunoselection without permanent changes to the genome. Epigenetic plasticity of tumor and immune cells allows dynamic adaptation to microenvironment, leading to immune adaptation and immune resistance. Important: unlike genetic mutations, epigenetic states can be changed in a way that is reversible; this presents a therapeutic opportunity as epigenetic modifications can be pharmacologically reversed, potentially reinvigorating antitumor immunity [1], [2].

The tumour-immune interface is a dynamic ecosystem in which there are multiple bidirectional communication channels between malignant cells and immune cells. Both of these compartments are under the control of epigenetic regulators, including cytokines, chemokines, metabolites and extracellular signals. The signalling network can help to reshape the epigenetic programme of a tumour when it is attacked by the immune system, and it can help immune cells to survive in the hostile tumour environment. In the course of tumour evolution, the immune landscape is dynamically remodelled, where epigenetic changes contribute to the functional state, polarisation and composition of immune cells. Epigenetic modifications play a role in regulating every aspect of the cancer-immunity cycle, including tumour antigen release; antigen capture and presentation by APCs; T-cell priming and activation; trafficking of activated T-cells and infiltration into the tumour microenvironment;

and killing of tumour cells by CTLs [3]. Although immune checkpoint inhibitors (ICIs) are transformative drugs, most patients do not respond to these drugs for long. Primary resistance occurs from the beginning, and acquired resistance happens after an initial response, many times because of a process called 'immune editing' that causes a decrease in the immunogenicity of the tumour or an increase in immunosuppression. Two large non-responsive phenotypes have been described: immune-excluded tumours, in which T cells are trapped in the tumour stroma and are unable to enter the tumour parenchyma; and immune-desert tumours, lacking T cell infiltration. Persistent immunosuppression in the TME causes the emergence of these phenotypes, which are indicative of T-cell dysfunction such as exhaustion, senescence and anergy. It is usual to classify tumours as "cold" (low infiltration of T cells, weak immunogenicity, poor response to ICI) or "hot" (high infiltration of T cells, strong immunogenicity, good response to ICI), and epigenetic mechanisms are central to this classification [1], [4].

Epigenetics allows tumours to evolve in response to the selective forces of immunotherapy with mechanisms that are more easily acquired than genetic mutations. Epigenetic silencing of antigen-presentation machinery (MHC-I, MHC-II, β_2 -microglobulin, TAP1/TAP2) to suppress the display of tumour antigens to T cells is a key adaptive mechanism. Failure of inflammatory signalling pathways, especially the JAK-STAT axis and interferon-stimulated genes, may be epigenetically regulated to render a tumour refractory to inflammatory cues. Tumours and immune cells are able to dynamically control immune inhibitory signalling through the use of chromatin. Epigenetic adaptation is an important driver of therapeutic resistance and highlights a need for therapeutic strategies targeting the epigenetic underpinnings of immune evasion [5].

This review incorporates the epigenetic control of not only the tumour cells themselves but also the epigenetic control of cells in the tumour microenvironment and how these are related to the therapy response and clinical translation. The epigenetic regulation of the tumour microenvironment, the epigenetic modification of tumour cells and the epigenetic regulation of anti-tumour immunity are explored. We next review epigenetic approaches as immune sensitizers, in combination with immunotherapies, and recent advances into new directions in precision epigenetic immunotherapy, with a focus on unsolved questions and areas for future exploration.

2. Epigenetic Architecture of Antitumor Immunity

2.1 DNA Methylation and Demethylation

DNA methylation, which refers to the addition of a methyl group to cytosine residues in CpG dinucleotides, is performed by three DNA methyltransferases: DNMT1, DNMT3A and DNMT3B. DNMT1 maintains the methylation patterns during replication, and DNMT3A and DNMT3B establish de novo methylation patterns. Active DNA demethylation is a process that is mediated by TET proteins (TET1, TET2, TET3) through the oxidation of 5-methylcytosine [6]. Immune-regulatory and tumour-associated genes are highly methylated, and this is reflected in the immune phenotype. CpG island methylation of promoter regions can silence tumour suppressor genes, cancer-testis antigens and components of the antigen-presentation machinery. Abnormal hypomethylation of DNA is a characteristic of cancer, which can result in increased genomic instability and an increase in the activation of endogenous retroviral elements, thus providing a viral mimicry signal that enhances immunogenicity. On the other hand, hypermethylation of certain loci can help in immune evasion due to silencing of genes needed for immune recognition. DNA methylation status could be used as predictive biomarkers for immune infiltration and response to immunotherapy [7].

2.2 Histone Modifications

The histone modifications are a complex code in regulating the structure of chromatin and the transcription of genes. Acetylation of histones, an activity carried out by histone acetyltransferases, and deacetylation, by histone deacetylases, usually leads to increased transcription because the acetylation neutralizes the positive charges on the histones, resulting in less compaction of the chromatin. Methylation of histones has context-dependent effects: promoters with H3K4me3 marks and active gene bodies with H3K36me3 marks and transcriptional repression with H3K27me3 and H3K9me3 marks [8]. The key enzymes are the H3K27me3 catalyst EZH2, LSD1/KDM1A and different classes of HDACs. These changes regulate the differentiation and activity of immune cells, and certain histone modifications define the "exhausted" and "effector" T-cell phenotypes and the polarisation of macrophages. During T-cell exhaustion in CD8⁺ T-cells, there is a corresponding decrease in H3K27ac (active chromatin) and an increase in H3K27me3 (repressive chromatin) [9].

2.3 ATP-Dependent Chromatin Remodeling

Nucleosome positioning and chromatin accessibility are governed by ATP-dependent chromatin remodelling complexes such as SWI/SNF, BAF, PBAF and others. Cancer-activating mutations in subunits like ARID1A and SMARCA4, which, when lost, result in a change in the transcription of immune genes. CD8⁺ T cell function and longevity are controlled by the SWI/SNF chromatin remodelling complex, otherwise called the BRG1/BRM-associated factor (BAF) complex. The BAF complex also plays an important role in terminal T-cell exhaustion, as confirmed by the finding that depletion of its members ARID1A or SMARCD2 prevents T-cell exhaustion and promotes memory T-cell generation. Depending on the context, chromatin remodelling defects can enhance or diminish the sensitivity to immunotherapy by affecting the accessibility of promoters and enhancers of immune genes, including the expression of MHC molecules, chemokines and immune checkpoints [10].

2.4 Non-Coding RNA-Mediated Epigenetic Regulation

In addition to the above epigenetic regulation, there is another layer of epigenetic regulation, which is provided by non-coding RNAs. Specific miRNAs regulate immune-cell states and immune checkpoints: microRNAs (miRNAs) control post-transcriptional degradation of transcripts and/or prevent translation through binding to the 3'-untranslated regions of mRNAs. lncRNAs are known to regulate gene expression on many different levels, such as nucleosome positioning and looping of chromosomes. circRNAs might function as miRNA sponges and impact the downstream gene expressions. There's evidence that non-coding RNA forms part of the regulatory network of RNA-chromatin interaction that also helps to regulate immune-gene transcription, and tumour cells have been shown to exploit non-coding RNA for immune-cell dysfunction and evasion. miRNAs are important regulators

of TIM-3, CTLA-4 and PD-1/PD-L1 expression in haematological malignancies and solid tumours [11], [12].

2.5 Epitranscriptomic Regulation of Antitumor Immunity

An additional layer of epigenetic control is brought in by RNA modifications, such as N6-methyladenosine (m6A). The m6A modification is added by METTL3-METTL14, stripped by demethylases FTO and ALKBH5, and read by proteins that belong to the YTH family. This alteration has an impact on the stability of RNA and the translation of immune-regulating transcripts. The epigenetic regulation of gene expression in the transcription products, or epitranscriptomic regulation, affects the functioning and signalling of immune checkpoint molecules and is a new area of research in the molecular regulation of anti-tumour immunity [13].

Cycle of Epigenetic Regulation in Antitumor Immunity

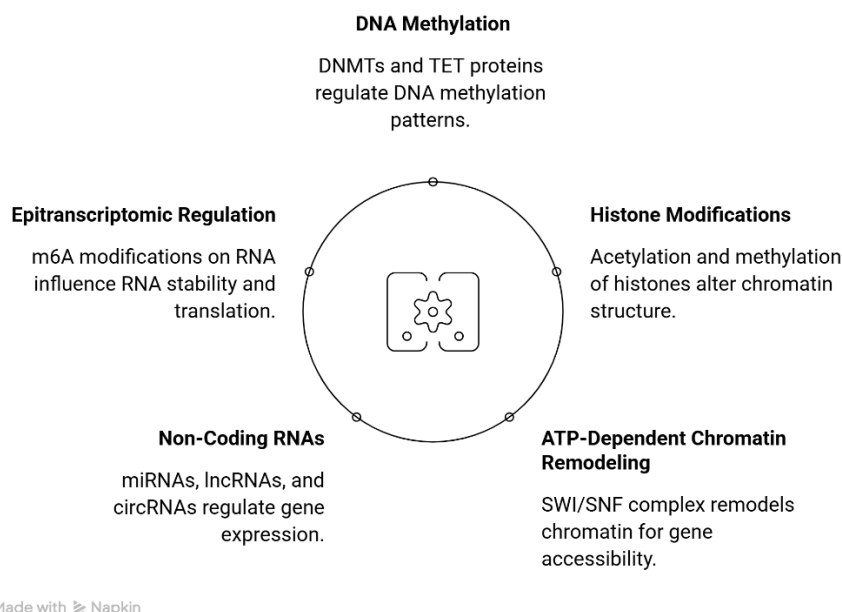


Figure 1.0 Illustrating the various layers of epigenetic regulation involved in antitumor immunity

3. Epigenetic Reprogramming of Tumor Cells to Escape Immune Surveillance

3.1 Epigenetic Silencing of Tumor Antigens

Epigenetic mechanisms have been used by the tumours as a way to silence the expression of

tumour antigens in order to minimise the immune system's ability to recognise them. The cancer-testis antigens (CTAs) are normally expressed in the germline but are often silenced by methylation of the promoter DNA and/or

regulation by non-coding RNAs (ncRNAs). Epigenetic silencing of families of CTA genes (such as MAGE, GAUGE, XAGE, BAGE, or PAGE) reduces the number of antigens presented to T cells. In the same way, DNA methylation is responsible for the silencing of genes that encode the "non-self" neoantigens so that cancer cells can decrease the expression of these neoantigens and evade detection by the immune system. Epigenetic control also acts on endogenous retroviral elements (EREs), which may act as a source of viral-like antigens and induce innate immune responses, and reactivation of these elements may constitute a mechanism by which tumour immunogenicity is increased [14].

3.2 Epigenetic Control of Antigen Processing and Presentation

Proper antigen processing is necessary for effective T-cell recognition, which involves both MHC-I and MHC-II molecules. This pathway is an important mechanism of immune evasion that is frequently disrupted epigenetically. The genes coding for MHC-I, MHC-II, β 2-microglobulin, and TAP1-TAP2 and other genes involved in antigen processing are silenced by promoter hypermethylation and/or repressive histone modifications. Recurrent tumors following PD-1 therapy could exhibit an adaptation resistance mechanism of transcriptional silencing of genes in the antigen presentation pathway. Epigenetic marks can be reverted by DNMT or HDAC inhibitors, which can then re-establish antigen presentation and increase immune recognition [1], [15].

3.3 Epigenetic Regulation of Interferon Signaling

Interferon (IFN) signalling is a key component of antitumor immunity, responsible for the induction of both MHC molecules and chemokines, as well as other immune-modulatory genes. Tumours epigenetically silence interferon response pathways such as IFN- γ signalling and the JAK-STAT cascade and are unresponsive to immune activation. Inflammatory signalling is dampened, and the tumour microenvironment is less immunogenic, by chromatin-dependent suppression of interferon-stimulated genes. SUV39H1 is a

histone methyltransferase which negatively regulates the expression of IFN γ and granzyme B in CD8⁺ effector T cells leading to T-cell exhaustion. The double treatment of SUV39H1 inhibitors with anti-PD-1 increases the ratio of effector CD8⁺ T cells and suppresses tumour growth, which holds therapeutic promise [16], [17].

3.4 Epigenetic Regulation of Immune Checkpoints

Epigenetic changes are involved in regulating expression of immune checkpoints, besides transcriptional changes. Epigenetic regulation of the PD-L1/PD-1 axis, CTLA-4-associated pathways and emerging checkpoints TIM-3, LAG-3, and TIGIT. Both pan-HDAC and specific HDAC inhibitors can enhance the expression of PD-L1 in lung cancer and melanoma. Studies on cervical cancer have shown that EZH2, H3K27me3 and DNA methyltransferase 3A are involved in the epigenetic regulation of TIM3 expression in the context of HPV18 infection. DNA and histone modifications are both responsible for the expression of multiple checkpoint molecules (PD-1, CTLA-4, TIM-3, and LAG-3) in cancer of the breast. HDAC3, in particular, promotes the expression of PD-L1 in B-cell lymphoma. Epigenetic therapies could be used to regulate checkpoint expression to sensitise tumours to checkpoint blockade or to identify new targets [18].

3.5 Epigenetic Remodeling of Tumor Immunogenicity

Epigenetic programming may transform an immunologically "cold" tumour into a more "hot" tumour that is sensitive to ICIs. Increased antigenicity of the tumour, restored production of chemokines, recruitment of dendritic cells and infiltration of T-cells during this transition. DNMT inhibitors may also lead to reactivation of endogenous retroviral elements, which in turn leads to a viral mimicry response, inducing interferon signalling, chemokine production, and thus immune cells in the tumour microenvironment. HDAC inhibitors can be used to increase MHC molecule and co-stimulatory ligand expression to facilitate immune recognition. Repressive H3K27me3

marks can be reversed by EZH2 inhibitors, leading to the re-expression of immune-related genes. The demethylation and re-expression of endogenous retroviral elements provide new tumour antigens and provoke a viral mimicry

response, which leads to activation of innate immune sensing pathways, type 1 interferon production, and a pro-inflammatory tumour microenvironment more receptive to immunotherapeutic approaches [19], [20].

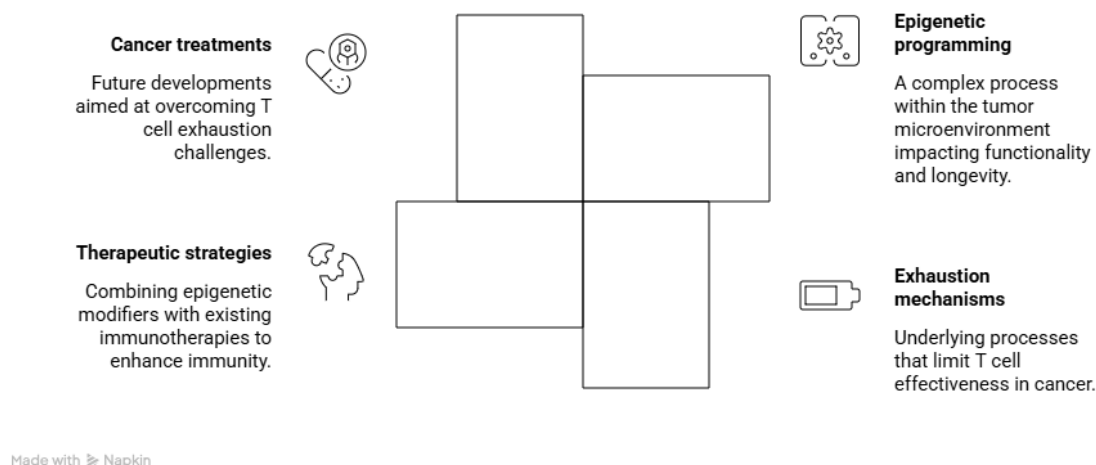


Figure 2.0 The epigenetic programming of CD8⁺ T cells within the tumor microenvironment

4. Epigenetic Programming of Immune Cells Within the Tumor Microenvironment

4.1 CD8⁺ T Cells: From Effector Function to Exhaustion

Epigenetic programmes that have been altered during the tumour microenvironment control the differentiation and functional state of CD8⁺ T cells. Disseminated antigen exposure during the progression of the tumour induces global epigenetic changes in T cells, resulting in a T cell “exhausted” state which is defined by impaired T cell proliferation, decreased production of pro-inflammatory cytokines and decreased self-renewal capacity. With T-cell exhaustion, there is a reduction in H3K27ac (active chromatin) and an increase in H3K27me3 (repressive chromatin) [21]. The SWI/SNF chromatin remodelling complex has an essential role in regulating CD8⁺ T-cell function and longevity; absence of BAF complex members ARID1A or SMARCD2 blocks terminal T-cell exhaustion and instead promotes memory T-cell generation. But immune checkpoint blockade treatment fails to completely reprogram the epigenetic landscape associated with the exhausted state, thus offering a mechanistic explanation for the inability to completely reinvigorate T-cells.

SUV39H1 inhibitors with anti-PD-1 therapy boost the percentage of effector CD8⁺ T cells (reinvigorate chromatin). cells, tumour programmes, and cells and suppress the growth of tumours, while the histone methyltransferase SUV39H1 suppresses the expression of IFN γ and granzyme B, leading to T-cell exhaustion [17].

4.2 Regulatory T Cells and Immune Suppression

Regulatory T cells are important for immune homeostasis and can inhibit anti-cancer immune responses in the TME. Epigenetics – epigenetic events mediated through FOXP3 control stability and function of Tregs; EZH2 is important for maintaining the Treg program. The EZH2⁺ subset among CD8⁺ T cells, cytotoxic after TCR stimulation, indicates context-dependent effects of EZH2 inhibition. Treg expansion and suppressive activity in tumours are influenced by the epigenetic landscape. Therapy to alter immunosuppressive Treg programmes is under investigation but will need to be safe in respect to mechanisms of action [22].

4.3 NK Cells and Innate Antitumor Immunity

For example, natural killer cells are important cells to fight the appearance of tumours by secreting perforin and granzymes when in contact with them and also regulate the immune response by secreting proinflammatory cytokines, like IFN γ and TNF- α . Maturation, expression of cytotoxic receptors and functional capacity of NK cells are regulated by epigenetic control. However, the activation of NK cells is suppressed in the immunosuppressive tumour microenvironment, enabling tumour progression. Epigenetic increase in killing by the NK cells is a promising line of treatment, and HDAC and DNMT inhibitors have been found to restore the killing function of the NK cells [23].

4.4 Myeloid Cells: TAMs and MDSCs

Tumour-associated macrophages (TAMs) are the major cells of the tumour microenvironment which affect the malignancy and immune evasion of tumours. In early cancer, TAMs present a proinflammatory M1 phenotype, possessing anti-tumour functions, but influenced by the tumour microenvironment, TAMs change into an immunosuppressive and angiogenic M2 phenotype, which promotes the depletion of T cells and leads to the inhibition of CTL viability and the decrease in the activity of ICI. Epigenetic regulation of macrophage polarisation entails modifications of the histone, DNA and accessibility of the chromatin at inflammatory and anti-inflammatory gene sites. The expansion and suppressive function of myeloid-derived suppressor cells is also epigenetically controlled, and several circuits (BET, HDAC, EZH2, and others) involved in chromatin control are targets for myeloid reprogramming. A combination of scRNA-seq and metabolomics has been used in the study of pancreatic ductal adenocarcinoma (PDAC) to uncover four primary patterns of TAMs, including one type of tissue-resident macrophages that express elevated levels of intracellular deoxycytidine and reduced levels of deoxycytidine kinase, which restricts gemcitabine uptake by the tumour and promotes a fibrotic tumour microenvironment that is immunosuppressive through secretion of TGF- β and collagen [24], [25], [26].

4.5 Dendritic Cells and B Cells

The most efficient APCs are dendritic cells, which can process and present antigens through the MHC-I and MHC-II molecules to initiate and maintain immune responses. DC maturation, antigen presentation capacity and T-cell priming ability are regulated by epigenetics. DC-10 cells are able to express HLA-G under the induction of tumours, which results in dysfunctional T-cells. The differentiation of B-cells and development of tertiary lymphoid organs are also epigenetically regulated with impact on anti-tumour humoral immunity. Epigenetic therapies could be used to improve the function of DCs and the induction of a productive anti-tumour antibody response [27].

5. Epigenetic Control of the Tumor Microenvironment: From Immune Suppression to Immune Activation**5.1 Epigenetic Regulation of Cytokine and Chemokine Networks**

Epigenetic regulation of the cytokine and chemokine networks which are important in the function and trafficking of immune cells. The expression of chemokines such as CXCL9, CXCL10 and CXCL11 affects the recruitment of T cells, and the recruitment of monocytes and T cells is influenced by CCL2 and CCL5. HDAC8 has been found to inhibit the infiltration of CD8 $^{+}$ T cells into the tumour tissue by decreasing the acetylation of H3K27 and silencing the chemokine gene CCL4 during the study of HCC. Epigenetic modifications are involved in the regulation of chemokine gene expression through a variety of mechanisms and can function as reversible switches that can have therapeutic value in reversing immune suppression and reprogramming the tumour microenvironment. The epigenetic therapies can reverse that process and allow chemokines to be produced again, creating hot tumours and making them infiltratable by immune cells [28], [29].

5.2 Metabolic-Epigenetic Crosstalk in the Tumor Microenvironment

There is a close connection between metabolism and epigenetics, as metabolites are substrates and cofactors for epigenetic modifications. Lactate may induce histone lactylation, thereby

leading to TAMs' M2 polarisation and PD-1 blockade resistance. Tumour glycolysis leads to increased H3K27ac and PD-L1 upregulation, fuelled by acetyl-CoA. S-adenosylmethionine provides the methylation reactions with methyl groups, and α -ketoglutarate is needed for the activity of histone demethylases, which remodel the chromatin. During obesity, the lipotoxic environment leads to up-regulation of the genes for PPAR α and δ , which signal for lipid accumulation in NK cells. The leptin-STAT3-FAO axis is one of the key players that connect obesity with impaired anti-tumour immunity. There is a complex microbiota-metabolism-epigenetics-immunology axis in cancer, with metabolites of gut microbiota, like butyrate and indole-3-lactic acid, being direct modulators of the epigenetics in immune cells, especially CD8⁺ T cells [30], [31].

5.3 Cancer-Associated Fibroblasts and Extracellular Matrix

Cancer-associated fibroblasts are important stromal players in immune exclusion via matrix remodelling and immunosuppressive signalling. Histone modifications and DNA methylation have been shown to be important epigenetic regulators of CAF activation that regulate enzymes associated with matrix remodelling and TGF- β expression. Multi-omics integration has identified 3 conserved CAF metaprogrammes (steady-state-like, mechano-responsive and immunomodulatory) across tissues, and mechanotransduction via YAP/TAZ is a mechanism which differentiates the mechano-responsive CAFs into immunomodulatory and steady-state-like subsets. Immune-checkpoint blockade disrupts this balance, and targeting CAF plasticity might be a therapeutic approach. The presence of dense stroma slows the clearance of metabolites, producing a build-up of lactate and acidification that affects T-cell function [32].

5.4 Hypoxia, Acidity, and Nutrient Stress

Epigenetic reprogramming of tumour and immune cells occurs through the direct impact of the hostile tumour microenvironment, which is defined by hypoxia, acidity and nutrient deprivation. Hypoxia-responsive chromatin-modifying enzymes are oxygen-dependent

histone demethylases, which are regulated by hypoxia-inducible factors. Metabolic stress is one factor that contributes to dysfunction of T cells: hypoxia has a profound effect on T-cell mitochondrial OXPHOS and ATP production, which affects actin cytoskeletal remodelling and, consequently, affects the ability of the cells to move. This epigenetic adaptation to these negative lots permits the endurance of both tumour and immune cells under negative circumstances and factors in a global immunosuppressive system [33].

5.5 Epigenetic Regulation of the "Cold-to-Hot" Tumor Transition

One of the most important therapeutic targets is to turn "cold tumours" into "hot tumours". This transition involves enhancing tumour antigenicity, re-establishing chemokine production, boosting dendritic-cell recruitment and inducing T-cell infiltration. Epigenetic therapies regulate this switch by a variety of means. DNMT inhibitor-induced viral mimicry leads to interferon signalling and production of chemokines [34]. HDAC inhibitors are promoters of MHC expression and co-stimulatory ligand display. Immune-related genes are repressed, releasing repression by EZH2 inhibitors. Clinical data from the phase II pembrolizumab and oral azacitidine combination in platinum-resistant ovarian cancer showed upregulation of interferon signalling, antigen presentation and immune cell adhesion and migration gene sets as well as an increase in CD8⁺ T-cell density, indicating that epigenetic modifiers can raise the level of tumour inflammation [35].

6. Epigenetic Therapies as Immune Sensitizers

6.1 DNA Methyltransferase Inhibitors

The DNMT inhibitors azacitidine and decitabine are FDA-approved for some haematological malignancies. In addition to their direct anti-tumour activity, DNMTIs may also reactivate silenced immune pathways. Loss of methylation at endogenous retroviral elements results in activation of these elements, leading to an interferon signal and the production of chemokines. DNMTis contribute to improved antigen presentation by demethylating promoters of both the antigen-

processing machinery and MHC genes [15]. Their immune-sensitising activity is due to effects on immune-cell function such as T-cell activation and macrophage polarisation. Such synergy has been directly demonstrated in several preclinical models of the combination of DNMT inhibition and ICI. Decitabine and camrelizumab were shown to be effective in refractory Hodgkin lymphoma in a phase II trial, which showed a twofold efficacy improvement over the monotherapy and was shown to be because of epigenetic enhancement of T-cell activation [36].

6.2 Histone Deacetylase Inhibitors

HDAC inhibitors are inhibitors of class I HDAC, class II HDAC and pan-HDAC, which show differential levels of inhibition between different types of tumour and immune cells. Through the modulation of the acetylation of histones at immune-related gene loci, HDACIs are involved in regulating the function of T-cells and myeloid-cells. They might increase the expression of tumour antigen and MHC presentation. The actions of HDAC inhibitors are, however, complex, with different HDAC subtypes having both opposing and overlapping roles in cancer and immunity. The HDAC3-selective inhibitor treatment can increase the CD8⁺ T-cell cytotoxic ability, and HDAC3 negatively regulates a cytotoxic effector-associated transcriptional programme in CD8⁺ T cells. However, the clinical activity of HDACIs as immune sensitisers has been disappointing to date, and combination clinical trials report only modest activity. The Phase II trial of entinostat plus pembrolizumab in metastatic uveal melanoma has an objective response rate of 14%, versus the previous 5% with anti-PD-1s alone [37], [38].

6.3 EZH2 and Histone Methylation Inhibitors

The catalytic part EZH2 of the Polycomb repressive complex 2 (PRC2) is responsible for the deposition of the repressive H3K27me3 mark. The Ezh2-mediated H3K27me3 helps to silence the immune-related genes of tumour cells and stabilise Treg. It is possible to reverse this suppression with EZH2 inhibitors, thereby making tumours more immunogenic and disrupting the Treg-mediated suppression.

However, the EZH2 can also have the opposite effect on the activation of T-cells after TCR stimulation, depending on the cellular situation. Its EZH2⁺ subset of CD8⁺ T cells shows enhanced cytotoxicity, indicating that an EZH2 inhibitor might enhance CD8⁺ T cell infiltration at the expense of CD8⁺ T cell functional activity. The effects of the treatment are sometimes context-dependent; that is, patient selection and combination strategies need to be carefully monitored [22], [39].

6.4 LSD1, BET, G9a, and Emerging Epigenetic Targets

LSD1 (KDM1A) is a histone demethylase which is able to demethylate H3K4me1/2 and H3K9me1/2. LSD1 inhibition has been demonstrated to have immune-activating properties, which may be an attractive opportunity to combine with ICIs. Immunoregulation of the expression of immune checkpoints and myeloid suppression can be achieved by BET bromodomain inhibition, which is directed against BET proteins that read the acetylation marks on histones. A cancer vaccination approach has been shown to be promising using G9a (EHMT2) inhibitors. Emerging regulators of the chromatin are studied as therapeutic targets, such as SETDB1. Using the CRISPR-based epigenomic screens, new targets such as EZH2 and BET have been identified, inhibition of which remodels myeloid suppression and increases NK cell killing [40].

6.5 Precision Epigenetic Editing

With CRISPR/dCas9, it is possible to target epigenetic marks to specific locations on the genome. These systems can specifically target DNA methylation or demethylation, editing of histone modifications and reactivation of locus-specific immune genes. Benefits compared to systemic epigenetic inhibition include greater specificity, minimal off-target effects and optimal fine tuning of gene expression concentrations. Activators could use dCas9 to activate markers of cell stress and neoplastic transformation to improve immune recognition. Currently, however, there are limitations for efficient in vivo delivery; existing platforms include viral

vectors, lipid nanoparticles and virus-like particles [41], [42].

7. Epigenetic-Immunotherapy Combinations: From Preclinical Synergy to Clinical Translation

7.1 Epigenetic Drugs Plus Immune Checkpoint Inhibitors

Epigenetic drugs in combination with ICIs are the most well-investigated option for epimmunotherapy. Preclinically, DNMTi + anti-PD-1/PD-L1 combinations have been proven to demonstrate synergy via various mechanisms such as increasing tumour antigenicity, viral mimicry and T-cell activation. Results of clinical trials have been inconsistent, and some trials have shown promise in the treatment of

refractory Hodgkin lymphoma and some activity in solid tumours. A phase II study of pembrolizumab plus oral azacitidine (OAZ) in platinum-resistant ovarian cancer showed that this combination is moderately well tolerated, with an overall response rate of 2.9% and a disease control rate of 50%; transcriptomic analysis showed increased expression of inflammatory and cytolytic genes, interferon signalling, and antigen presentation gene sets and an increase in the density of CD8⁺ T cells. The idea of the HDACi + checkpoint blockade combinations is to reduce the effect of immunosuppression and increase the function of T-cells. Table 1 summarises some of the most important clinical trials of epigenetic-immunotherapy combinations [43].

Table 1: Selected Clinical Trials of Epigenetic-Immunotherapy Combinations

Trial Identifier	Cancer Type	Epigenetic Drug	Immunotherapy	Phase	Key Findings
NCT03150329	Non-Hodgkin lymphoma	Vorinostat	Pembrolizumab	I	Improved objective remission rate and PFS
NCT03250962	Classical Hodgkin lymphoma	Decitabine	Camrelizumab	II	Twofold improvement in efficacy vs monotherapy
NCT02900560	Platinum-resistant ovarian cancer	Oral azacitidine	Pembrolizumab	II	ORR 2.9%, DCR 50%, increased CD8 ⁺ T-cell infiltration
NCT03820596	Relapsed/refractory NK/T-cell lymphoma	Chidamide	Sintilimab	Ib/II	Improved DOR/PFS
NCT02220842	R/R diffuse large B-cell lymphoma	Tazemetostat	Atezolizumab	Ib	Safe and tolerable, moderate antitumor activity

7.2 Epigenetic Modulation of CAR-T and TCR-T Cell Therapy

Epigenetic regulation plays important roles in directing T-cell differentiation, function and fate, which impact the success of T-cell-based immunotherapies. Epigenetic engineering of therapeutic lymphocytes is one of the approaches that can be adopted to improve CAR-T/TCR-T therapy that can increase persistence, reduce exhaustion and promote

memory formation. Epigenetic reprogramming factors have proved their effectiveness in preventing T-cell exhaustion, and reprogrammed T cells have been found to have higher durability, with the capacity to slow down the growth of tumours in vivo. Epigenetic modulation of the infused cells or modulation of the tumour microenvironment can be another way of overcoming the suppressive tumour microenvironment [1], [44].

7.3 Epigenetic Therapy and Cancer Vaccines

Epigenetic therapy can be used to augment cancer vaccination by increasing the expression of tumour antigens and through better antigen presentation. Epigenetic reprogramming targeting methylation can cause arrest of the cell cycle and apoptosis of the tumour cells and stimulate infiltration of dendritic cells. Both G9a and DNMT inhibition have been demonstrated to enhance the therapeutic potential of TCR-redirected T-cell and DC vaccination, leading to higher overall survival in melanoma models. To maximise antitumor immunity, integration with neoantigen vaccines, viral-vector and mRNA vaccine strategies is being explored [45].

7.4 Epigenetic Therapy with Radiotherapy, Chemotherapy, and Targeted Therapy

Other forms of combination therapy are targeted therapy, radiotherapy, and chemotherapy. Epigenetic agents have the potential to increase the extent of immunogenic cell death (ICD) resulting from radiotherapy or chemotherapy and have the potential to amplify DNA damage and innate immune activation. Another opportunity for rational multimodal treatment design is through PARP inhibitor-epigenetic interactions and targeted therapy-induced immune remodelling [46].

7.5 Clinical Evidence, Toxicity, and Translational Barriers

Today, there are many clinical trials underway that involve the use of epi-immunotherapy, and the majority of the data are from Phase 1 and 2 trials. Numerous regimens have not posed new safety issues, but dose and scheduling issues are important. Some of them are immune-related adverse events and off-target epigenetic effects, and some are causes for discrepancy between preclinical and clinical results. The early trial discontinuation of an epigenetic reprogramming trial using a combination of decitabine and carboplatin due to excessive toxicity (60% grade 3/4 neutropenia) and diminished efficacy is a testament to the complexity of balancing epigenetic reprogramming with acceptable pharmacokinetics. A key dilemma is the limited selectivity of epigenetic inhibitors widely

distributed and intercellularly heterogeneous. Additionally, clinical translation is hindered by the translation difficulties between mouse models and human diseases [47].

8. Emerging Frontiers in Precision Epigenetic Immunotherapy

8.1 Single-Cell and Spatial Epigenomics

Single-cell and spatial epigenomics are reshaping epigenetic views of the tumour's epigenetic heterogeneity. Combining scRNA-seq with scATAC-seq uncovers links between chromatin accessibility and immune-cell states and functions. In colorectal tissue, single-cell ATAC-seq shows increasing chromatin accessibility at T-cell exhaustion-associated regions. Epigenetic subpopulations in tumours can be identified by single-cell DNA methylome profiling. Spatial transcriptomics and spatial epigenomics characterise epigenetic variations in the context of the tissue, uncovering the cellular niche of therapy resistance and spatial distribution of immune-excluded cells. Single-cell technologies like scATAC-seq and spatial CUT&Tag can be used to profile the epigenetic landscape at single-cell resolution, which helps understand the role of chromatin accessibility, histone modifications, and RNA interactions in immune cell trafficking, exhaustion, and plasticity [48].

8.2 Multi-Omic Biomarkers for Patient Stratification

Predictive biomarkers are needed for epi-immunotherapy. An integration of epigenomic signatures, transcriptomic profiles, and proteomic data can provide identification of patients likely to benefit. Tumour mutational burden and epigenetic phenotype may predict response to epigenetic-ICI combinations. Immune-cell infiltration signatures, such as T-cell exclusion or inflammation patterns, can provide input for patient selection. In clear cell renal cell carcinoma (ccRCC), FBLN2 promoter hypermethylation was found to be a negative prognosis biomarker and an anti-PD-1 response predictor. Although MSI-H endometrial cancer and a high tumour mutational burden correlate with better responses to ICIs, epigenetic biomarkers are under-investigated, and their use for clinical decision-making is still in its infancy

[44]. Emerging epigenetic biomarkers and their clinical applications are summarised in Table 2.

Table 2: Emerging Epigenetic Biomarkers for Immunotherapy Response [1], [49], [50], [51], [52]

Biomarker Type	Example	Cancer Type	Clinical Application
DNA methylation signature	FBLN2 promoter hypermethylation	Clear cell renal cell carcinoma	Negative prognostic marker, anti-PD-1 response predictor
CpG island methylation profile	Genome-wide methylation patterns	Multiple	Predicts immune infiltration and immunotherapy efficacy
Blood-based DNA methylation	Peripheral blood methylation profile	Multiple	Minimally invasive predictor of ICI response
Chromatin accessibility	scATAC-seq profiles	Colorectal, others	Identifies T-cell exhaustion states and immune exclusion
Integrated multi-omic signatures	cfDNA methylation + EV-miRNAs	NSCLC	Identifies immunotherapy-outcome molecular subtypes

8.3 Temporal Epigenomics and Evolution of Treatment Resistance

To understand the temporal behaviour of epigenetic modifications during therapy would be crucial to overcome resistance. Pre-therapy epigenetic status can be a predictor of initial response to, or resistance to, therapy. During immunotherapy, remodelling occurs and causes secondary resistance. Epigenetic biomarkers might be used for longitudinal monitoring to allow adaptive modification of treatment. A differential gene expression analysis of pembrolizumab and azacitidine combination therapy in ovarian cancer (OC) identified increased gene expression of both inflammatory and cytolytic genes and co-inhibitory checkpoints (ICs) after 6 weeks of pembrolizumab treatment, showing dynamic epigenetic remodelling in response to pembrolizumab [53].

8.4 Artificial Intelligence and Epigenetic Precision Oncology

Epigenetic precision oncology is sped up by artificial intelligence and machine learning. AI can foresee epigenetic weakness, combine multi-omic information and direct drug mix discovery. Derivation of digital biomarkers for immunotherapy response from the epigenomic information. The genetic treatment modelling can be used to select the treatment for a patient individually on the basis of his/her epigenetic status. Harmonisation of multi-omics datasets at the scale of spatial tissue has started to clear up CAF-TAM organisation at the tissue level,

showing a strong association with spatial topology, especially cell deposition, community architecture and intercellular communication with patient outcome [54].

8.5 Next-Generation Epigenetic Immunotherapy

Better specificity and delivery of epigenetic immunotherapy are the future. Improved targeting with cell-selective epigenetic delivery, nanoparticle-based epidrug delivery and PROTAC-mediated degradation of epigenetic regulators. Targeted epigenome editing is the most specific type of editing. Combination strategies, utilising tumour-specific epigenetic vulnerabilities, will increase the therapeutic efficacy and reduce the off-target effects. Next-generation epidrugs are expected to be more specific; e.g., selective HDAC6 inhibitors or EZH2 inhibitors will reduce the off-target effects and increase the therapeutic indices. Current limitations with epidrug delivery could be overcome by innovative drug delivery solutions that achieve increased tumour penetration with lower systemic toxicity, for example, via nanoparticle-based carriers [55].

9. Conclusion and Future Perspectives

Epigenetic modification has become a major factor influencing anti-tumour immunity, with impact both on the immunogenicity of tumour cells and on the functional status of immune cells in the tumour microenvironment. While genetically determined, the epigenetic modifications are reversible, creating a specific

possibility for therapeutic intervention: pharmacological or genetic manipulation of the epigenome can lead to the restoration of anti-tumour immunity and the reversal of resistance to immunotherapy. Epigenetic interventions are thus no longer adjunct therapy in cancer immunotherapy but are now being looked into as a core treatment approach due to the dual function of epigenetic machinery as an immune evasion mechanism and therapeutic target. Epigenetic regulation involves interconnected layers that coordinately control the epigenetic landscape of tumours and immune cells, including DNA methylation, histone modifications, chromatin remodelling, non-coding RNAs, and epitranscriptomic modifications that control immune recognition, T-cell functions, and the tumour immunosuppressive context. Epigenetic therapies like DNMTis, HDACis, EZH2 inhibitors, BET inhibitors and LSD1 inhibitors have shown promise as immune sensitisers, while in various stages of clinical development are combinations of the above with ICIs, cellular therapies, vaccines and other modalities. But there are plenty of issues to resolve. Heterogeneous results have been obtained so far, and some combinations of epi-immunotherapy have been showing modest activity while others appear to have not added any beneficial activity, thus limiting its clinical translation. Potential factors contributing to the discrepancy between preclinical and clinical outcomes include mechanisms by which epigenetic regulation is complicated, the limited selectivity of current epigenetic agents, the mixed nature of the tumour and immune cell population, the use of mouse models different from the human disease, suboptimal dosing and delivery, and the absence of robust biomarkers to predict response to epigenetic drugs. The metabolic pathways and epigenetic profiles of various tumour types are different, which creates complex compensatory mechanisms that could help explain therapeutic resistance.

There is a way out with emerging technologies. Identification of epigenetic heterogeneity and therapy-resistant niches in single-cell and spatial epigenomics. The multi-omic biomarkers can be used to stratify patients. AI speeds up drug discovery and forecasting drug response. With

precision epigenetics editing, locus-specific manipulation can be achieved with better specificity. Combining high-throughput sequencing, spatial transcriptomics and multi-omics provides unprecedented opportunities to map epigenetic landscapes and how these regulate the immune component, identify new biomarkers, and discover new therapeutic targets. The ultimate goal is to design rational and biomarker-based epi-immunotherapy strategies that prioritise therapeutic benefit and limit toxicity. To achieve this requires ongoing research into the molecular mechanisms that link epigenetics and immunity, development of more selective epigenetic modulators, finding predictive epigenetic biomarkers, and incorporation of state-of-the-art technologies to accurately map and manipulate epigenetic landscapes. As we learn more about the role of the epigenetic modification dimension of anti-tumour immunity, the more we will be able to leverage this to the benefit of our patients with cancer.

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REFERENCES

- E. Dai, Z. Zhu, S. Wahed, Z. Qu, W. J. Storkus, and Z. S. Guo, "Epigenetic modulation of antitumor immunity for improved cancer immunotherapy," *Molecular Cancer*, vol. 20, no. 1, Dec. 2021, doi: 10.1186/s12943-021-01464-x.
- X. Li, C. Shao, Y. Shi, and W. Han, "Lessons learned from the blockade of immune checkpoints in cancer immunotherapy," *Journal of Hematology & Oncology*, vol. 11, no. 1, pp. 31–31, Feb. 2018, doi: 10.1186/s13045-018-0578-4.

- H. González, C. Hagerling, and Z. Werb, "Roles of the immune system in cancer: from tumor initiation to metastatic progression," *Genes & Development*, vol. 32, pp. 1267–1284, Oct. 2018, doi: 10.1101/gad.314617.118. [4]
- P. Bonaventura *et al.*, "Cold Tumors: A Therapeutic Challenge for Immunotherapy," *Frontiers in Immunology*, vol. 10, pp. 168–168, Feb. 2019, doi: 10.3389/fimmu.2019.00168.
- J. D. Licht and R. L. Bennett, "Leveraging epigenetics to enhance the efficacy of immunotherapy," *Clinical Epigenetics*, vol. 13, no. 1, pp. 115–115, May 2021, doi: 10.1186/s13148-021-01100-x.
- M. Kim and J. Costello, "DNA methylation: an epigenetic mark of cellular memory," *Experimental & Molecular Medicine*, vol. 49, no. 4, Apr. 2017, doi: 10.1038/emmm.2017.10.
- R. R. Walker, Z. Rentia, and K. B. Chiappinelli, "Epigenetically programmed resistance to chemo- and immuno-therapies," *Advances in cancer research*, vol. 158, pp. 41–71, Jan. 2023, doi: 10.1016/bs.acr.2022.12.001.
- A. Jambhekar, A. Dhall, and Y. Shi, "Roles and regulation of histone methylation in animal development," *Nature Reviews Molecular Cell Biology*, vol. 20, no. 10, pp. 625–641, Jul. 2019, doi: 10.1038/s41580-019-0151-1.
- T cells," *Scientific Reports*, vol. 15, no. 1, pp. 17359–17359, May 2025, doi: 10.1038/s41598-025-99804-0.
- A. I. Osman *et al.*, "Microplastic sources, formation, toxicity and remediation: a review," *Environmental Chemistry Letters*, vol. 21, no. 4, pp. 2129–2169, Apr. 2023, doi: 10.1007/s10311-023-01593-3.
- M. T. D. Martino *et al.*, "miRNAs and lncRNAs as Novel Therapeutic Targets to Improve Cancer Immunotherapy," *Cancers*, vol. 13, no. 7, pp. 1587–1587, Mar. 2021, doi: 10.3390/cancers13071587.
- L. Liu *et al.*, "Noncoding RNAs: the shot callers in tumor immune escape," *Signal Transduction and Targeted Therapy*, vol. 5, no. 1, pp. 102–102, Jun. 2020, doi: 10.1038/s41392-020-0194-y.
- X. Zhao and S. Guan, "Epitranscriptomic control of cancer immunity and therapy resistance," *Frontiers in Immunology*, vol. 16, pp. 1706557–1706557, Nov. 2025, doi: 10.3389/fimmu.2025.1706557.
- Y. Kong *et al.*, "Transposable element expression in tumors is associated with immune infiltration and increased antigenicity," *Nature Communications*, vol. 10, no. 1, pp. 5228–5228, Nov. 2019, doi: 10.1038/s41467-019-13035-2.
- S. Gomez, T. Tabernacki, J. Kobyra, P. Roberts, and K. B. Chiappinelli, "Combining epigenetic and immune therapy to overcome cancer resistance," *Seminars in Cancer Biology*, vol. 65, pp. 99–113, Dec. 2019, doi: 10.1016/j.semcancer.2019.12.019.
- L. L. Niborski *et al.*, "CD8+T cell responsiveness to anti-PD-1 is epigenetically regulated by Suv39h1 in melanomas," *Nature Communications*, vol. 13, no. 1, pp. 3739–3739, Jun. 2022, doi: 10.1038/s41467-022-31504-z.
- L. Pang *et al.*, "Epigenetic regulation of tumor immunity," *Journal of Clinical Investigation*, vol. 134, no. 12, Jun. 2024, doi: 10.1172/jci178540.
- S. Deng, Q. Hu, H. Zhang, F. Yang, C. Peng, and C. Huang, "HDAC3 Inhibition Upregulates PD-L1 Expression in B-Cell Lymphomas and Augments the Efficacy of Anti-PD-L1 Therapy," *Molecular Cancer Therapeutics*, vol. 18, no. 5, pp. 900–908, Mar. 2019, doi: 10.1158/1535-7163.mct-18-1068.
- I. Cañadas *et al.*, "Tumor innate immunity primed by specific interferon-stimulated endogenous retroviruses," *Nature Medicine*, vol. 24, no. 8, pp. 1143–1150, Jul. 2018, doi: 10.1038/s41591-018-0116-5.

- C. A. Ishak and D. D. D. Carvalho, "Reactivation of Endogenous Retroelements in Cancer Development and Therapy," *Annual Review of Cancer Biology*, vol. 4, no. 1, pp. 159–176, Nov. 2019, doi: 10.1146/annurev-cancerbio-030419-033525.
- A. Baessler and D. A. A. Vignali, "T Cell Exhaustion," *Annual Review of Immunology*, vol. 42, no. 1, pp. 179–206, Jan. 2024, doi: 10.1146/annurev-immunol-090222-110914.
- A. Christofides, T. Karantanos, K. Bardhan, and V. A. Boussiotis, "Epigenetic regulation of cancer biology and anti-tumor immunity by EZH2," *Oncotarget*, vol. 7, no. 51, pp. 85624–85640, Oct. 2016, doi: 10.18632/oncotarget.12928.
- A. Ben-Shmuel, G. Biber, and M. Barda-Saad, "Unleashing Natural Killer Cells in the Tumor Microenvironment-The Next Generation of Immunotherapy?," *Frontiers in Immunology*, vol. 11, pp. 275–275, Feb. 2020, doi: 10.3389/fimmu.2020.00275.
- S. Hsu, M. Jadhao, W. Liao, W. Chang, C.-T. Hung, and C. Chiu, "Culprits of PDAC resistance to gemcitabine and immune checkpoint inhibitor: Tumour microenvironment components," *Frontiers in Molecular Biosciences*, vol. 9, pp. 1020888–1020888, Oct. 2022, doi: 10.3389/fmolb.2022.1020888.
- Y. Fu *et al.*, "Unbiasedly decoding the tumor microenvironment with single-cell multiomics analysis in pancreatic cancer," *Molecular Cancer*, vol. 23, no. 1, pp. 140–140, Jul. 2024, doi: 10.1186/s12943-024-02050-7.
- J. Zhang *et al.*, "Multi-omics analysis reveals the chemoresistance mechanism of proliferating tissue-resident macrophages in PDAC via metabolic adaptation," *Cell Reports*, vol. 42, no. 6, pp. 112620–112620, Jun. 2023, doi: 10.1016/j.celrep.2023.112620.
- G. Godoy-Tena and E. Ballestar, "Epigenetics of Dendritic Cells in Tumor Immunology," *Cancers*, vol. 14, no. 5, pp. 1179–1179, Feb. 2022, doi: 10.3390/cancers14051179.
- J. Dunn and S. Rao, "Epigenetics and immunotherapy: The current state of play," *Molecular Immunology*, vol. 87, pp. 227–239, May 2017, doi: 10.1016/j.molimm.2017.04.012.
- Z. Xie, Z. Zhou, S. Yang, S. Zhang, and B. Shao, "Epigenetic regulation and therapeutic targets in the tumor microenvironment," *Molecular Biomedicine*, vol. 4, no. 1, pp. 17–17, Jun. 2023, doi: 10.1186/s43556-023-00126-2.
- Z. Li *et al.*, "Critical role of the gut microbiota in immune responses and cancer immunotherapy," *Journal of Hematology & Oncology*, vol. 17, no. 1, pp. 33–33, May 2024, doi: 10.1186/s13045-024-01541-w.
- B. Ren, Y. Fang, M. Gu, L. You, T. Zhang, and Y. Zhao, "Microbiota-metabolism-epigenetics-immunity axis in cancer," *Frontiers in Immunology*, vol. 15, pp. 1449912–1449912, Jul. 2024, doi: 10.3389/fimmu.2024.1449912.
- E. Sahai *et al.*, "A framework for advancing our understanding of cancer-associated fibroblasts," *Nature reviews. Cancer*, vol. 20, no. 3, pp. 174–186, Jan. 2020, doi: 10.1038/s41568-019-0238-1.
- X. Cao *et al.*, "m6A methylation: a process reshaping the tumour immune microenvironment and regulating immune evasion," *Molecular Cancer*, vol. 22, no. 1, pp. 42–42, Mar. 2023, doi: 10.1186/s12943-022-01704-8.
- W. Huang *et al.*, "Dual inhibitors of DNMT and HDAC induce viral mimicry to induce antitumour immunity in breast cancer," *Cell Death Discovery*, vol. 10, no. 1, pp. 143–143, Mar. 2024, doi: 10.1038/s41420-024-01895-7.

- M. L. Stone *et al.*, "Epigenetic therapy activates type I interferon signaling in murine ovarian cancer to reduce immunosuppression and tumor burden," *Proceedings of the National Academy of Sciences*, vol. 114, no. 51, Dec. 2017, doi: 10.1073/pnas.1712514114.
- J. Nie *et al.*, "Addition of Low-Dose Decitabine to Anti-PD-1 Antibody Camrelizumab in Relapsed/Refractory Classical Hodgkin Lymphoma," *Journal of Clinical Oncology*, vol. 37, no. 17, pp. 1479–1489, Apr. 2019, doi: 10.1200/jco.18.02151.
- J. Wang, Z. Li, and H. Yin, "The Future of Checkpoint Inhibitors in Uveal Melanoma: A Narrative Review," *Ophthalmology and Therapy*, vol. 13, no. 5, pp. 1103–1123, Mar. 2024, doi: 10.1007/s40123-024-00913-2.
- L. Ny *et al.*, "The PEMDAC phase 2 study of pembrolizumab and entinostat in patients with metastatic uveal melanoma," *Nature Communications*, vol. 12, no. 1, pp. 5155–5155, Aug. 2021, doi: 10.1038/s41467-021-25332-w.
- C. J. Stairiker, G. D. Thomas, and S. Salek-Ardakani, "EZH2 as a Regulator of CD8⁺ T Cell Fate and Function," *Frontiers in Immunology*, vol. 11, pp. 593203–593203, Oct. 2020, doi: 10.3389/fimmu.2020.593203.
- W. Dai *et al.*, "Epigenetics-targeted drugs: current paradigms and future challenges," *Signal Transduction and Targeted Therapy*, vol. 9, no. 1, pp. 332–332, Nov. 2024, doi: 10.1038/s41392-024-02039-0.
- P. J. Rice-Boucher, X. Cui, and D. T. Curiel, "Adenoviral vectors for in vivo delivery of CRISPR-Cas gene editors," *Journal of Controlled Release*, vol. 327, pp. 788–800, Sep. 2020, doi: 10.1016/j.jconrel.2020.09.003.
- P. Zhang, G. Zhang, and X. Wan, "Challenges and new technologies in adoptive cell therapy," *Journal of Hematology & Oncology*, vol. 16, no. 1, pp. 97–97, Aug. 2023, doi: 10.1186/s13045-023-01492-8.
- immunotherapy: a new avenue to improve anticancer efficacy," *Clinical Epigenetics*, vol. 9, no. 1, pp. 59–59, May 2017, doi: 10.1186/s13148-017-0358-y.
- J. Yang, J. Xu, W. Wang, B. Zhang, X. Yu, and S. Shi, "Epigenetic regulation in the tumor microenvironment: molecular mechanisms and therapeutic targets," *Signal Transduction and Targeted Therapy*, vol. 8, no. 1, pp. 210–210, May 2023, doi: 10.1038/s41392-023-01480-x.
- S. Zhu *et al.*, "Combination strategies to maximize the benefits of cancer immunotherapy," *Journal of Hematology & Oncology*, vol. 14, no. 1, pp. 156–156, Sep. 2021, doi: 10.1186/s13045-021-01164-5.
- D. Bhamidipati, J. Haro-Silerio, T. A. Yap, and N. Y. L. Ngoi, "PARP inhibitors: enhancing efficacy through rational combinations," *British Journal of Cancer*, vol. 129, no. 6, pp. 904–916, Jul. 2023, doi: 10.1038/s41416-023-02326-7.
- L. Zhong *et al.*, "Small molecules in targeted cancer therapy: advances, challenges, and future perspectives," *Signal Transduction and Targeted Therapy*, vol. 6, no. 1, pp. 201–201, May 2021, doi: 10.1038/s41392-021-00572-w.
- P. Guruprasad, Y. G. Lee, K. H. Kim, and M. Ruella, "The current landscape of single-cell transcriptomics for cancer immunotherapy," *The Journal of Experimental Medicine*, vol. 218, no. 1, Dec. 2020, doi: 10.1084/jem.20201574.
- E. HÄ©ninger, T. Krueger, and J. M. Lang, "Augmenting Antitumor Immune Responses with Epigenetic Modifying Agents," *Frontiers in Immunology*, vol. 6, pp. 29–29, Feb. 2015, doi: 10.3389/fimmu.2015.00029.

- Y. Xu *et al.* , “Epigenetic modulations of immune cells: from normal development to tumor progression,” *International Journal of Biological Sciences* , vol. 19, no. 16, pp. 5120–5144, Jan. 2023, doi: 10.7150/ijbs.88327.
- P. Darvin, S. M. Toor, V. S. Nair, and E. Elkord, “Immune checkpoint inhibitors: recent progress and potential biomarkers,” *Experimental & Molecular Medicine* , vol. 50, no. 12, pp. 1–11, Dec. 2018, doi: 10.1038/s12276-018-0191-1.
- C. Pilard, M. Ancion, P. Delvenne, G. Jérusalem, P. Hubert, and M. Herfs, “Cancer immunotherapy: it’s time to better predict patients’ response,” *British Journal of Cancer* , vol. 125, no. 7, pp. 927–938, Jun. 2021, doi: 10.1038/s41416-021-01413-x.
- B. V. Landon *et al.* , “Pembrolizumab and epigenetic modification with azacitidine reshapes the tumor microenvironment of platinum-resistant epithelial ovarian cancer: a phase 2 non-randomized clinical trial,” *Communications Medicine* , vol. 6, no. 1, Feb. 2026, doi: 10.1038/s43856-026-01404-0.
- T. Fu *et al.* , “Spatial architecture of the immune microenvironment orchestrates tumor immunity and therapeutic response,” *Journal of Hematology & Oncology* , vol. 14, no. 1, pp. 98–98, Jun. 2021, doi: 10.1186/s13045-021-01103-4.
- S. Patnaik and S. Anupriya, “Drugs Targeting Epigenetic Modifications and Plausible Therapeutic Strategies Against Colorectal Cancer,” *Frontiers in Pharmacology* , vol. 10, pp. 588–588, Jun. 2019, doi: 10.3389/fphar.2019.00588.

