Open Access Full Text Article

REVIEW

DNA Methyltransferase Inhibitors: Catalysts For Antitumour Immune Responses

This article was published in the following Dove Press journal:
OncoTargets and Therapy

Huimin Dan
Shanshan Zhang
Yongning Zhou
Quanlin Guan

Gansu Province Key Laboratory of
Gastrointestinal Diseases, The First
Hospital of Lanzhou University, Lanzhou
University, Lanzhou, Gansu Province,
People's Republic of China

Abstract: Epigenetics is a kind of heritable change that involves the unaltered DNA sequence and can have effects on gene expression. The regulatory mechanism mainly includes DNA methylation, histone modification and non-coding RNA regulation. DNA methylation is currently the most studied aspect of epigenetics. It is widely present in eukaryotic cells and is the most important epigenetic mark in the regulation of gene expression in the cell. DNA methyltransferase inhibitors (DNMTi) have been increasingly recognized in the field of cancer immunotherapy, have been approved for the treatment of acute myeloid leukaemia (AML) and are widely being used in clinical trials of cancer immunotherapies. DNMTi promote the reactivation of tumour suppressor genes, enhance tumour immunogenicity, and stimulate a variety of immune cells to secrete cytokines that exert cytotoxic effects, promote tumour cell death, including macrophages, natural killer (NK) cells and CD8+ T cells, and upregulate major histocompatibility complex (MHC) class I expression levels. Here, we mainly summarize the epigenetics related to DNMTi and their regulation of the antitumour immune response and DNMTi combined with immuno-therapeutics or histone deacetylase inhibitors to demonstrate the great development potential and clinical application value of DNMTi.

Keywords: DNA methyltransferase inhibitors, histone deacetylase inhibitor, immunomodulation, immune cells, immunotherapy, DNA methylation, epigenetics

Introduction

In recent years, the epigenetic therapies used in cancer have made significant progress, mainly due to the rapid development of genome-wide high-throughput sequencing technology. Investigators can use sequencing technology to detect all changes in gene expression associated with epigenetic modifications, and these technologies are rapidly translating into tools for cancer treatment and prevention.[1] Epigenetic regulation of genes can modulate gene expression, the alterations of which can be used by tumour cells to disrupt immunogenic and immune recognition mechanisms, thereby acquiring an immune escape phenotype.[2–5]

Immune escape is an important factor in the development and evolution of tumours.[6] One of the most effective escape strategies adopted by cancer cells is disruption of the antigen presentation process. Epigenetic silencing affects almost all antigen processing and presentation processes.[7] The important role of epigenetics in tumour immune escape provides a solid theoretical foundation for the use of epigenetic-related drugs to improve the immune targeting of tumour cells.

Some studies have found that tumour epigenetic drugs can improve the antitumour immune response, and DNA methyltransferase inhibitors (DNMTi) can upregulate the

Correspondence: Quanlin Guan
Gansu Province Key Laboratory of
Gastrointestinal Diseases, The First
Hospital of Lanzhou University, Lanzhou
University, Lanzhou, Gansu Province,
People's Republic of China
Tel +8613893473086
Email guanquanlin@163.com

expression level of MHC class I molecules, increase the presentation level of tumour-associated antigens, and ultimately enhance the immunogenicity of tumours by removing DNA hypermethylation modifications in the promoter region of MHC class I molecules.[8,9] DNMTi can induce the expression of tumour-associated antigens and regulate the activity of immune cells to improve the antitumour immune response. In this review, we introduce epigenetic and DNA methyltransferase inhibitors and summarize the effects of DNMTi on regulating antitumour immunity and improving the efficacy of immunotherapy.

Tumour Epigenetics And DNA Methylation

Epigenetics can regulate gene expression by abnormally modifying and controlling the spatial structure of genomic DNA sequence and participates in the process of tumorigenesis and development. These epigenetic abnormalities are reversible because they do not alter the properties of the genomic DNA sequence, thus providing a basis for epigenetic therapy in cancer. DNA methylation usually refers to the addition of a methyl group to the base of a DNA molecule by the action of DNA methyltransferases (DNMTs), most

commonly provided by S-adenosyl methionine (SAM), and the hydrogen at the 5′ position of cytosine is replaced by a methyl group to become 5-methylcytosine.[10,11] DNA methylation, which exists widely in eukaryotic cells, is the most important epigenetic mark for regulating gene expression. DNA methylation plays a key role in gene silencing, X chromosome inactivation, genome stability, and imprinting, and it is a chromatin modification that has been extensively studied.[12–14] (Figure 1 summarizes the components of epigenetic modulation.)

Abnormalities in DNA methylation play an important role in processes such as cancer initiation, progression, invasion, and metastasis.[15–17] The relationship between DNA methylation and cancer was first discovered in 1983: DNA methylation levels in cancer cells have been found to be significantly reduced genome-wide.[18] Detection of genome-wide hypomethylation levels in peripheral blood has been reported in many tumorigenic diseases; for example, in patients with brain tumours, gastric cancer, liver cancer, and breast cancer, the genome-wide DNA in peripheral blood is hypomethylated.[19] The main cause of reduced methylation levels in cancer cells is demethylation of repetitive sequence regions of the
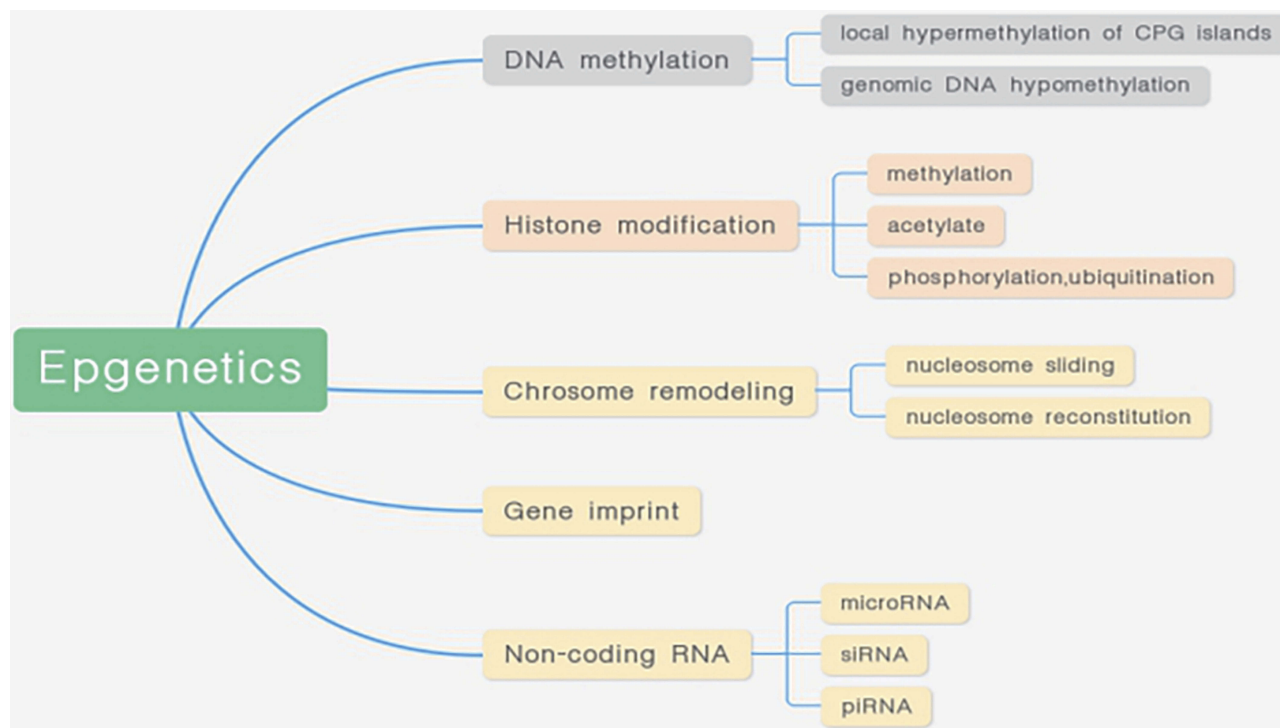
Figure 1 Basic composition of epigenetics.
Notes: Epgenetics includes: DNA methlation, histone modification, chrosome remodeling, gene imprint, non-coding RNA.The changes of DNA methylation in tumors are manifested in the decrease of global methylation level of the genome and the increase of methylation level of CpG islands in the promoter regions of some genes.

genome.[20] However, it has been shown that both low and high levels of DNA methylation coexist in cancer cells. Low levels of DNA methylation are associated with the activation of proto-oncogenes, which leads to genomic instability, while high levels of DNA methylation silence the promoters of tumour suppressor genes, which results in the inactivation of tumour suppressor genes.[21,22]

Studies have shown that the proportion of methylated CpG islands in clear cell renal cell carcinoma (ccRCC) and papillary renal cell carcinoma (pRCC) is as high as 31%[23] and 7%[24] respectively. The WNT pathway is one of the key pathways in cancer. Through this pathway, the expression of its downstream target, β-catenin, is suppressed, and the expression of some proto-oncogenes is inhibited.[25] Therefore, inhibiting the activity of DNMTs and blocking the hypermethylation of DNA in cancer cells can inhibit the growth of tumour cells or kill tumour cells, which may stimulate new ideas for cancer therapy.[26] The study of DNMTi has also become a hot topic in cancer drug development.

DNA Methyltransferase Inhibitors

The two most classic drug classes used in epigenetic therapy are DNMTi and histone deacetylase inhibitors (HDACi).[27] DNMTs are key enzymes that catalyse DNA methylation, mainly DNMT1, DNMT3A, and DNMT3B. These three enzymes catalyse the formation of 5mC from cysteines in DNA CpG islands and ultimately suppress gene expression.[28] DNMTi constitute a class of cytidine analogues that are divided into two classifications; in one class, a nucleotide analogue binds to DNA to form a covalent complex that promotes the degradation of DNMT.[29] In the other class is the non-nucleotide analogue DNMTi, which binds directly to the methylated region of the DNMT.[30] Representative nucleic acid analogues are decitabine (DAC) and azacitidine (AZA). They are currently approved by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for the treatment of acute myeloid leukaemia (AML), chronic myelomonocytic leukaemia (CMML), and myelodysplastic syndromes (MDS).[31–33] Due the severe cytotoxicity induced by these drugs, several research teams have successively found that such drugs can exert their demethylation-related antitumour effects only at low doses, a finding that has pioneered epigenetic therapy.[34] Non-nucleoside analogues such as procainamide, SGI-110 and quinazoline, propiophenone, pyrrolopyridine derivatives, and other similar drugs are still under development.[35]

DNMTi can restore the expression activity and function of tumour suppressor genes by inhibiting the activation of DNA methylation, thereby inhibiting the growth of tumour cells and inducing their apoptosis; thus, DNMTi can be used as potential anticancer drugs in cancer therapy.[36–38] Further studies[39] found that, although DNMTi exhibit great clinical promise in blood-borne tumours, they are less effective as treatments for solid tumours. Compared with first-generation DNMTi, second-generation DNMTi such as SGI110 were confirmed to have greater stability and to induce less toxicity in normal tissues in vivo.[40,41] For example, SGI-1027 is a novel small molecule inhibitor of DNMT[42,43] that does not inhibit DNMT activity by binding to either RNA or DNA but rather achieves demethylation by inducing the degradation of DNMT. Targeting DNA hypermethylation using nucleoside analogues is an effective way to reprogramme the epigenome of cancer cells, thereby inhibiting cancer cell proliferation, promoting cancer cell differentiation, enhancing immune system recognition of cancer cells, and ultimately leading to cancer cell death, providing a new theoretical and experimental basis for the future application of demethylation drugs in the treatment of cancer. (Common DNA methyltransferase inhibitors and their mechanisms of action are summarized in Table 1.)

DNA Methyltransferase Inhibitors Regulate Tumour Immunity

The interaction of anticancer drugs with the host immune system has been implicated in therapeutic response.[44] The major histocompatibility complex (MHC) class I is at the core of antigen presentation, and the expression of MHC class I molecules in tumour cells is often inhibited by irreversible mutations or reversible hypermethylation, resulting in downregulation.[45] DNMTi can upregulate MHC class I levels in a variety of cancer tissues, as has been demonstrated in breast, lung, colon, and thyroid histotypes, as well as in human papilloma virus (HPV)-related cancers, sarcomas, and gliomas,[46–50] and they promote the release of interferon-γ from tumour-specific cytotoxic T lymphocytes (CTLs), which kill target cells.[51] In addition, similar results have been observed in ovarian cancer cells and xenograft melanoma models.[52] In addition to promoting MHC class I expression in tumour cells, DNMTi can also induce the expression of tumour-associated antigens. Experiments have shown that DNMTi can upregulate almost all antigen processing and presentation machinery components in

Table 1 Representative DNA Methyltransferase Inhibitors

Therapeutic Agent	Nth Generation	Drug Name	Mechanism Of Action
Nucleosides	First-generation	Azacitidine	It is involved in the synthesis of RNA or DNA at high concentrations Inhibition of DNMT blocks methylation.
		Decitabine	At high concentrations it can lead to blocked DNA synthesis and cytotoxicity; at low concentrations it leads to changes in gene expression profiles.
	Second-generation	Guanosine decitabine	It enhances stability in the aqueous phase, improves resistance to cytidine deaminase (CDA) degradation, and prolongs half-life
		Zebularine	It forms a covalent complex with DNMT to inhibit DNA methylation
Non-nucleoside	First-generation	Procainamideandprocaine	It binds tightly to CpG island dense regions of DNA,thereby interfering with the binding of DNMT to DNA.
		RG108	Non-covalent binding to the DNMT1 active site to achieve a block to DNA methylation.
		EGCG	It binds non-covalently to the catalytic active site of DNMT to inhibit the methylation catalytic activity of DNMT.
		MG-98	Acts on the mRNA of DNMT1 and radically inhibits the expression and synthesis of DNMT1.
		SGI110	The constitutive methylation level of the CTA promoter in cancer cells treated for treatment induction was significantly reduced
		SGI1027	Induction of Degraded DNMTs for Demethylation.

mouse and human tumour cells, including the expression level and intra-tumoural distribution of the tumour-associated antigens (TAA) and LMP2 and LMP7 proteasome subunits. In addition, DNMTi can also improve the costimulatory properties of tumour cells by upregulating the expression of surface molecules such as CD40, CD80, CD86, and ICAM1, as well as by restoring the sensitivity of tumour cells to the apoptosis triggered by immune cells using the enhanced expression of death-inducing receptors such as FAS.[53–56] After treatment with AZA, non-small cell lung carcinoma (NSCLC) cells showed significantly enhanced expression of antigen presentation-related genes and interferon signalling; additionally, the apoptosis rate and the viral defence protein and immune-related transcription factor expression levels were significantly increased.[57,58]

Cancer-testis antigens (CTAs) constitute a family of antigens that are closely related to tumour development. CTAs are expressed in testis, placenta and tumour tissues and are mainly regulated by DNA methylation levels. DNMTi are able to promote the overexpression of CTAs by tumour cells, thereby assisting host CTL in distinguishing tumour cells from healthy cells while being able to upregulate the levels of multiple oncogenes of various CTAs, including the extremely immunogenic oesophageal squamous epithelial tumour-testis antigen 1B (CTAG1B/NY-ESO1), thereby intensifying the antigen presentation process.[59] Elevation in CTA level can be found in most cancers,[47,48,60] such as mesothelioma,[61] renal, oesophageal, pleural, and liver cancers.[62] In addition, the hypomethylating agent SGI-110 was found to induce hypomethylation and CTA gene expression and enhance the expression of MHC I and intercellular cell adhesion molecule 1 (ICAM-1).[63]

Rouloisan et al found that DNMTi activates the classical IFN signaling pathway in ovarian cancer cell lines,which activates the cytosolic dsRNA sensors TLR3 and MDA5 through an increase in dsRNA, thereby inducing IFNB and JAK/STAT signalling. One RNA that triggers this response is transcribed from hypermethylated endogenous retroviruses (ERVs).[64] The involvement is similar for dsRNA and MDA5 sensors in colon cancer cells, and the canonical IFN response is critical for the suppression of colon cancer stem cells by DNMTi.[65] Decitabine can activate the NOTCH1 signalling pathway, which in turn inhibits cancer cell proliferation and affects the immune system in patients

with muscle-invasive bladder cancer.[66] DAC and AZA not only kill targeted cells directly through their cytotoxic effects but also affect antigen presentation in blood cells: on the one hand, DAC promotes the expression of MHC class I and II molecules for the treatment of chronic lymphocytic leukaemia (CLL);[67] on the other hand, AZA is used for the treatment of Hodgkin lymphoma (HL), which can generate more abundant antitumour T cells than are generated in patients treated with HDACi, indicating that AZA can effectively activate the antigen presentation process.[68]

DNA Methyltransferase Inhibitors Are Regulators Of Immune Cells

Maturation and activation of immune cells are regulated at the epigenetic level. From the onset of lineage formation, immune cells are regulated by DNA methylation.[69] (The regulation of immune cells by DNMTi is summarized in Figure 2.) For example, epigenetic changes are closely associated with lymphocytes, macrophage polarization, myeloid-derived suppressor cell function, and regulatory T cell (Treg cell) development and function.[70–73] The effects of DNMTi on multiple immune cell functions are addressed below.

CD8+ And CD4+ T Cells

The generation of memory T cells against cancer-specific neoantigens is a key factor in achieving sustainable responses to immunotherapy. Memory T cells are usually multipotent T cells that maintain long-term plasticity and survival. In contrast, effector T cells have limited survival times; they heavily depend on the presence of antigen but are prone to exhaustion after prolonged exposure to antigen. The lineage of effector
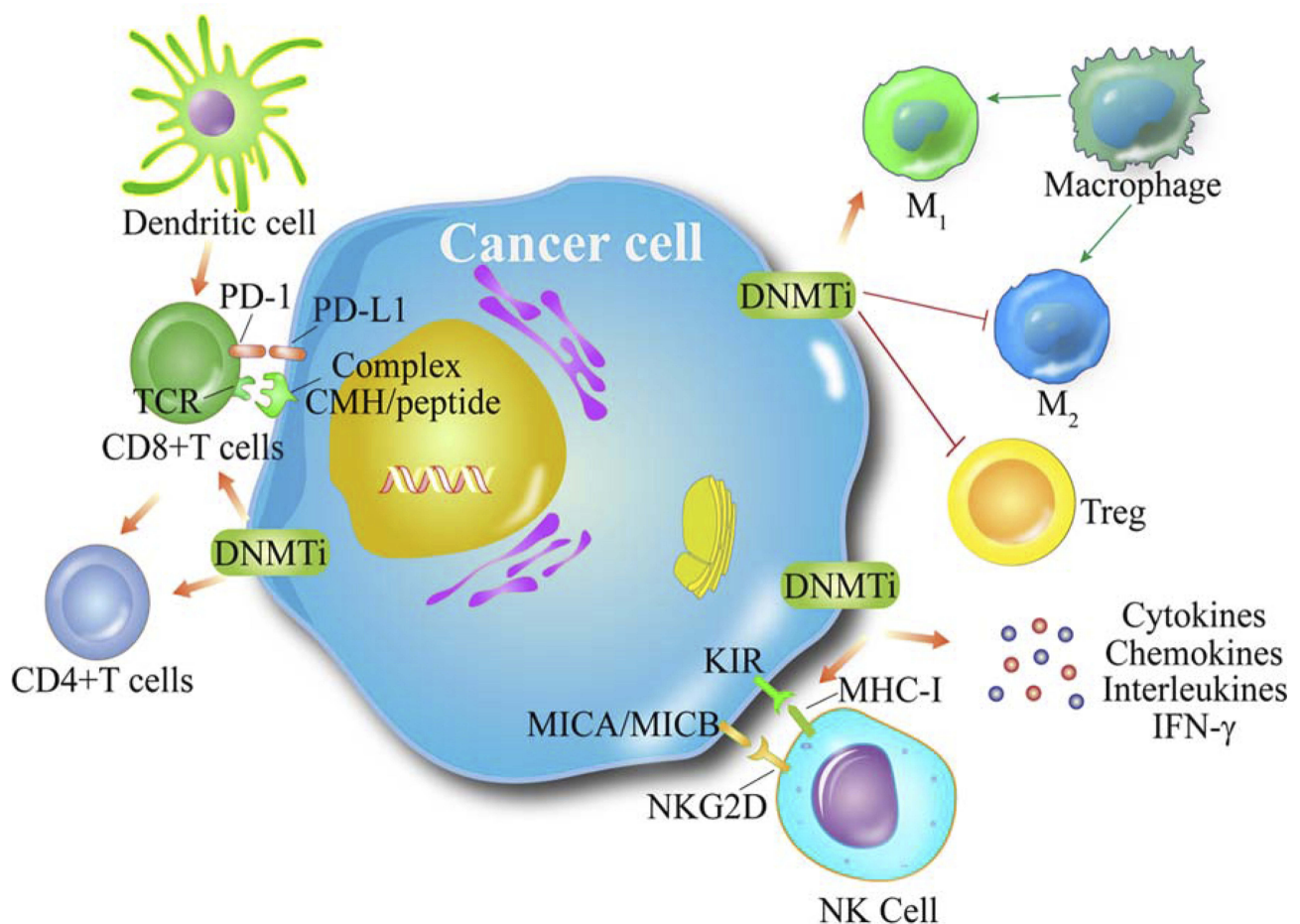
Figure 2 The regulation of DNMTi on immune cells.
Notes: While enhancing the cytotoxicity of CD8 + T cells, DNMTi can assist CD4 + T cells by inducing the expression of key immunostimulatory cytokines. DNMTi inhibits the expression of Treg cells; it can inhibit M1 and promote M2 to regulate macrophages. Promotes KIR expression on NK cell surface, binds to MHC class I molecules to recognize abnormal cells, and increases NKG2D-dependent NK cell-mediated killing of these cells in vitro.

or memory T cells is tightly regulated by histone modification-promoting memory gene silencing or DNA methylation.[74,75] The role of DNA methylation in T cell status is as follows: in terms of their developmental trajectory, CD8+ T cells can be broadly classified as "naive" prior to exposure to antigen and, following exposure to antigen, as "effectors" that mount a response against cells bearing the cognate antigen.[76] Sustained expression of some checkpoint inhibitors, such as PD-1 and TIM3, and exhaustion markers, which deplete T cells, is associated with specific epigenetic profiles.[77,78]

Interestingly, pretreatment with DNMTi rejuvenates tumour-infiltrating CD8+ T cells and reverses drug resistance in an ICB-resistant model.[79] DNMTi are capable of promoting CTL action by mediating the transcription of antitumour cytokines. In non-proliferating T lymphocytes, interleukin 2 (IL2) transcription has been closely linked to the enhanced effects of demethylation at the promoter, specifically in the enhancer region of IL2.[80] When CD8+ T cells are exposed to antigen, the IL2 locus is significantly demethylated, which results in the expression of large amounts of IL2.[81] In immature CD8+ T cells, there are three highly methylated CpG islands in the upstream regulatory sequence of IFN-γ; in effector T lymphocytes, these three CpG islands are demethylated, enabling T cells to produce large amounts of IFN-γ. In addition, in memory T cells, these sites are partially methylated and rapidly demethylated upon certain types of stimulation. These phenomena suggest that DNMTi enhance the function of CTLs by mediating demethylation to enhance and maintain the expression levels of IL2, IFN-γ, and other antitumour cytokines during tumour immunity. DNA methyltransferase 1 (DNMT1)-mediated DNA methylation inhibits tumour production of the T helper 1 (TH1)-type chemokines CXCL9 and CXCL10, which in turn affect the transport of effector T cells to the tumour microenvironment.[82] These processes further define the pathway by which epigenetic therapy may remodel the TME to induce an antitumour state.

Epigenetic therapy with HDACi and DNMTi has been demonstrated to regulate the expression of the chemokine CCL5 by reducing Myc levels,[83] possibly through the demethylation of gene bodies,[84] and a key requirement for an immune response driven by CD8+ T cells is antigen presentation via MHC class I. Without this step, binding between the TCR and the cognate antigen on the CD8+ T cells cannot occur. Differences in chromatin accessibility also distinguish dysfunctional T cells from functional memory T cells, suggesting that epigenetic programmes

also mediate cellular exhaustion.[77] CD4+ T cells comprise a diverse family of helper T cells with opposing activities against tumour cells: Th1 CD4+ T cells have antitumour properties, whereas Th2 CD4+ T cells have pro-tumorigenic function.[85] The functional relevance of the epigenetic pathways involved in the differentiation and maturation of cell subsets remains unclear, and it has been found experimentally that CD4+ T cells isolated from 68 patients with MDS were able to secrete large amounts of IL17 after AZA treatment.[86] Furthermore, the number of IL17A-secreting CD4+ T cells in the peripheral blood of AML and MDS patients was significantly increased after AZA treatment.[87] These results suggest that DNMTi can enhance the cytotoxic effect of CD8+ T cells and help CD4+ T cells by inducing the expression of key immune-stimulatory cytokines.

Regulatory T Cells

Regulatory T cells (Treg cells) can be divided into natural regulatory T cells (nTerg cells) and inducible regulatory T cells (iTreg cells), according to different sources, and they can be divided into resting Tregs (rTreg cells), activated Tregs (aTreg cells) and cytokine-secreting Treg cells, according to different functions.[88–90] As regulatory T cells in cancer immunosuppression-implants for anticancer therapy, Treg cells are characterized by the expression of the FOXP3 transcription factor, which plays an essential role in immune suppression.[91,92] Epigenetic modification, as an important way to regulate FoxP3 gene expression, plays an important role in its stable expression, including through DNA methylation and histone phthalation.[93] It was found that the number of Tregs was significantly reduced in the AZA-treated group compared with the control group after AZA treatment using peripheral blood samples from 68 MDS patients containing regulatory T cells (Treg), indicating that DNMTi can inhibit the expression of Treg cells.[94]

Macrophages

As heterogeneous innate immune cells, macrophages have important theoretical research and clinical application prospects. M1 macrophages display antitumour phagocytic properties, whereas M2 macrophages have pro-tumorigenic properties.[95] These dual roles have also been described in tumours.[96] Experimentally, it has been found that DNMT3b is aberrantly expressed in obese mice, causing an increase in DNA methylation in the promoter region of peroxisome proliferator-activated receptor

gamma 1 (PPARγ1, a nuclear receptor and a key transcription factor involved in M2 polarization whose promoter region is rich in CpG and prone to epigenetic regulation), which suppresses its expression, leading to restricted macrophage polarization to M2 and chronic inflammation in adipose tissue; knocking down DNMT3b shows the opposite trend.[97] It has also been shown that DNMT1 causes an increase in the methylation level of the suppressor of the cytokine signalling 1 (SOCS1) promoter, enabling its continual suppression. After DNMT1 silencing using the DNMT inhibitor 5-azadC, the degree of methylation in the promoter region of the SOCS1 gene was reduced, thereby blocking the LPS-induced activation of the JAK2/START3 pathway in macrophages and reducing the pro-inflammatory phenotype.[98] Thus, the use of DNMTi targeting DNMT can be used to control the pro-inflammatory M1 phenotype and promote an anti-inflammatory M2 response. In summary, the effects of reducing M1 and promoting M2 can be achieved by applying HDACi and DNMTi alone or in combination. This finding lays the foundation for the future discovery and application of new epigenetic modifying drugs. These examples support epigenetic strategies that may allow modulation of the state of macrophages and thus antitumour immunity.

Myeloid-Derived Suppressor Cells (MDSCs) And Dendritic Cells (DCs)

The important function of various myeloid cells, including MDSCs and DCs, is antigen presentation, and their antitumour potential is largely determined by their ability to activate T cells. Maturation of DCs is also controlled by chromatin regulators, such as special AT-rich binding protein 1 (SATB1), which regulates MHC class II expression and modulates its antitumour potential.[99] Epigenetic modifiers, such as HDACi and DNMTi, have been shown to directly increase MHC II and costimulatory molecule (CD40 and CD86) expression in peripheral MDSCs from breast and lung cancer patients.[100–102] The percentage of MDSCs in the tumour microenvironment and spleens of mice bearing TRAMP-C2 prostate cancer cells or TC1/A9 primary lung epithelial tumour cells was significantly reduced after the mice were subcutaneously injected with AZA, and the number of cyclophosphamide-induced MDSCs in the mice accumulated with increasing doses of AZA. The percentage of CD11b+/Gr1+ MDSCs was significantly reduced and accompanied by an increase in the percentage of CD11c+ and CD86+/CD8+ DCs after AZA treatment in vitro cultured tumor-infiltrated CD11b myeloid cells. indicating that DNMTi could partially induce MDSCs to differentiate into dendritic cells (DCs);[100] thus, DNMTi can inhibit the negative regulatory cells of tumour immunity.

NK Cells

Killer cell immunoglobulin-like receptors (KIR) on the surface of NK cells recognize abnormal cells by binding to MHC class I molecules. In the tumour microenvironment, modification of the DNA regulatory sequences of KIR by hypermethylation is a common tumour escape mechanism. Therefore, promoting the expression of KIR using DNMTi would be an effective approach for cancer immunotherapy.[103] Some studies[104] have shown that the development and function of immune cells are regulated by DNA methylation. Different concentrations of the DNMT inhibitor decitabine on NK cells affect cell viability, proliferation, cytotoxicity and activation performance. Demethylation agents can be used to treat acute myeloid leukaemia (AML) by modulating NK cell activity. NK cells directly kill tumour cells, and in the presence of IFNγ, NK cells are usually activated and are relatively more cytotoxic.[105] Although DNA methylation is an epigenetic mechanism regulating KIR expression in NK cells, the effects of hypomethylating agents on NK cell function have not been well characterized. Decitabine has been shown to increase cell surface expression of recombinant UL16 binding protein (ULBP)[106] and MHC class I-related molecule B (MICB)[107] in AML cells, increasing natural-killer group 2 member D (NKG2D)-dependent sensitivity of these cells to NK-mediated killing in vitro. When applied to NK cells under non-proliferative conditions, 5-azacytidine increases KIR expression, which results in reduced NK cytolytic activity,[108,109] whereas decitabine was shown to improve the responsiveness of human NK cells in vitro. However, it has been found that low-dose decitabine in tumour-bearing mice reduced the antitumour response of NK cells.[110] How DNMTi affect NK cell activity requires further basic experimental studies in the future.

DNMTi: Contributor To Cancer Immunotherapy

Epigenetic therapies show advantages when used in concert with novel immunotherapies. In a phase I dose-escalation experiment of 5-AZA-CdR in 12 patients with recurrent epithelial ovarian cancer, Odunsi et al observed

increased T cell responses in most patients.[111] The mRNA expression of testicular cancer antigens involved in NK and T cell signalling and recruitment, immune checkpoint blocking molecules, immunostimulatory cytokines, and genes involved in the interferon pathway was higher after treatment with guadecitabine(SGI-110) and DAC compared with the immunomodulatory effects of AZA treatment.[112] In addition, this combination reduced the number of MDSCs, indicating that the immunomodulatory effects of DNMTi may be useful for immunotherapy.[113] In melanoma, 5-azacytidine can induce specific double-stranded RNA production for host viral defence mechanisms, upregulate the transcription of interferon-β and elevate malignant cell sensitivity to CTLA-4 inhibitors.[114] However, in the melanoma B16 mouse model, low-dose 5-azacytidine with anti-CTLA4 showed the same effect in

controlling tumour growth in vitro and in vivo.[64] 5-AZA-CdR has been reported to regulate the expression of CTA and class I human leukocyte antigen (HLA), thereby improving tumour cell immunogenicity.[115] 5-AZA has been found to upregulate PD-L1 in EOC and NSCLC cell lines and can activate cytosolic dsRNA sensing in colorectal cancer, ultimately activating the viral/IFN response,[65,116] which demonstrates that DNMTi can induce cancer cells to behave as virus-infected cells and trigger dsRNA sensing. Importantly, improved viral defence pathway signalling levels correlate with improved immune checkpoint inhibitor treatment response and long-term survival of cancer patients.[117] SGI-110 has been found to reactivate ERVs to stimulate cancer cell immune response pathways, which provides the rationale for combinatorial therapy with immune checkpoint therapies.[118]
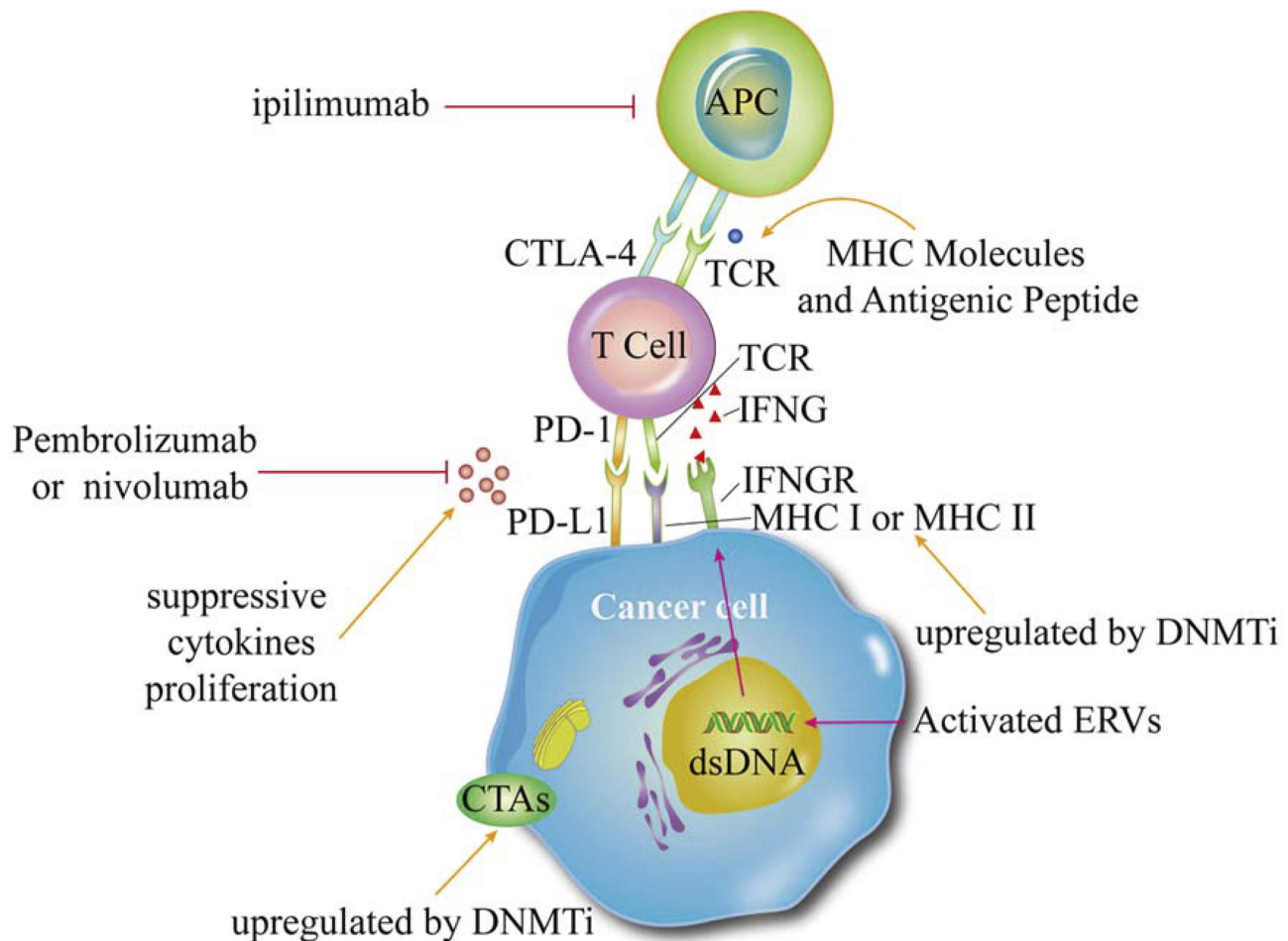
Figure 3 Advantages of Combining DNMTi with immune checkpoint inhibitors.
Notes: T cell stimulation is driven by antigen and requires the coordinated engagement of several other receptors and molecules expressed on the T cell surface as well as antigen-presenting cells (APCs) or tumor cells. DNMTi can inhibit different signaling pathways involved in adaptive immune responses and enhance antitumor effects by combining with immune checkpoint inhibitors.

(The advantages of combining DNMTi with immunotherapy are summarized in Figure 3).

Notably, DNMTi-resistant patients were found to have elevated levels of PD-L1, PD-L2, and CTLA-4. The combination of DNMTi and PD-1/PD-L1 inhibition may solve the problem of resistance to AZA or DAC. Regarding the addition of immune checkpoint therapy to a small number of patients with advanced NSCLC who progressed after low-dose DNMTi therapy, approximately 20% of patients responded to immune checkpoint therapy, did not progress at 24 weeks, and, in general, achieved a standard response,[113] which was a surprising result. In a single-centre trial of azacitidine in combination with nivolumab in relapsed/refractory acute myeloid leukaemia (AML), the overall response rate (ORR) to treatment was 33%, including 15 (22%) complete responses, 1 partial response, 7 haematologic improvements maintained for > 6 months, and 6 patients (9%) with stable disease for > 6 months, and the response rate and OS results of the azacitidine and nivolumab regimens were also encouraging.[119] In addition, a randomized clinical trial compared pembrolizumab plus azacitidine with pembrolizumab plus placebo in patients with advanced non-small cell lung cancer, but no significant difference in PFS was observed.[120] When idarubicin, cytarabine and nivolumab are used to treat newly diagnosed AML or high-risk MDS, the median relapse-free survival time of the responders was 18.54 months, and the median overall survival time was 18.54 months. The rationality of the combined medication has been shown, and the study is still in progress (NCT02464657). We expect positive clinical results.[121] At present, clinical experimental data from the use of DNMTi combined with immunotherapy are limited, and such studies are still in progress. The advantages of combination therapy have been initially shown, but there are still some uncertainties. This makes us wonder whether the sample size is too small to show experimental deviation. What is the best time to use combination drugs? Are there any other potential therapeutic molecular biomarkers? DNMTi combined with immunotherapy still presents many challenges, and a large number of preclinical or clinical experiments are required.

DNMTi Combination HDACi Therapy

Histone deacetylase inhibitors (HDACi) induce cell cycle arrest, differentiation and cell death in cancer cells, reduce angiogenesis, and modulate the immune response.[122] The activity of HDACs can affect the expression of MHC (major histocompatibility complex) and co-stimulatory molecules.[123,124] Histone acetylation may play an important role in regulating T cell development, differentiation, and cell function[125] and, in combination with DNMTi, can also increase the response of antitumour CD8+ T cells.[126] Class I/IIa HDACi combination enhances class I MHC cell surface expression and the expression of co-stimulatory molecules CD40 and CD86 in tumour cells.[127,128] In addition, we found that class II HDACi enhance Treg cell number and function, and class I HDAC inhibitors enhance the function of NK cells and CD8 T cells.[129] However, the molecular mechanisms by which HDACi regulate genes involved in immune recognition are not fully understood. Upregulation of MAGE-A gene in cancer cells by 5-AZA-CdR/TSA combination has been reported.[130] While the use of a combination of decitabine/HDAC inhibitors can induce an increase in CTA and PD-L1 expression, the results suggest that the CTA expression and the epigenetic regulation of PD-L1 may be correlated. In the future, anti-PD-1/PD-L1 combination therapy with decitabine and HDACi will be considered to overcome the possible induction of PD-L1 expression. DAC and HDACi (panobinostat or valproic acid) downregulate the expression of epigenetic modifiers (e.g., KDM2B and SUV39H1) when used in combination to treat acute myeloid leukaemia cells.[131] These findings are beneficial for understanding the mechanism of action in combined epigenetic drug therapy.

In a randomized clinical study in which 184 patients with HR-MDS or CMML were randomly assigned to AZA ± vorinostat or AZA monotherapy with a median follow-up of 23 months, the ORR was 38% in patients treated with AZA monotherapy compared with 27% in the AZA plus vorinostat arm,[132] showing no advantage. Panobinostat in combination with AZA was used for previously untreated AML or high-risk MDS, and 27.5% of patients treated with PAN + AZA were in CR, compared with 14.3% of patients receiving AZA, but there was no significant difference in the 1-year OS rate.[133] AZA plus pracinostat improved OS compared with AZA monotherapy. In contrast, AZA monotherapy in HR-MDS patients showed no improvement in overall patient survival after treatment with VS AZA plus pracinostat.[134] It remains unclear whether the combination of HDACi and HMA is beneficial in patients with MDS and AML. In addition,

studies have demonstrated that the addition of vorinostat does not improve the efficacy of azacitidine in the treatment of acute myeloid leukaemia; when 217 adults with AML were randomly selected to receive AZA monotherapy or AZA plus vorinostat (VOR), there was no improvement in overall response rate or overall survival.[135] There is still some uncertainty regarding HDACi combined with DNMTi, which we intend to further explore in the future.

Summary

Research on DNMT inhibitors has become a hot topic in the field of anticancer drug research. Recently, some DNMT inhibitors were in the preclinical and clinical research evaluation stage, and their inherent cellular toxic side effects limited the clinical application of demethylating drugs. Big data show that DNMTi can effectively stimulate the expression of the major histocompatibility complex (MHC), significantly improve the immunogenicity of tumours, and enhance the killing of tumours by effector T cells. Preclinical studies have confirmed that both decitabine and azacitidine promote the expression of genes involved in the immune system,[9,136] and DNMTi can regulate a variety of immune cells, such as lymphocytes, NK cells, macrophages, dendritic cells and so on. DNA methyltransferase inhibitors are expected to play an important role in cancer immunotherapy. However, DNMTi modulation of immune cells is closely related to the state of cell activity, and the drug dose and regulatory mechanism need to be further elaborated in basic experiments.

In addition, the combination of methylase inhibitor and immune checkpoint inhibitor has initially shown advantages, and the combination of these with HDACi is uncertain. Currently, the experimental clinical data on combined drugs are lacking, and the clinical sample sizes are small; therefore, the findings cannot be generalized. Further research is needed. We found that DNMTi are also synergized with other classes of epigenetic drugs. For example, dual inhibition of DNMT and LSD1 was shown to synergistically reactivate epigenetically silenced genes in cancer cells.[137] DNMTi are also used in combination with isocitrate dehydrogenase (IDH) inhibitors, lenalidomide (LEN), nucleoside analogues sapacitabine, venetoclax, and other combinations to treat tumours.[138] Do these novel therapeutic strategies also have an impact on the body's antitumour immunity? Ways they may be applied more widely in the clinical treatment of cancer are directions for follow-up

research. More epigenetic drugs will be approved for marketing in the next few years. Such emerging drugs are expected to inject new vitality into the treatment of cancer and have bright prospects for development.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Shen H, Laird PW. Interplay between the cancer genome and epigenome. *Cell.* 2013;153(1):38–55. doi:10.1016/j.cell.2013.03.008
2. Wu X, Chen H, Xu H. The genomic landscape of human immune-mediated diseases. *J Hum Genet.* 2015;60(11):675–681. doi:10.1038/jhg.2015.99
3. Maio M, Grob JJ, Aamdal S, et al. Five-year survival rates for treatment-naive patients with advanced melanoma who received ipilimumab plus dacarbazine in a phase III trial. *J Clin Oncol.* 2015;33(10):1191–1196. doi:10.1200/JCO.2014.56.6018
4. McDermott D, Lebbe C, Hodi FS, et al. Durable benefit and the potential for long-term survival with immunotherapy in advanced melanoma. *Cancer Treat Rev.* 2014;40(9):1056–1064. doi:10.1016/j.ctrv.2014.06.012
5. Goldberg AD, Allis CD, Bernstein E. Epigenetics: a landscape takes shape. *Cell.* 2007;128(4):635–638. doi:10.1016/j.cell.2007.02.006
6. Fouad YA, Aanei C. Revisiting the hallmarks of cancer. *Am J Cancer Res.* 2017;7(5):1016–1036.
7. Zhang Q, Cao X. Epigenetic regulation of the innate immune response to infection. *Nat Rev Immunol.* 2019. doi:10.1038/s41577-019-0151-6
8. Gallagher SJ, Shklovskaya E, Hersey P. Epigenetic modulation in cancer immunotherapy. *Curr Opin Pharmacol.* 2017;35:48–56. doi:10.1016/j.coph.2017.05.006
9. Sigalotti L, Fratta E, Coral S, et al. Epigenetic drugs as immunomodulators for combination therapies in solid tumors. *Pharmacol Ther.* 2014;142(3):339–350. doi:10.1016/j.pharmthera.2013.12.015
10. Menezo YJ, Silvestris E, Dale B, et al. Oxidative stress and alterations in DNA methylation: two sides of the same coin in reproduction. *Reprod Biomed Online.* 2016;33(6):668–683. doi:10.1016/j.rbmo.2016.09.006
11. Schubeler D. Function and information content of DNA methylation. *Nature.* 2015;517(7534):321–326. doi:10.1038/nature14192
12. Baylin SB, Jones PA. A decade of exploring the cancer epigenome - biological and translational implications. *Nat Rev Cancer.* 2011;11(10):726–734. doi:10.1038/nrc3130
13. Robertson KD. DNA methylation and human disease. *Nat Rev Genet.* 2005;6(8):597–610. doi:10.1038/nrg1655
14. Bird A. DNA methylation patterns and epigenetic memory. *Genes Dev.* 2002;16(1):6–21. doi:10.1101/gad.947102
15. Morris MR, Latif F. The epigenetic landscape of renal cancer. *Nat Rev Nephrol.* 2017;13(1):47–60. doi:10.1038/nrneph.2016.168
16. Jones PA. Functions of DNA methylation: islands, start sites, gene bodies and beyond. *Nat Rev Genet.* 2012;13(7):484–492. doi:10.1038/nrg3230
17. Wolffe AP, Matzke MA. Epigenetics: regulation through repression. *Science.* 1999;286(5439):481–486. doi:10.1126/science.286.5439.481

18. Feinberg AP, Tycko B. The history of cancer epigenetics. *Nat Rev Cancer*. 2004;4(2):143–153. doi:10.1038/nrc1279

19. Goyos A, Sowa J, Ohta Y, et al. Remarkable conservation of distinct nonclassical MHC class I lineages in divergent amphibian species. *J Immunol*. 2011;186(1):372–381. doi:10.4049/jimmunol.1001467

20. Yoder JA, Walsh CP, Bestor TH. Cytosine methylation and the ecology of intragenomic parasites. *Trends Genet*. 1997;13(8):335–340. doi:10.1016/S0168-9525(97)01181-5

21. Qazi TJ, Quan Z, Mir A, et al. Epigenetics in alzheimer's disease: perspective of DNA methylation. *Mol Neurobiol*. 2018;55(2):1026–1044. doi:10.1007/s12035-016-0357-6

22. Akhavan-Niaki H, Samadani AA. DNA methylation and cancer development: molecular mechanism. *Cell Biochem Biophys*. 2013;67(2):501–513. doi:10.1007/s12013-013-9555-2

23. Tian Y, Arai E, Gotoh M, et al. Prognostication of patients with clear cell renal cell carcinomas based on quantification of DNA methylation levels of CpG island methylator phenotype marker genes. *BMC Cancer*. 2014. 14:772. doi:10.1186/1471-2407-14-772

24. Linehan WM, Spellman PT, Ricketts CJ, et al. Comprehensive molecular characterization of papillary renal-cell carcinoma. *N Engl J Med*. 2016;374(2):135–145. doi:10.1056/NEJMoa1505917

25. Kruck S, Eyrich C, Scharpf M, et al. Impact of an altered Wnt1/beta-catenin expression on clinicopathology and prognosis in clear cell renal cell carcinoma. *Int J Mol Sci*. 2013;14(6):10944–10957. doi:10.3390/ijms140610944

26. Mikeska T, Craig JM. DNA methylation biomarkers: cancer and beyond. *Genes (Basel)*. 2014;5(3):821–864. doi:10.3390/genes5030821

27. Chiappinelli KB, Zahnow CA, Ahuja N, et al. Combining epigenetic and immunotherapy to combat cancer. *Cancer Res*. 2016;76(7):1683–1689. doi:10.1158/0008-5472.CAN-15-2125

28. Da CE, McInnes G, Beaudry A, et al. DNA methylation-targeted drugs. *Cancer J*. 2017;23(5):270–276. doi:10.1097/ppo.0000000000000278

29. Ghoshal K, Datta J, Majumder S, et al. 5-Aza-deoxycytidine induces selective degradation of DNA methyltransferase 1 by a proteasomal pathway that requires the KEN box, bromo-adjacent homology domain, and nuclear localization signal. *Mol Cell Biol*. 2005;25(11):4727–4741. doi:10.1128/mcb.25.11.4727-4741.2005

30. Brueckner B, Garcia BR, Siedlecki P, et al. Epigenetic reactivation of tumor suppressor genes by a novel small-molecule inhibitor of human DNA methyltransferases. *Cancer Res*. 2005;65(14):6305–6311. doi:10.1158/0008-5472.can-04-2957

31. Erdmann A, Halby L, Fahy J, et al. Targeting DNA methylation with small molecules: what's next? *J Med Chem*. 2015;58(6):2569–2583. doi:10.1021/jm500843d

32. Steensma DP, Baer MR, Slack JL, et al. Multicenter study of decitabine administered daily for 5 days every 4 weeks to adults with myelodysplastic syndromes: the alternative dosing for out-patient treatment (ADOPT) trial. *J Clin Oncol*. 2009;27(23):3842–3848. doi:10.1200/jco.2008.19.6550

33. Kaminskas E, Farrell AT, Wang YC, Sridhara R, Pazdur R. FDA drug approval summary: azacitidine (5-azacytidine, Vidaza) for injectable suspension. *Oncologist*. 2005;10(3):176–182. doi:10.1634/theoncologist.10-3-176

34. Egger G, Liang G, Aparicio A, et al. Epigenetics in human disease and prospects for epigenetic therapy. *Nature*. 2004;429(6990):457–463. doi:10.1038/nature02625

35. Pechalrieu D, Etievant C, Arimondo PB. DNA methyltransferase inhibitors in cancer: from pharmacology to translational studies. *Biochem Pharmacol*. 2017;129:1–13. doi:10.1016/j.bcp.2016.12.004

36. Park JW, Han JW. Targeting epigenetics for cancer therapy. *Arch Pharm Res*. 2019;42(2):159–170. doi:10.1007/s12272-019-01126-z

37. Dedeurwaerder S, Defrance M, Calonne E, et al. Evaluation of the infinium methylation 450K technology. *Epigenomics*. 2011;3(6):771–784. doi:10.2217/epi.11.105

38. Kondo Y. Epigenetic cross-talk between DNA methylation and histone modifications in human cancers. *Yonsei Med J*. 2009;50(4):455–463. doi:10.3349/ymj.2009.50.4.455

39. Stewart DJ, Issa JP, Kurzrock R, et al. Decitabine effect on tumor global DNA methylation and other parameters in a phase I trial in refractory solid tumors and lymphomas. *Clin Cancer Res*. 2009;15(11):3881–3888. doi:10.1158/1078-0432.ccr-08-2196

40. Maio M, Covre A, Fratta E, et al. Molecular pathways: at the crossroads of cancer epigenetics and immunotherapy. *Clin Cancer Res*. 2015;21(18):4040–4047. doi:10.1158/1078-0432.CCR-14-2914

41. Covre A, Coral S, Di Giacomo AM, et al. Epigenetics meets immune checkpoints. *Semin Oncol*. 2015;42(3):506–513. doi:10.1053/j.seminoncol.2015.02.003

42. Gros C, Fleury L, Nahoum V, et al. New insights on the mechanism of quinoline-based DNA methyltransferase inhibitors. *J Biol Chem*. 2015;290(10):6293–6302. doi:10.1074/jbc.M114.594671

43. Datta J, Ghoshal K, Denny WA, et al. A new class of quinoline-based DNA hypomethylating agents reactivates tumor suppressor genes by blocking DNA methyltransferase 1 activity and inducing its degradation. *Cancer Res*. 2009;69(10):4277–4285. doi:10.1158/0008-5472.can-08-3669

44. Galluzzi L, Senovilla L, Zitvogel L, et al. The secret ally: immunostimulation by anticancer drugs. *Nat Rev Drug Discov*. 2012;11(3):215–233. doi:10.1038/nrd3626

45. Furi I, Sipos F, Spisak S, et al. Association of self-DNA mediated TLR9-related gene, DNA methyltransferase, and cytokeratin protein expression alterations in HT29-cells to DNA fragment length and methylation status. *ScientificWorldJournal*. 2013; 293–296. doi:10.1155/2013/293296

46. Wachowska M, Gabrysiak M, Muchowicz A, et al. 5-Aza-2′-deoxycytidine potentiates antitumour immune response induced by photodynamic therapy. *Eur J Cancer*. 2014;50(7):1370–1381. doi:10.1016/j.ejca.2014.01.017

47. Krishnadas DK, Bao L, Bai F, et al. Decitabine facilitates immune recognition of sarcoma cells by upregulating CT antigens, MHC molecules, and ICAM-1. *Tumour Biol*. 2014;35(6):5753–5762. doi:10.1007/s13277-014-1764-9

48. Gunda V, Frederick DT, Bernasconi MJ, et al. A potential role for immunotherapy in thyroid cancer by enhancing NY-ESO-1 cancer antigen expression. *Thyroid*. 2014;24(8):1241–1250. doi:10.1089/thy.2013.0680

49. Simova J, Pollakova V, Indrova M, et al. Immunotherapy augments the effect of 5-azacytidine on HPV16-associated tumours with different MHC class I-expression status. *Br J Cancer*. 2011;105(10):1533–1541. doi:10.1038/bjc.2011.428

50. Natsume A, Wakabayashi T, Tsujimura K, et al. The DNA demethylating agent 5-aza-2′-deoxycytidine activates NY-ESO-1 antigenicity in orthotopic human glioma. *Int J Cancer*. 2008;122(11):2542–2553. doi:10.1002/ijc.23407

51. Lucarini V, Buccione C, Ziccheddu G, et al. Combining type I interferons and 5-Aza-2′-deoxycitidine to improve anti-tumor response against melanoma. *J Invest Dermatol*. 2017;137(1):159–169. doi:10.1016/j.jid.2016.08.024

52. Wang L, Amoozgar Z, Huang J, et al. Decitabine enhances lymphocyte migration and function and synergizes with CTLA-4 blockade in a murine ovarian cancer model. *Cancer Immunol Res*. 2015;3(9):1030–1041. doi:10.1158/2326-6066.CIR-15-0073

53. Yang H, Lan P, Hou Z, et al. Histone deacetylase inhibitor SAHA epigenetically regulates miR-17-92 cluster and MCM7 to upregulate MICA expression in hepatoma. *Br J Cancer*. 2015;112(1):112–121. doi:10.1038/bjc.2014.547

54. Srivastava P, Paluch BE, Matsuzaki J, et al. Immunomodulatory action of SGI-110, a hypomethylating agent, in acute myeloid leukemia cells and xenografts. *Leuk Res*. 2014;38(11):1332–1341. doi:10.1016/j.leukres.2014.09.001

55. Coral S, Parisi G, Nicolay HJ, et al. Immunomodulatory activity of SGI-110, a 5-aza-2′-deoxycytidine-containing demethylating dinucleotide. *Cancer Immunol Immunother*. 2013;62(3):605–614. doi:10.1007/s00262-012-1365-7

56. Murakami T, Sato A, Chun NA, et al. Transcriptional modulation using HDACi depsipeptide promotes immune cell-mediated tumor destruction of murine B16 melanoma. *J Invest Dermatol*. 2008;128(6):1506–1516. doi:10.1038/sj.jid.5701216

57. Zhang J, Fu J, Pan Y, et al. Silencing of miR-1247 by DNA methylation promoted non-small-cell lung cancer cell invasion and migration by effects of STMN1. *Onco Targets Ther*. 2016;9:7297–7307. doi:10.2147/ott.s111291

58. Li XY, Wu JZ, Cao HX, et al. Blockade of DNA methylation enhances the therapeutic effect of gefitinib in non-small cell lung cancer cells. *Oncol Rep*. 2013;29(5):1975–1982. doi:10.3892/or.2013.2298

59. Klar AS, Gopinadh J, Kleber S, et al. Treatment with 5-Aza-2′-deoxycytidine induces expression of NY-ESO-1 and facilitates cytotoxic T lymphocyte-mediated tumor cell killing. *PLoS One*. 2015;10(10):e0139221. doi:10.1371/journal.pone.0139221

60. Ayyoub M, Taub RN, Keohan ML, et al. The frequent expression of cancer/testis antigens provides opportunities for immunotherapeutic targeting of sarcoma. *Cancer Immun*. 2004;4:7. doi:10.1084/jem.155.6.1823

61. Sigalotti L, Coral S, Altomonte M, et al. Cancer testis antigens expression in mesothelioma: role of DNA methylation and bioimmunotherapeutic implications. *Br J Cancer*. 2002;86(6):979–982. doi:10.1038/sj.bjc.6600174

62. Guo ZS, Hong JA, Irvine KR, et al. De novo induction of a cancer/testis antigen by 5-aza-2′-deoxycytidine augments adoptive immunotherapy in a murine tumor model. *Cancer Res*. 2006;66(2):1105–1113. doi:10.1158/0008-5472.can-05-3020

63. Srivastava P, Paluch BE, Matsuzaki J, et al. Immunomodulatory action of the DNA methyltransferase inhibitor SGI-110 in epithelial ovarian cancer cells and xenografts. *Epigenetics*. 2015;10(3):237–246. doi:10.1080/15592294.2015.1017198

64. Chiappinelli KB, Strissel PL, Desrichard A, et al. Inhibiting DNA methylation causes an interferon response in cancer via dsRNA including endogenous retroviruses. *Cell*. 2016;164(5):1073. doi:10.1016/j.cell.2015.10.020

65. Roulois D, Loo YH, Singhania R, et al. DNA-demethylating agents target colorectal cancer cells by inducing viral mimicry by endogenous transcripts. *Cell*. 2015;162(5):961–973. doi:10.1016/j.cell.2015.07.056

66. Ramakrishnan S, Hu Q, Krishnan N, et al. Decitabine, a DNA-demethylating agent, promotes differentiation via NOTCH1 signaling and alters immune-related pathways in muscle-invasive bladder cancer. *Cell Death Dis*. 2017;8(12):3217. doi:10.1038/s41419-017-0024-5

67. Wang LX, Mei ZY, Zhou JH, et al. Low dose decitabine treatment induces CD80 expression in cancer cells and stimulates tumor specific cytotoxic T lymphocyte responses. *PLoS One*. 2013;8(5):e62924. doi:10.1371/journal.pone.0062924

68. Li B, Zhu X, Sun L, et al. Induction of a specific CD8+ T-cell response to cancer/testis antigens by demethylating pre-treatment against osteosarcoma. *Oncotarget*. 2014;5(21):10791–10802. doi:10.18632/oncotarget.2505

69. Schuyler RP, Merkel A, Raineri E, et al. Distinct trends of DNA methylation patterning in the innate and adaptive immune systems. *Cell Rep*. 2016;17(8):2101–2111. doi:10.1016/j.celrep.2016.10.054

70. Daniel B, Nagy G, Czimmerer Z, et al. The nuclear receptor PPARgamma controls progressive macrophage polarization as a ligand-insensitive epigenomic ratchet of transcriptional memory. *Immunity*. 2018;49(4):615–626.e616. doi:10.1016/j.immuni.2018.09.005

71. Rodriguez-Ubreva J, Catala-Moll F, Obermajer N, et al. Prostaglandin E2 leads to the acquisition of DNMT3A-dependent tolerogenic functions in human myeloid-derived suppressor cells. *Cell Rep*. 2017;21(1):154–167. doi:10.1016/j.celrep.2017.09.018

72. Albrengues J, Bertero T, Grasset E, et al. Epigenetic switch drives the conversion of fibroblasts into proinvasive cancer-associated fibroblasts. *Nat Commun*. 2015;6:102–104. doi:10.1038/ncomms10204

73. Lal G, Zhang N, van der Touw W, et al. Epigenetic regulation of Foxp3 expression in regulatory T cells by DNA methylation. *J Immunol*. 2009;182(1):259–273. doi:10.4049/jimmunol.182.1.259

74. Yu B, Zhang K, Milner JJ, et al. Epigenetic landscapes reveal transcription factors that regulate CD8(+) T cell differentiation. *Nat Immunol*. 2017;18(5):573–582. doi:10.1038/ni.3706

75. Abdelsamed HA, Moustaki A, Fan Y, et al. Human memory CD8 T cell effector potential is epigenetically preserved during in vivo homeostasis. *J Exp Med*. 2017;214(6):1593–1606. doi:10.1084/jem.20161760

76. Youngblood B, Hale JS, Kissick HT, et al. Effector CD8 T cells dedifferentiate into long-lived memory cells. *Nature*. 2017;552(7685):404–409. doi:10.1038/nature25144

77. Sen DR, Kaminski J, Barnitz RA, et al. The epigenetic landscape of T cell exhaustion. *Science*. 2016;354(6316):1165–1169. doi:10.1126/science.aae0491

78. Scott-Browne JP, Lopez-Moyado IF, Trifari S, et al. Dynamic changes in chromatin accessibility occur in CD8(+) T cells responding to viral infection. *Immunity*. 2016;45(6):1327–1340. doi:10.1016/j.immuni.2016.10.028

79. Ghoneim HE, Fan Y, Moustaki A, et al. De novo epigenetic programs inhibit PD-1 blockade-mediated T cell rejuvenation. *Cell*. 2017;170(1):142–157.e119. doi:10.1016/j.cell.2017.06.007

80. Li X, Zhang Y, Chen M, et al. Increased IFNgamma(+) T cells are responsible for the clinical responses of low-dose DNA-demethylating agent decitabine antitumor therapy. *Clin Cancer Res*. 2017;23(20):6031–6043. doi:10.1158/1078-0432.CCR-17-1201

81. Qiu H, Hu X, Gao L, et al. Interleukin 10 enhanced CD8+ T cell activity and reduced CD8(+) T cell apoptosis in patients with diffuse large B cell lymphoma. *Exp Cell Res*. 2017;360(2):146–152. doi:10.1016/j.yexcr.2017.08.036

82. Peng D, Kryczek I, Nagarsheth N, et al. Epigenetic silencing of TH1-type chemokines shapes tumour immunity and immunotherapy. *Nature*. 2015;527(7577):249–253. doi:10.1038/nature15520

83. Topper MJ, Vaz M, Chiappinelli KB, et al. Epigenetic therapy ties MYC depletion to reversing immune evasion and treating lung cancer. *Cell*. 2017;171(6):1284–1300.e1221. doi:10.1016/j.cell.2017.10.022

84. Yang X, Han H, De Carvalho DD, et al. Gene body methylation can alter gene expression and is a therapeutic target in cancer. *Cancer Cell*. 2014;26(4):577–590. doi:10.1016/j.ccr.2014.07.028

85. Haghshenas MR, Khademi B, Ashraf MJ, et al. Helper and cytotoxic T-cell subsets (Th1, Th2, Tc1, and Tc2) in benign and malignant salivary gland tumors. *Oral Dis*. 2016;22(6):566–572. doi:10.1111/odi.12496

86. Allan RS, Zueva E, Cammas F, et al. An epigenetic silencing pathway controlling T helper 2 cell lineage commitment. *Nature*. 2012;487(7406):249–253. doi:10.1038/nature11173

87. Jennane S, El HM, Mahtat EM, et al. Successful treatment of donor cell derived myelodysplastic syndrome with 5-azacytidine. *Ann Biol Clin (Paris)*. 2017;75(6):713–714. doi:10.1684/abc.2017.1293

88. Togashi Y, Shitara K, Nishikawa H. Regulatory T cells in cancer immunosuppression - implications for anticancer therapy. *Nat Rev Clin Oncol*. 2019. doi:10.1038/s41571-019-0175-7

89. Togashi Y, Nishikawa H. Regulatory T cells: molecular and cellular basis for immunoregulation. *Curr Top Microbiol Immunol*. 2017;410:3–27. doi:10.1007/82_2017_58

90. Sakaguchi S, Miyara M, Costantino CM, et al. FOXP3+ regulatory T cells in the human immune system. *Nat Rev Immunol*. 2010;10(7):490–500. doi:10.1038/nri2785

91. Lu L, Barbi J, Pan F. The regulation of immune tolerance by FOXP3. *Nat Rev Immunol*. 2017;17(11):703–717. doi:10.1038/nri.2017.75

92. Speiser DE, Ho PC, Verdeil G. Regulatory circuits of T cell function in cancer. *Nat Rev Immunol*. 2016;16(10):599–611. doi:10.1038/nri.2016.80

93. Delacher M, Imbusch CD, Weichenhan D, et al. Genome-wide DNA-methylation landscape defines specialization of regulatory T cells in tissues. *Nat Immunol*. 2017;18(10):1160–1172. doi:10.1038/ni.3799

94. Costantini B, Kordasti SY, Kulasekararaj AG, et al. The effects of 5-azacytidine on the function and number of regulatory T cells and T-effectors in myelodysplastic syndrome. *Haematologica*. 2013;98(8):1196–1205. doi:10.3324/haematol.2012.074823

95. Ginhoux F, Schultze JL, Murray PJ, et al. New insights into the multidimensional concept of macrophage ontogeny, activation and function. *Nat Immunol*. 2016;17(1):34–40. doi:10.1038/ni.3324

96. Mantovani A, Marchesi F, Malesci A, et al. Tumour-associated macrophages as treatment targets in oncology. *Nat Rev Clin Oncol*. 2017;14(7):399–416. doi:10.1038/nrclinonc.2016.217

97. Yang X, Wang X, Liu D, et al. Epigenetic regulation of macrophage polarization by DNA methyltransferase 3b. *Mol Endocrinol*. 2014;28(4):565–574. doi:10.1210/me.2013-1293

98. Cheng C, Huang C, Ma TT, et al. SOCS1 hypermethylation mediated by DNMT1 is associated with lipopolysaccharide-induced inflammatory cytokines in macrophages. *Toxicol Lett*. 2014;225(3):488–497. doi:10.1016/j.toxlet.2013.12.023

99. Tesone AJ, Rutkowski MR, Brencicova E, et al. Satb1 overexpression drives tumor-promoting activities in cancer-associated dendritic cells. *Cell Rep*. 2016;14(7):1774–1786. doi:10.1016/j.celrep.2016.01.056

100. Zhou J, Yao Y, Shen Q, et al. Demethylating agent decitabine disrupts tumor-induced immune tolerance by depleting myeloid-derived suppressor cells. *J Cancer Res Clin Oncol*. 2017;143(8):1371–1380. doi:10.1007/s00432-017-2394-6

101. Adeegbe DO, Liu Y, Lizotte PH, et al. Synergistic immunostimulatory effects and therapeutic benefit of combined histone deacetylase and bromodomain inhibition in non-small cell lung cancer. *Cancer Discov*. 2017;7(8):852–867. doi:10.1158/2159-8290.CD-16-1020

102. Tomita Y, Lee MJ, Lee S, et al. The interplay of epigenetic therapy and immunity in locally recurrent or metastatic estrogen receptor-positive breast cancer: correlative analysis of ENCORE 301, a randomized, placebo-controlled phase II trial of exemestane with or without entinostat. *Oncoimmunology*. 2016;5(11):e1219008. doi:10.1080/2162402x.2016.1219008

103. Kucuk C, Hu X, Gong Q, et al. Diagnostic and biological significance of KIR expression profile determined by RNA-seq in natural killer/T-cell lymphoma. *Am J Pathol*. 2016;186(6):1435–1441. doi:10.1016/j.ajpath.2016.02.011

104. Kopp LM, Ray A, Denman CJ, et al. Decitabine has a biphasic effect on natural killer cell viability, phenotype, and function under proliferative conditions. *Mol Immunol*. 2013;54(3–4):296–301. doi:10.1016/j.molimm.2012.12.012

105. Netea MG, Joosten LA, Latz E, et al. Trained immunity: a program of innate immune memory in health and disease. *Science*. 2016;352(6284):aaf1098. doi:10.1126/science.aaf1098

106. Rohner A, Langenkamp U, Siegler U, et al. Differentiation-promoting drugs up-regulate NKG2D ligand expression and enhance the susceptibility of acute myeloid leukemia cells to natural killer cell-mediated lysis. *Leuk Res*. 2007;31(10):1393–1402. doi:10.1016/j.leukres.2007.02.020

107. Grimm EA, Mazumder A, Zhang HZ, et al. Lymphokine-activated killer cell phenomenon. Lysis of natural killer-resistant fresh solid tumor cells by interleukin 2-activated autologous human peripheral blood lymphocytes. *J Exp Med*. 1982;155(6):1823–1841. doi:10.1084/jem.155.6.1823

108. Schmiedel BJ, Arelin V, Gruenebach F, et al. Azacytidine impairs NK cell reactivity while decitabine augments NK cell responsiveness toward stimulation. *Int J Cancer*. 2011;128(12):2911–2922. doi:10.1002/ijc.25635

109. Gao XN, Lin J, Wang LL, et al. Demethylating treatment suppresses natural killer cell cytolytic activity. *Mol Immunol*. 2009;46(10):2064–2070. doi:10.1016/j.molimm.2009.02.033

110. Triozzi PL, Aldrich W, Achberger S, et al. Differential effects of low-dose decitabine on immune effector and suppressor responses in melanoma-bearing mice. *Cancer Immunol Immunother*. 2012;61(9):1441–1450. doi:10.1007/s00262-012-1204-x

111. Odunsi K, Matsuzaki J, James SR, et al. Epigenetic potentiation of NY-ESO-1 vaccine therapy in human ovarian cancer. *Cancer Immunol Res*. 2014;2(1):37–49. doi:10.1158/2326-6066.cir-13-0126

112. Fazio C, Covre A, Cutaia O, et al. Immunomodulatory properties of DNA hypomethylating agents: selecting the optimal epigenetic partner for cancer immunotherapy. *Front Pharmacol*. 2018;9:1443. doi:10.3389/fphar.2018.01443

113. Kim K, Skora AD, Li Z, et al. Eradication of metastatic mouse cancers resistant to immune checkpoint blockade by suppression of myeloid-derived cells. *Proc Natl Acad Sci U S A*. 2014;111(32):11774–11779. doi:10.1073/pnas.1410626111

114. Chiappinelli KB, Strissel PL, Desrichard A, et al. Inhibiting DNA methylation causes an interferon response in cancer via dsRNA including endogenous retroviruses. *Cell*. 2017;169(2):361. doi:10.1016/j.cell.2017.03.036

115. Coral S, Sigalotti L, Altomonte M, et al. 5-aza-2′-deoxycytidine-induced expression of functional cancer testis antigens in human renal cell carcinoma: immunotherapeutic implications. *Clin Cancer Res*. 2002;8(8):2690–2695.

116. Li H, Chiappinelli KB, Guzzetta AA, et al. Immune regulation by low doses of the DNA methyltransferase inhibitor 5-azacitidine in common human epithelial cancers. *Oncotarget*. 2014;5(3):587–598. doi:10.18632/oncotarget.1782

117. Chiappinelli KB, Strissel PL, Desrichard A, et al. Inhibiting DNA methylation causes an interferon response in cancer via dsRNA including endogenous retroviruses. *Cell*. 2015;162(5):974–986. doi:10.1016/j.cell.2015.07.011

118. Liu M, Zhang L, Li H, et al. Integrative epigenetic analysis reveals therapeutic targets to the DNA methyltransferase inhibitor guadecitabine (SGI-110) in Hepatocellular Carcinoma. *Hepatology*. 2018;68(4):1412–1428. doi:10.1002/hep.30091

119. Daver N, Garcia-Manero G, Basu S, et al. Efficacy, safety, and biomarkers of response to azacitidine and nivolumab in relapsed/refractory acute myeloid leukemia: a nonrandomized, open-label, phase II study. *Cancer Discov*. 2019;9(3):370–383. doi:10.1158/2159-8290.CD-18-0774

120. Levy BP, Giaccone G, Besse B, et al. Randomised phase 2 study of pembrolizumab plus CC-486 versus pembrolizumab plus placebo in patients with previously treated advanced non-small cell lung cancer. *Eur J Cancer*. 2019;108:120–128. doi:10.1016/j.ejca.2018.11.028

121. Ravandi F, Assi R, Daver N, et al. Idarubicin, cytarabine, and nivolumab in patients with newly diagnosed acute myeloid leukaemia or high-risk myelodysplastic syndrome: a single-arm, phase 2 study. *Lancet Haematol*. 2019. doi:10.1016/S2352-3026(19)30114-0

122. Eckschlager T, Plch J, Stiborova M, et al. Histone deacetylase inhibitors as anticancer drugs. *Int J Mol Sci*. 2017;18(7):1414. doi:10.3390/ijms18071414

123. Setiadi AF, Omilusik K, David MD, et al. Epigenetic enhancement of antigen processing and presentation promotes immune recognition of tumors. *Cancer Res*. 2008;68(23):9601–9607. doi:10.1158/0008-5472.can-07-5270

124. Balliu M, Guandalini L, Romanelli MN, et al. HDAC-inhibitor (S)-8 disrupts HDAC6-PP1 complex prompting A375 melanoma cell growth arrest and apoptosis. *J Cell Mol Med*. 2015;19(1):143–154. doi:10.1111/jcmm.12345

125. Reiner SL. Inducing the T cell fates required for immunity. *Immunol Res*. 2008;42(1–3):160–165. doi:10.1007/s12026-008-8054-9

126. Goodyear O, Agathanggelou A, Novitzky-Basso I, et al. Induction of a CD8+ T-cell response to the MAGE cancer testis antigen by combined treatment with azacitidine and sodium valproate in patients with acute myeloid leukemia and myelodysplasia. *Blood*. 2010;116(11):1908–1918. doi:10.1182/blood-2009-11-249474

127. Khan AN, Gregorie CJ, Tomasi TB. Histone deacetylase inhibitors induce TAP, LMP, Tapasin genes and MHC class I antigen presentation by melanoma cells. *Cancer Immunol Immunother*. 2008;57(5):647–654. doi:10.1007/s00262-007-0402-4

128. Magner WJ, Kazim AL, Stewart C, et al. Activation of MHC class I, II, and CD40 gene expression by histone deacetylase inhibitors. *J Immunol*. 2000;165(12):7017–7024. doi:10.4049/jimmunol.165.12.7017

129. Kroesen M, Gielen P, Brok IC, et al. HDAC inhibitors and immunotherapy; a double edged sword? *Oncotarget*. 2014;5(16):6558–6572. doi:10.18632/oncotarget.2289

130. Sigalotti L, Fratta E, Coral S, et al. Epigenetic drugs as pleiotropic agents in cancer treatment: biomolecular aspects and clinical applications. *J Cell Physiol*. 2007;212(2):330–344. doi:10.1002/jcp.21066

131. Blagitko-Dorfs N, Schlosser P, Greve G, et al. Combination treatment of acute myeloid leukemia cells with DNMT and HDAC inhibitors: predominant synergistic gene downregulation associated with gene body demethylation. *Leukemia*. 2019;33(4):945–956. doi:10.1038/s41375-018-0293-8

132. Sekeres MA, Othus M, List AF, et al. Randomized phase II study of azacitidinealone or in combination with lenalidomide or with vorinostat in higher-risk myelodysplastic syndromes and chronic myelomonocytic leukemia: North American Intergroup Study SWOG S1117. *J Clin Oncol*. 2017;35:2745–2753. doi:10.1200/jco.2015.66.2510

133. Garcia-Manero G, Sekeres MA, Egyed M, et al. A phase 1b/2b multicenter study of oral panobinostat plus azacitidine in adults with MDS, CMML or AML with 30% blasts. *Leukemia*. 2017;31(12):2799–2806. doi:10.1038/leu.2017.159

134. Garcia-Manero G, Montalban-Bravo G, Berdeja JG, et al. Phase 2, randomized, double-blind study of pracinostat in combination with azacitidine in patients with untreated, higher-risk myelodysplastic syndromes. *Cancer*. 2017;123(6):994–1002. doi:10.1002/cncr.30533

135. Craddock CF, Houlton AE, Quek LS, et al. Outcome of azacitidine therapy in acute myeloid leukemia is not improved by concurrent vorinostat therapy but is predicted by a diagnostic molecular signature. *Clin Cancer Res*. 2017;23(21):6430–6440. doi:10.1158/1078-0432.CCR-17-1423

136. Wrangle J, Wang W, Koch A, et al. Alterations of immune response of non-small cell lung cancer with azacytidine. *Oncotarget*. 2013;4(11):2067–2079. doi:10.18632/oncotarget.1542

137. Han H, Yang X, Pandiyan K, et al. Synergistic re-activation of epigenetically silenced genes by combinatorial inhibition of DNMTs and LSD1 in cancer cells. *PLoS One*. 2013;8(9):e75136. doi:10.1371/journal.pone.0075136

138. Figueroa ME, Abdel-Wahab O, Lu C, et al. Leukemic IDH1 and IDH2 mutations result in a hypermethylation phenotype, disrupt TET2 function, and impair hematopoietic differentiation. *Cancer Cell*. 2010;18(6):553–567. doi:10.1016/j.ccr.2010.11.015