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REVIEW

Epigenome-Driven Strategies for Personalized Cancer Immunotherapy

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Abstract: Fighting cancer remains one of the greatest challenges for science in the 21st century. Advances in immunotherapy against different types of cancer have greatly contributed to the treatment, remission, and cure of patients. In this context, knowledge of epigenetic phenomena, their relationship with tumor cells and how the immune system can be epigenetically modulated represent some of the greatest advances in the development of anticancer therapies. Epigenetics is a rapidly growing field that studies how environmental factors can affect gene expression without altering DNA sequence. Epigenomic changes include DNA methylation, histone modifications, and non-coding RNA regulation, which impact cellular function. Epigenetics has shown promise in developing cancer therapies, such as immunotherapy, which aims to stimulate the immune system to attack cancer cells. For example, PD-1 and PD-L1 are biomarkers that regulate the immune response to cancer cells and recent studies have shown that epigenetic modifications can affect their expression, potentially influencing the efficacy of immunotherapy. New therapies targeting epigenetic modifications, such as histone deacetylases and DNA methyltransferases, are being developed for cancer treatment, and some have shown promise in preclinical studies and clinical trials. With growing understanding of epigenetic regulation, we can expect more personalized and effective cancer immunotherapies in the future. This review highlights key advances in the use of epigenetic and epigenomic tools and modern immuno-oncology strategies to treat several types of tumors.

Keywords: epigenetics, epigenomics, PD-1, PD-L1, epigenetic biomarkers, oncology, immunotherapy

Introduction

Epigenetics is the study of changes in the expression of genes that occur without changes to the underlying DNA sequence.[1] These changes can be caused by a variety of factors, including environmental exposures, aging, and disease.[2] Oncology is the branch of medicine that deals with the study and treatment of cancer.[2] In recent years, there has been increasing interest in the intersection of these two fields, as epigenetic changes have been found to play a role in the development and progression of many types of cancer.[3]

One way in which epigenetics contributes to cancer is through changes in the way that genes are expressed. For example, certain genes known as tumor suppressors are responsible for keeping cell growth in check.[1] These genes can be turned off or "silenced" through a process known as methylation, which involves the addition of a methyl group to the DNA.[3] When tumor suppressor genes are silenced, cells can continue to divide uncontrollably, leading to the formation of a tumor.[4]

Another way in which epigenetics contributes to cancer is through changes in the way that chromatin, the material that makes up chromosomes, is structured. Chromatin is composed of DNA and proteins called histones. Changes in the way that histones are modified, known as histone modifications, can lead to changes in the way that genes are expressed. For example, certain histone modifications, such as acetylation, can make genes more easily accessible and therefore

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more likely to be transcribed into RNA.[5] Conversely, other histone modifications, such as methylation, can make genes less accessible and therefore less likely to be transcribed.[5]

Epigenetic changes have also been linked to drug resistance, a major problem in cancer treatment. In many cases, cancer cells acquire changes in their epigenetic machinery that allows them to resist the effects of chemotherapy and other cancer treatments.[1] This is a complex and multifactorial process that can involve changes in multiple genes and pathways and is only beginning to be understood.[4]

Another epigenetic mechanism recently elucidated is the participation of several types of non-coding RNAs in epigenetic mechanisms. Non-coding RNAs (ncRNAs) are RNA molecules that are not translated into proteins but play important roles in gene regulation.[2,4] Aberrant expression of ncRNAs, such as microRNAs and long non-coding RNAs, has been observed in various types of cancer and can contribute to oncogenesis by promoting cell proliferation, invasion, and metastasis. Epigenetic changes, including DNA methylation and histone modifications, can also alter the expression of cancer-related genes and contribute to tumor initiation and progression.[6–8] Understanding the roles of ncRNAs and epigenetic modifications in cancer may lead to the development of new diagnostic and therapeutic approaches for this complex disease.

A growing body of research suggests that epigenetic changes may also play a role in the development of cancer caused by exposure to environmental toxins, such as tobacco smoke and air pollution.[9] For example, it has been shown that exposure to tobacco smoke leads to changes in the methylation of genes involved in cell growth and repair, which can increase the risk of cancer. For a complete overview of the epigenetic changes and how they affect cancer cells please check Figure 1.

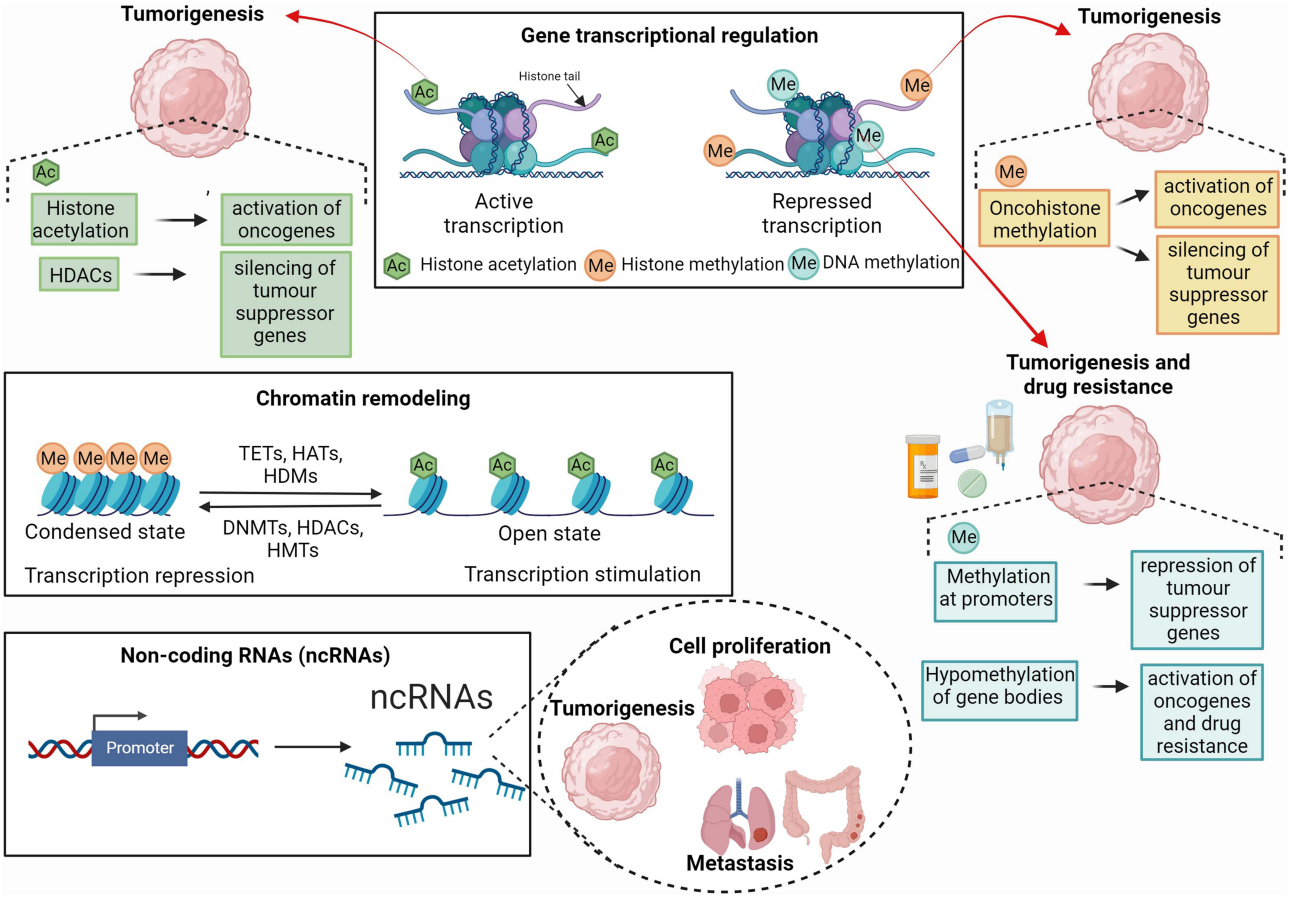

Figure 1 General overview of the epigenetic mechanisms involved in gene expression associated with cancer. Epigenetic modifications in DNA, histones, and the biosynthesis of Non-coding RNAs (ncRNAs) are closely related to the genesis of several types of tumors. These modifications also contribute to regulating other key events in the establishment of the disease, such as cell proliferation, cell cycle changes, apoptosis, invasion, metastasis, DNA damage and senescence. DNA and histone methylation, histone acetylation and ncRNA biosynthesis can activate oncogenes and repress tumor suppressor genes, often related to tumorigenesis. Thus, enzymes that catalyze epigenetic modifications, such as DNA methyltransferases (DNMTs), ten-eleven translocation enzymes (TETs), histone acetylases (HATs), histone deacetylases (HDACs), histone methyltransferases (HMTs) and histone demethylases (HDMs), are the main agents that promote epigenetic modifications in DNA and histones, capable of inducing reversible chromatin remodeling, and activating or repressing the transcription of cancer-related genes.

Many of the current therapeutic approaches for cancer targets the genetic mutations that drive the growth of the cancer. However, in recent years, researchers began to realize that epigenetic changes also play a crucial role in cancer. There are a few therapeutic drugs that are targeting these changes as well, such as Histone deacetylase inhibitors (HDACIs) and DNA methyltransferase inhibitors (DNMTIs) that have shown promise in preclinical and clinical studies.[2,10–12]

HDACIs and DNMTIs are two classes of drugs that have shown promise in preclinical and clinical studies for the treatment of cancer.[10] HDACIs work by inhibiting the activity of enzymes called histone deacetylases (HDACs), which play a role in regulating gene expression. By inhibiting HDACs, HDACIs can cause cancer cells to undergo cell death or differentiation, making them a potential treatment option for certain types of cancer.[10,13] HDACIs have been studied in several types of cancer, including leukemia, lymphoma, and solid tumors, and several HDACIs have been approved by regulatory agencies for the treatment of certain types of cancer. DNMTIs, on the other hand, inhibit the activity of enzymes called DNA methyltransferases (DNMTs), which play a role in regulating gene expression by adding methyl groups to DNA.[13,14] By inhibiting DNMTs, DNMTIs can cause cancer cells to undergo cell death or differentiation, making them a potential treatment option for certain types of cancer.[14] DNMTIs have been studied in several types of cancer, including leukemia, lymphoma, and solid tumors, and several DNMTIs have been approved by regulatory agencies for the treatment of certain types of cancer.[2,10,14]

The synergy between epigenetic therapeutic approaches and immunotherapies has been recently demonstrated by the de-repression of endogenous retroviral element (ERV)-encoded promoters in several types of cancer, such as lung cancer, acute myeloid leukemia, glioblastoma, and colon cancer.[15] The treatment of cancer cell lines from these tumors with DNMTi and/or HDACi, allowed the identification of several thousand ERV-derived, treatment-induced novel polyadenylated transcripts (TINPATs), obtained from the assembly of a de novo transcriptome of tumor cell lines.[15] Using Immunopeptidomics, Goyal and colleagues obtained 45 spectra-validated treatment-induced neopeptides (t-neopeptides) arising from TINPATs and induced human leukocyte antigen (HLA) presentation to elicit a T-cell response to effectively target cancer cells in patients with acute myeloid leukemia (AML).[15]

Both HDACIs and DNMTIs have shown promise in preclinical and clinical studies, with some drugs from both classes showing strong efficacy in certain types of cancer.[14,16] However, both classes of drugs are still in the early stages of development and more research is needed to fully understand their potential as cancer treatments. Additionally, both classes of drugs have potential side effects, and more research is needed to understand their long-term safety.

In summary, the field of epigenetics is rapidly advancing and holds significant promise for improving our understanding of cancer and developing new strategies for its prevention, diagnosis, and treatment. While we still have much to learn, the insights gained from studying the epigenetics of cancer are already providing new avenues for research and new opportunities for the development of targeted therapies, especially in immunotherapeutics.

Epigenetics and Epigenomics in Complex Diseases

One of the keyways in which epigenetics can contribute to disease is through the alteration of gene expression. For example, certain genetic variations can increase a person's risk of developing a certain disease, but whether the person develops the disease may depend on the presence of certain environmental factors or lifestyle choices.[5] These factors can cause changes to the epigenetic marks on the person's DNA, leading to altered gene expression and an increased risk of disease. Epigenomics is the study of the complete set of epigenetic modifications on the genetic material of a cell, known as the epigenome, and the effects these modifications have on gene expression and cell behavior.[13]

Another important aspect of epigenetic modifications in disease is the role of methylation, a process by which methyl groups are added to the DNA molecule. Methylation can silence genes by physically blocking the transcription machinery from accessing the genetic information.[10] This process has important implications in cancer. For example, the inactivation of tumor suppressor genes through methylation is a common mechanism of cancer development. Abnormal methylation patterns have been identified in various types of cancer, and drugs that target these patterns are being developed as a potential cancer therapy.[13]

Epigenetics also plays a role in aging and age-related diseases. As cells divide, the chromosomes can become damaged, leading to the accumulation of genetic mutations over time. Epigenetic changes can also accumulate over time,

and these changes can contribute to aging and age-related diseases. Recent studies have shown that epigenetic changes can also influence the development of neurodegenerative diseases such as Alzheimer's and Parkinson's.[17]

Another area of growing interest in epigenetics is the study of how environmental exposures and lifestyle factors can affect the epigenome and contribute to disease. For example, research has shown that prenatal exposure to certain pollutants, such as lead and bisphenol A (BPA), can lead to epigenetic changes that increase the risk of disease later in life. Similarly, diet and nutrition can also affect the epigenome, with studies showing that a poor diet can lead to changes in gene expression that contribute to the development of obesity and other metabolic diseases.[18]

In disease, epigenomic changes can lead to the silencing of genes that normally protect against the disease, or the activation of genes that promote the disease. For example, in cancer, epigenomic changes can lead to the silencing of tumor suppressor genes, allowing cancer cells to grow and spread. Similarly, in neurodegenerative diseases such as Alzheimer's, epigenomic changes can lead to the activation of genes that promote the buildup of toxic protein aggregates in the brain.[13,14,17]

Epigenomic changes can also play a role in the development of certain diseases that have a strong environmental component. For example, epigenomic changes have been linked to the development of obesity and diabetes, which are often caused by poor diet and lack of exercise.[17] The study of epigenomics in disease is still in its early stages, but researchers are working to better understand the underlying mechanisms and to develop new therapies that target these mechanisms.[14,19] Some of the current research focuses on the development of drugs that target the enzymes that are responsible for making these epigenetic changes.[2,13] Overall, epigenomic changes play a key role in the development and progression of many diseases, and understanding these changes is crucial for the development of effective treatments. As research in this field continues to advance, it is likely that we will see the development of new therapies that target the underlying epigenomic mechanisms of disease, leading to more effective treatments and improved outcomes for patients.[13]

In summary, epigenetics and epigenomics are rapidly growing fields that are providing new insights into the complex mechanisms underlying disease. By understanding how changes in the epigenome can contribute to disease, researchers may be able to develop new therapies and strategies for preventing and treating a wide range of diseases.

PD-1, PD-L1, Epigenetics and Diseases

PD-1 and PD-L1 are proteins involved in the regulation of the immune system's response to cancer cells. PD-1 (programmed cell death 1) is a protein found on the surface of T cells, which are a type of white blood cell important for fighting infection and cancer. PD-L1 (programmed cell death ligand 1) is a protein found on the surface of cancer cells and some normal cells. When PD-L1 binds to PD-1 on T cells, it suppresses the immune response and allows cancer cells to evade detection and destruction by the immune system. Drugs that block the interaction between PD-1 and PD-L1, known as checkpoint inhibitors, have been developed and are used to treat certain types of cancer.[20–22]

PD-1 and PD-L1 are molecules that play a role in the regulation of the immune response to cancer cells. PD-1 is a protein that is expressed on the surface of T cells, a type of white blood cell that is important for the immune response. When PD-1 binds to its ligand, PD-L1, it can inhibit the activity of the T cell and reduce the immune response to the cancer cells. PD-L1, on the other hand, is a protein that is expressed on the surface of many types of cancer cells, as well as on some normal cells. When PD-L1 binds to PD-1, it can inhibit the activity of T cells and prevent them from attacking the cancer cells. Inhibitors of the PD-1/PD-L1 pathway has now been used for cancer treatment. Such inhibitors are called immune checkpoint inhibitors, are intended to block the cancer's ability to evade the immune system.[13,14,20–22]

Research has shown that cancer cells can overexpress PD-L1, allowing them to evade the immune response and promote their growth.[21] These findings have led to the development of drugs that target the PD-1/PD-L1 pathway as a means of treating cancer.[20] Some examples of these drugs are nivolumab, pembrolizumab and atezolizumab, now FDA-approved for some cancer types such as lung cancer, bladder cancer, melanoma, head and neck cancer, among others.[23–25]

In the context of HIV infection in AIDS, the regulation of PD-1 expression plays a pivotal role in immune responses. PD-1, encoded by the Pdcd1 gene, undergoes epigenetic regulation, specifically through the addition of 5-methylcytosine (5mC) at two CpG sites in the gene's promoter region. Studies in mice infected with the lymphocytic choriomeningitis virus (LCMV) demonstrated a dynamic methylation pattern in the Pdcd1 promoter.[25] Notably, methylation occurs in CD8 T cells, leading to transcriptional silencing of Pdcd1. This silencing was inversely correlated with viral load, highlighting the significance of Pdcd1 methylation in modulating immune responses during viral infections.[26]

In another example of peptide-mediated immunotherapy in mouse models, demethylation of Pdcd1 facilitated the accumulation of PD-1 in tolerizing T cells.[27] This process contributes to antigen tolerance, emphasizing the regulatory role of epigenetic modifications in modulating immune tolerance mechanisms. In patients with chronic HIV, differential methylation patterns were observed in cells expressing varying levels of PD-1. In another example, high concentrations of PD-1 (PD-1^{hi} cells) underwent serial demethylation in the Pdcd1 locus, whereas naïve and non-exhausted cells with low PD-1 expression (PD-1^{lo} cells) did not exhibit similar methylation changes. Interestingly, patients undergoing antiretroviral treatment with low viral load did not show remethylation in Pdcd1, indicating a lack of remethylating mechanisms in exhausted CD8 T cells. These findings underscore the complex interplay between PD-1 regulation, epigenetic modifications, and immune exhaustion in the context of chronic HIV infection.[28]

Regarding epigenetic histone modifications associated with PD-1 expression, *Pdcd1* transcription was activated in CD8 T cells in vitro by the following histone modifications at the Conserved Region C proximal cis-regulatory element (CR-C): acetylation of lysine 9 of histone H3 (H3K9^{ac}) and H3K27^{ac}.[29] Other regulatory cis-elements 23.7 and 117.1 did not present histone modifications in cytokine-free cells and only under T cell receptor stimulation (TCR stimulation).[30] However, when ex vivo CD8 T cells were subjected to treatment with the cytokines IL-6 or IL-12 without TCR stimulation, there was extensive monomethylation of lysine 4 of histone 3 (H3K4^{me1}) in both 23.7 and 117.1 sites, without H3K27^{ace}, with no PD-1 biosynthesis. In contrast, by combining cytokine and TCR stimulation there was an enrichment of H3K4^{me1} and H3K27^{ac} marks and an increase in PD-1 expression, indicating that both stimuli are important for the epigenetic activation of *Pdcd1*.[30]

The epigenetic regulation of PD-L1 is extensively described in the literature, especially in solid tumors.[31] The epigenetic activation and inactivation of PD-L1 biosynthesis are basically the result of 4 classes of mechanisms: histone acetylation, histone methylation, histone phosphorylation, regulation mediated by non-coding RNAs - microRNAs (miRNAs), long non-coding RNAs (lncRNAs) and circular RNAs (circRNAs).[31] Acetylation of histone H3 tail lysine in the PD-L1 promoter is related to increased PD-L1 expression in several drug-resistant tumor cells, mainly in breast cancer, hepatocellular carcinoma and lung cancer.[32] Histone acetylation/deacetylation in PD-L1 is mediated by the balance between HDACs and histone acetyl transferases (HATs).[31] Among HDACs, HDAC3 is the most active isoform in solid tumors.[33] PD-L1 and IFN γ levels in the tumor microenvironment and in dendritic cells tend to increase as a function of HDAC3 inhibition,[33,34] while in breast, lung and hepatocellular carcinoma tumor cells HDCA3 appears to be related to drug-resistant tumors.[32]

Another histone methyltransferase that regulates PD-L1 expression in some types of tumors, such as melanoma and lung cancer, is EZH2, of the polycomb repressive complex 2.[35] In the first case, inactivation of EZH2 leads to a decrease in PD-L1 transcription in melanoma. In lung tumors, EZH2 activation tends to increase PD-L1 levels.[36]

In addition to histone acetylation and methylation in PD-L1, histone phosphorylation appears to be related to multiple targets associated with tumor proliferation and metastasis.[37] One of the most active kinases in this context is Pyruvate kinase isoform M2 (PKM2), capable of moving to the nucleus after its phosphorylation is induced by epidermal growth factor (EGF).[37] In hepatocellular carcinoma cells, PKM2 phosphorylated at Ser37 can translocate to the nucleus and catalyze the phosphorylation of H3 at Thr^{11} in the PD-L1 locus, activating its transcription.[38]

PD-L1 biosynthesis can also be regulated by several types of ncRNAs. miRNAs are a class of ncRNAs with 19–22 nucleotides, capable of regulating cellular processes such as cell proliferation, metastasis, and apoptosis by pairing their bases at scattered sites in the 3' untranslated region (3' UTR) of mRNA targets.[39] In lung tumors, aberrantly expressed miRNAs of the miR-3127-5p type are related to the induction of PD-L1 expression and the processes of tumorigenesis, invasion, migration and chemoresistance.[40,41] Other miRNAs, such as miR-377-3p and miR-155-5p, when downregulated, lead to an increase in the production of PD-L1.[42]

Additionally, the microRNAs miR-3127-5p, miR-377-3p miR-55-5p, and miRNAs from the miR-200 family (miR-200a, miR-200b, miR-200c, miR-429 and miR-14 miRNAs) are involved in the regulation of PD-L1 expression in lung cancer.[42] Biosynthesis of miR-200b in patients appears to inhibit PD-L1 expression in patients, leading to inhibition of tumor cell proliferation and migration.[43]

lncRNAs (>200 nucleotides) are tissue-specific transcripts synthesized at low levels, capable of targeting multiple genetic targets and aberrantly produced in tumor cells.[44] lncRNAs can downregulate PD-L1 expression in the tumor

microenvironment, with immunotherapeutic potential.[45] In breast tumors, lncRNA KRT19P3 inhibits PD-L1 expression and activates CD8 T cells, reducing disease progression.[46] The production of the lncRNA GATA3 AS1 appears to be associated with PD L1 deubiquitination, with immune evasion of breast cancer cells.[47]

The last class of PD-L1 regulatory ncRNAs is made up of circRNAs. These "back spliced" RNAs present covalently closed loops and are independent of the linear transcripts encoded by the same gene of origin.[48] The biosynthesis of circRNAs is related to the regulation of tumor development, metastasis and the expression of multidrug resistance associated proteins in liver cancer and glioma.[49] In NSCLC mice, anti-PD-L1 therapy associated with hsa_circ_0003222 inhibition contributed to reducing both tumor volume and cellular resistance to anti-PD L1 therapy in NSCLC cells.[50]

Epigenomics and Immunotherapies

Cancers are characterized by some genetic mutations that carry multiple epigenetic modifications that can be - probably - recognizable to the immune system, especially in humans. This tolerance of the immune system develops due to the resistance that tumors acquired, whether by systemic dysfunction of T-cell signaling, local immune suppression and others.[51–53] It has been studied for many years that the manipulation of the immune system can fight tumor growth, therefore, the role of immunotherapy aims to fight multiple types of cancer, such as lung cancer, colorectal cancer and others.[53,54] Through the years some immunotherapy combo drugs have been approved by the American Food and Drug Administration (FDA), such as Nivolumab or Opdivo (developed by Bristol-Myers Squibb – BMS – NASDAQ: BMY) and Pembrolizumab or Keytruda (developed by Merck – NASDAQ: MRK).[55]

Both Nivolumab and Pembrolizumab consist of a IgG4 monoclonal antibody inhibitor of Programmed Death-1 (PD-1). The binding between PD1 and one of its two ligands (PD-L1 or PD-L2) results in the emission of an inhibitory signal that decreases T-cell proliferation, cytotoxic activity, and cytokine synthesis.[56,57] Therefore, both combo drugs act to block PD1 binding to its ligands mediated by a monoclonal antibody, acting as an anti-PD1 ligand, thus having antitumor activity.[56]

In fact, both were approved in the same time frame by the FDA in the end of 2014. However, Pembrolizumab is projected to generate at least U$22.5 billion in revenue by 2025, according to an analysis by Global Data, a data and analytics company. In 2019, Pembrolizumab generated more than U$7 billion in revenue and, based on more than U $5 billion it earned in the first half of 2019 it could hit U$10 billion or more by the end of 2020.[24,58,59]

Other combo drugs have also been approved by the FDA, such as Bevacizumab (Avacin®) and Isatuximab (Sarclisa®). Bevacizumab consists of a humanized IgG1 monoclonal antibody that binds to VEGF and blocks its binding to the receptor on cancer cells, inhibiting the formation and growth of tumor blood cells.[60] This drug has been approved by the FDA for treatment of women with advanced ovarian cancer in 2018, being considered the first-line treatment for this type of cancer.[61]

Isatuximab consists of a chimeric IgG1 that binds to CD38 expressed on the surface of tumor cells, inducing apoptosis of the tumor cells and activation of immune mechanisms.[55,62] This drug was recently approved by the FDA for use against multiple myeloma and hematological malignancies. This drug also demonstrated potent antitumor action against non-Hodgkin's lymphoma and acute lymphoblastic leukemia.[62,63]

Advances in Immunotherapy Success Based on Reliable Epigenetic Biomarkers

In the context of immunotherapy, biomarkers are epigenetic modification events with the potential to act as molecular predictors of cancer response to immunotherapy. Biomarkers are often used in personalized approaches to cancer immunotherapy.[13]

Among the main biomarkers we can mention the occurrence of genetic alterations that are associated with treatment response, the biosynthesis of tumor-associated antigens (TAAs),[64] the biosynthesis of Programmed death-ligand 1 (PD-L1) and abnormal human leukocyte antigen (HLA) expression,[65,66] tumor mutational trend and neoantigen identification,[67] changes in the TCR repertoire,[68] mismatch repair deficiency,[69] the accumulation of Tumor-infiltrating lymphocytes (TILs),[70] and the presence of cells - mainly regulatory T cells (Tregs), tumor-associated macrophages

(TAMs), and polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs)] that inhibit antitumor immune responses within the tumor microenvironment (TME).[71,72]

The elucidation of the epigenetic mechanisms that control these events has already been widely demonstrated, as well as their relationship with the development of tumors and the chances for successful treatments. Examples of cellular epigenetic changes leading to carcinogenesis,[73,74] tumor growth and branching,[75,76] and treatment resistance and low efficacy have already been demonstrated in cancer types such as B-cell lymphoma and leukemia.[77] As a response to such changes, other epigenetic modifications may even be caused after tumor treatment, such as the methylation of HHLA2, CD96, CXCR5, CCR5 and CCL5 genes in purified CD8+ T cells from immune-treated healthy donors.[78]

The epigenetic biomarkers, taken together, provide an interesting overview of the habits and health of patients, with relatively low invasiveness, since DNA methylation, for example, can be detected in body fluids and by liquid biopsies.[14] In addition, for certain types of cancer, such as lung tumors, biomarkers can indicate the origin and probable evolution of the disease, being one of the parameters for the choice of personalized immunotherapy treatment.[79,80] This highlights the great importance of epigenetic biomarkers not only for diagnosis but also for prognosis and precision therapy.[79]

Despite the intrinsic potential that some epigenetic modifications have as tumor biomarkers, a good biomarker requires precision and accuracy in homogeneous and reliable tests in patient populations to consolidate itself as a useful tool in anticancer therapies.[13,79] Another requirement is the development of precise and highly sensitive methods for the detection of both consolidated and putative biomarkers, as well as the need for clinical analysis laboratories to be adapted to the execution of these methods.[79]

Regarding immunotherapy based on the identification and measurement of epigenetic changes in DNA, preclinical studies in mice, cell lines and patient samples have pointed to potential epigenetic biomarkers associated with immune evasion and response marks or signatures, which can be used as predictors of immunotherapy efficacy (Table 1).

Most studies reported in the literature to date describe epigenetic biomarkers in tissue samples, however additional information on biomarkers at the cellular level or tumor microenvironment is still preliminary. The deconvolution analysis of massive transcriptomic and epigenomic data from the differential expression of genes in tumor tissues is

Table 1 Some of the Main Epigenetic Biomarkers Involved in the Immune Control of Tumors

Biomarker	Source	Modification	Regulation	Cancer type	Ref.
Antigen presentation	TCGA	Hypomethylation of *HLA* and hypermethylation of *CD40, CD80, CD86*	Hypermethylation of *CD40, CD80, CD86* genes and hypomethylation of *HLA* immune checkpoint gene, which were resulted in lower T cell recruitment to the tumor microenvironment, and immunogenicity	Pan-cancer	[81]
	Human Human cell lines Mouse	H3K27me3 and H3K27ac at *MHCI*	Inhibition of H3K27me3 and stimulation of H3K27ac enhance the MHC-I antigen-processing pathway (MHC-I APP) and T cell-mediated antitumor immunity	Small cell lung cancer (SCLC)	[82]
	Mouse	H3K27me3	Histone hypomethylation reduced tumor resistance to immunotherapy and suppressed melanoma growth	Melanoma	[11]
	Human	H3K27me3 H3K27ac	Trimethylation or acetylation of H3K27 led to dysfunctional antigen presentation and tumor depletion of immunogenic peptides, reducing tumor antigenicity and increasing immune evasion from immunotherapy	Gastric adenocarcinoma	[83]
Novel immune checkpoint blockades (ICBs)	Human	*TNFRSF9* hypomethylation	Increase in gene expression, higher progression-free survival (PFS) and robust response to anti-PD-1 Ab therapy	Melanoma	[84]
	TCGA	*ADORA2A* hypomethylation	Increase in gene expression, immune cell infiltration and patient survival	HNSCC	[85]
$CD8^+$ T cell exhaustion	Mouse	$CD8^+$ T cell genome-wide methylation	Inhibition of De Novo DNA-Methylation synergizes with PD-1 blockade and enhance rejuvenation of $CD8^+$ T cells	Pan-cancer	[86]
	Human (ex vivo)	*PRF1* hypomethylation	High expression of the perforin protein, restoration of Trm antitumoral cytotoxic activity stimultated by IL-15	Urothelial bladder cancer (UBC)	[87]

(*Continued*)

Table 1 (Continued).

Biomarker	Source	Modification	Regulation	Cancer type	Ref.
$CD8^+$ T cell infiltration	TCGA Singapore cohort	*PDCD1LG2* hypermethylation	Increase of CD8+ T cell tumor infiltration and tumor mutational burden by methylation of several CpGs sites within the locus *PDCD1LG2*	Gastric adenocarcinoma	[88]
	Human, mouse	*CCL5* methylation	*CCL5* hypomethylation in humans leads to $CD8^+$ T cell tumor infiltration and longer patient survival and response to anti-PD-1 Ab ICB. In ID8 mouse ovarian cancer tissue, hypermethylation of *CCL5* led to impaired TIL recruitment and CXCL9 secretion by TAMs, decreasing animal survival and the response to anti-PD-1 Ab.	Melanoma, breast, ovarian, colon, and lung	[89]
	Human Mouse	H3K27me3 at *CXCL9*	Epigenetic silencing of CXCL9 triggers CD8+ T cell tumor infiltration tumor regression, immunological memory and an increase in overall survival	Glioblastoma Ovarian cancer	[90]
	Mouse	H3K27me3 at Th-1 genes	Trimethylation of H3K27 at Th-1 genes repress the production of T helper 1 (TH1)-type chemokines CXCL9 and CXCL10 by tumor cells. Increases T-cell trafficking to the tumor microenvironment, slows down tumor progression and improves the efficacy of PD-L1 checkpoint blockade	Ovarian cancer	[6]
	TCGA Human cell lines Mouse	Nuclear receptor-binding SET domain protein 1 inactivation	Downregulation of chemoattractant cytokines and tumor immune desertification	HNSCC	[91]
	Mouse	Lysine demethylase 1 (LSD1) inhibition	LSD1 depletion enhances tumor immunogenicity and T cell infiltration	Melanoma	[92]

a promising tool for the characterization and identification of cell types with deregulation of gene expression, with the potential to convert into tumors and, if this has already occurred, to suggest more effective immunotherapies with fewer side effects.[81]

In cancerous fibroblasts, for example, aberrant activation of the transforming growth factor (TGF)-β biosynthesis pathway, observed by DNA methylation-based deconvolution analysis, can lead to tumor immunosuppression, and decrease the response to novel immune checkpoint blockade (ICB).[82]

An interesting epigenetic mediator is EZH2, a component of the PRC2 H3K27me3 chromatin writer complex. This gene encodes a histone-lysine N-methyltransferase that acts as one of the main biochemical regulators of transcriptional silencing associated genes that lead to terminally differentiated CD8+ effector T cells.[83] Epigenetic silencing by histone methylation mediated by the activation of EZH2 and by DNA methylation by DNA methylase 1 (DNMT1) may constitute mechanisms of induction of tumor formation. In mice bearing ID8 ovarian cancer tumor cells, inhibition of EZH2 and DNMT1 markers suggests increased expression of the Th1 chemokine-encoding genes Cxcl9 and Cxcl10 in ovarian tumor cells.[84] To corroborate the role of both markers in the success of the treatment of this type of cancer, epigenetic therapy combined with immunotherapy in ID8 positive mice promoted an increase both in CD8+ T cell infiltration and the anti-PD-L1 Ab therapeutic effect in compared with the control submitted only to immunotherapy.[6] Thus, immune evasion mediated by epigenetic silencing of Th1 chemokine-encoding genes can be partially reversed by epigenetic modulation of these same target genes.[6]

Another example of an epigenetic mediator active in the immunotherapy of ovarian cancer is the leukemia inhibitory factor (LIF). LIF-mediated epigenetic silencing of the Cxcl9 gene in TAMs isolated from ID8 ovarian cancer mice induced a lower infiltration of CD8+ T cells into the tumor and, after LIF blockade, there was a decrease in the efficacy of anti-PD Ab −1.[84] In human ovarian tumors, the overexpression of genes encoding CCL5 and CXCL9 was shown to be correlated with an increase in tumor infiltration of CD8+ T cells, better prognosis of patient survival, in addition to increasing the antitumor action of anti-PD-1 Ab ICB.[85] Regarding ovarian cancer in ID8 mice, in tumors that express Cxcl9, methylation of the gene encoding Ccl5 led to decreased recruitment of Tumor infiltrating lymphocytes (TILs) and secretion of CXCL9 by TAMs, in contrast to tumors with low Cxcl9 expression levels.[85] As a result, there was a decrease in both the response to anti-PD-1 Ab and the survival of the animals.[85]

In the case of head and neck squamous cell carcinoma (HNSCC), DNA hypomethylation and deleterious structural alterations in histone methyltransferases seem to present a direct correlation with the reduction of immunotherapeutic effects.[86] The combination of DNA hypomethylation and inactivation of protein 1 of the SET domain binding to the histone methyltransferase nuclear receptor (NSD1), in a subtype of HNSCC, generated a decrease in both the infiltration of immune cells and the expression of PD-1 in cells. T CD8+ in relation to tumors that have active NSD1.[86] In an HNSCC xenograft mouse model, the suppression of NSD1 only in tumor cells resulted in the inhibition of the biosynthesis of chemoattractant cytokines and in the absence of CD8+ T cells both in the tumor and in its microenvironment when compared to the WT control.[86] Specifically, the adenosine A2A receptor (ADORA2A) promoter methylation in HNSCC tumors was correlated with immune cell infiltration and increased overall patient survival.[87]

Tumor recruitment of lymphocytes promoted by epigenetic mechanisms, mainly DNA extensive methylation, also has the potential to increase the positive effects of immunotherapy based on ICBs. The super methylation of CpGs at the PDCD1LG2 gene *locus*, encoding the Programmed cell death 1 ligand 2 (PD-L2) seems to modulate the infiltration of CD8+ T cells and to promote multiple mutations in gastric adenocarcinomas.[88] In other tumors, mainly immunogenic tumors such as melanoma, hypomethylation of immunological synapse genes such as HLA, CD40 and CD80 seems to increase the tolerogenicity of the tumor microenvironment.[89]

Another enzyme active in the epigenetic control of melanoma is histone lysine demethylase 1 (LSD1). Inactivation of the LSD1 encoding gene in B16 melanoma tumors in mutant mice, promoted greater infiltration of CD4+ and CD8+ T cells and increased immunogenicity of cancer cells compared to non-mutant controls.[90] Still in the field of methyltransferases, the overexpression of the gene encoding Enhancer of zeste homolog 2 (EZH2), a histone-lysine N-methyltransferase enzyme in three different murine models of melanoma (B16-F10, RIM3 and $Nras^{Q61KInk4a-/-}$), both treated with anti-CTLA-4 Ab showed epigenetic repression of the tumor immunogenic genes MHC-I and Cxcl9, inhibiting their presentation to immune cells when compared to controls.[11]

These different scenarios of correlation between the presence of a specific collection of biomarkers, the personalized immunotherapy indicated for each type of tumor and the expectation of survival of patients after treatment begin to be better understood as researchers look at massive data from epigenomic studies. Some of these correlations mentioned above can be seen on Table 2.

Using Epigenetic Modifications to Eliminate Tumor Cells and Tumor-Associated Immune Cells

Drugs with epigenetic action capable of modulating the biosynthesis of immune signaling components are being extensively studied, both structurally and functionally.[91,92] Among the best characterized are histone deacetylase inhibitors (HDACi) and DNA methyltransferase inhibitors (DNMTi).[97]

Table 2 Some Immunotherapy Treatments, Their Associated Epigenetic Hallmarks Found in Cancer Patients and Clinical Outcomes

Cancer	Treatment	Epigenetic Mark	Results	Ref.
Lung cancer	Use of anti-PD-1 monoclonal Ab	Systemic low methylation	Lower tumor immunity	[93]
Gastric cancer	Use of anti-PD-1 monoclonal Ab	H3K27ac/H3K4me3	Resistance to anti-PD-1 Ab	[8]
Melanoma	Use of anti-PD-1 and anti-CTLA-4 monoclonal Abs	Low CTLA-4 methylation	Good response to therapy	[94]
NSCLC	Use of anti-PD-1 monoclonal Ab	*FOXP1* demethylation and EPIMMUNE epigenetic signature	Clinical benefit with PD-1 blockade	[80]
NSCLC	Use of anti-PD-1 monoclonal Ab	PD-L1 methylation	Anti-PD-1 Ab resistance	[95]
CLL	Use of CART-19 cell therapy	*TET2* disruption	Enhanced CAR-T cell activity and complete remission	[96]

DNMTi, known as demethylating agents, are the most widely used epigenetic therapy for the treatment of various types of cancer. These inhibitors are cytidine nucleoside analogues capable of binding specifically to DNMT DNA proteins, irreversibly sequestering them. With DNMTs blocked, extensive DNA hypomethylation and modulation of genes associated with tumor development occur.[97]

HDACis, in turn, inhibit the function of histone deacetylases and cause changes in the degree of rigidity of compacted and condensed DNA in nucleosomes. Treatment of tumors with DNMTi, HDACi, or both may make immunosuppressive TME more attractive for and enhance lymphocyte infiltration.[19,98] These effects are a consequence of increased expression of tumor antigens or greater efficiency in their presentation, emergence of "viral mimicry" effects, inhibition of exhausted T cell biosynthesis, induction of inflammation by increased expression of chemokines or a combination of two or more of these factors.[98,99]

Methylation of DNMTis can lead to cycles of recurrent Cancer-testis antigens (CTAs) biosynthesis in many different solid tumors, and the use of 5-azacytidine, the chemical analog of cytidine - widely used for the treatment of leukemias, can increase the amount and the recruitment of antitumor T-lymphocytes in patients with Hodgkin's lymphoma. Thus, apparently, the inhibition of DNMT tends to increase the efficiency of the presentation of new antigens and potentiate the immunogenicity in tumor cells[93,94].

Epigenetic drugs can regulate other TAAs in addition to the CTA. Some of the most tested TAAs are those associated with high molecular weight melanoma (HMW-MAAs). The nucleotide analog 5-AZA-CdR, a prodrug that requires activation via phosphorylation by deoxycytidine kinase, demethylates the promoter of the gene encoding HMW-MAAs in melanoma cells, resulting in the re-expression of HMW-MAAs that triggers lymphocyte recruitment- anti-tumor drugs and increased tumor immunogenicity.[95]

The literature has shown that DNMTis are able to induce greater up-regulation of CTA than by HDACi, however, the use of HDACi can activate a greater expression of major histocompatibility complex (MHC) class I and boost antigen presentation. In a mouse melanoma cell model, the use of romidepsin, the selective inhibitor of histone deacetylase, approved in the US in 2009 for the treatment of cutaneous T-cell lymphoma (CTCL) for the inhibition of HDACi led to an increase in the expression of MHC-I and CD8+ T cell death, in addition to up-regulation of genes involved in the processing and presentation of MHC class I antigens, such as TAP1, TAP2, LMP2, LMP7 and B2M.[7,96,100,101]

One of the most important roles of DNMTis is the upregulation of immune signaling in cancer and, in this context, the main route by which this goal is achieved is the viral mimicry pathway. DNMTis actively promote the transcription of dsRNA-encoding genes in ovarian cancer cell lines, repressing the silent expression of hypermethylated endogenous retroviruses (ERVs). The presence of high concentration of specific dsRNA activates dsRNA cytoplasmic protein sensors, which trigger downstream signaling pathways, inducing the biosynthesis of inflammatory chemokines such as IFN-β.[16] In this context, the viral mimicry pathway would act as a potent activator of antitumor immunogenicity mediated by the production of type I and type III interferons, increasing antigen presentation and neutralization of cancer cells in the TME.[102]

Other genomic components that can be exploited in the epigenetic fight against cancer are the Endogenous retroviruses (ERVs). ERVs are repetitive elements abundantly dispersed in the human genome that act as methylation silencing island sites.[98] Treatment of tumors with DNMTis induces tumor cells to enter a previously mentioned "viral mimicry" state, in which their metabolism is programmed as if a viral invasion had successfully occurred, triggering the activation of the interferon pathway and considerably increasing the effectiveness of immune checkpoint inhibitors.[16,102] In addition to ERVs, histone epigenetic modification may relate to tumor immunotherapy via the interferon pathway. The use of histone deacetylases (HDACs) and the H3K4me1/2 eraser, KDM1A appear to have similar effects in inhibiting ERV and ERV-induced interferon pathway activation.[98,103]

A third point to be explored in the treatment of cancer mediated by epigenetic alterations is T cell exhaustion, one of the major causes of immune evasion. T cell exhaustion is the metabolic consequence of the induction of T cell differentiation by continuous antigen stimulation, resulting in impaired cell function.[104] Exhausted T cells show over-expression of multiple inhibitory membrane receptors, including PD-1, and reduced production of effector molecules.[105] The accumulation of these cells may represent a drawback in the effectiveness of immunotherapies, since they seem to be related to non-responsiveness in patients medicated with checkpoint inhibitors and even in tumor recurrence. Exhausted

T cells that have their PD-1 blocked can be reverted, partially and temporarily, to normal T cells, an effect that can be prolonged using complementary epigenetic therapies. Exhausted T cells treated with HDAC inhibitors can completely return to their native functional state.[12,106]

Another cellular strategy, at the epigenetic level, to stimulate the immune system to fight tumors is to suppress the production of some chemokines, such as Thelper1 (Th1) type chemokines CXCL9 and CXCL10.[6,107] Recent studies have shown that the biosynthesis of these chemokines can protect the tumor from the responsive action of the immune system, inhibiting the T cells trafficking to the tumor adjacent regions and immune infiltration of TME.[6]

In ovarian tumors, regulation of CXCL9 and CXCL10 synthesis is performed by the addition of H3K27me3 by DNMT1.[6] Using DNMTi, it is possible to reverse the inhibition of the genes involved in the biosynthesis of CXCL9 and CXCL10, increasing their concentrations and infiltration of Th1 tumors.[6] Similar effects can be observed epigenetically in lung tumors, in which increased expression of T cell chemokines by the action of HDACis may lead to increased TME infiltration.[108] An overview of the main intracellular targets of epigenetic changes for modifying the TME to treat cancer can be seen in Figure 2.

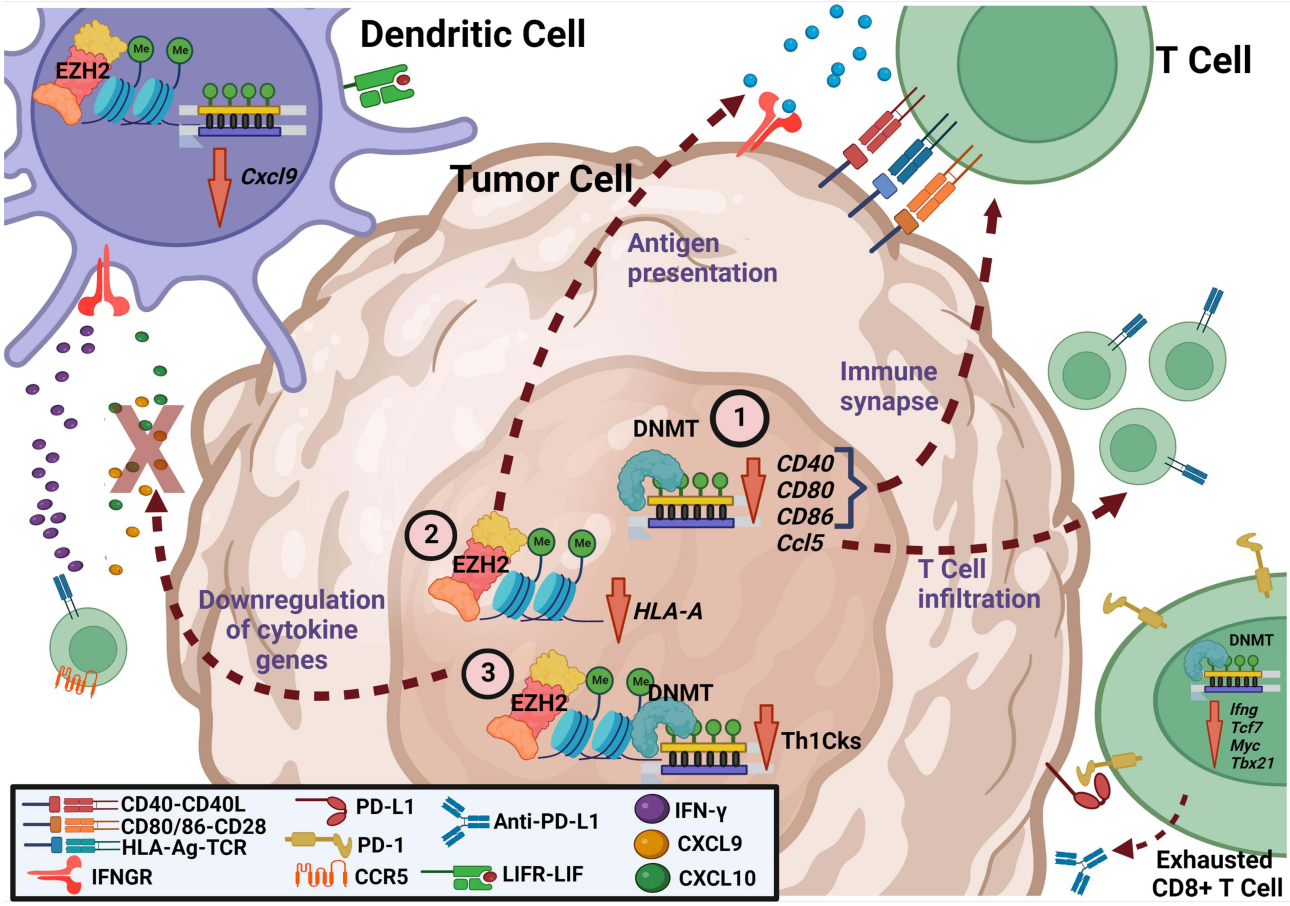

Figure 2 Main immunotherapy targets and therapeutic effects for the tumor microenvironment (TME) at the cellular level. The expression of genes related to the immune system can be modulated by DNA and histone methylation. 1) Hypomethylation of immunological synapse genes (*CD40, CD80* and *CD86*) of 20 human solid tumors, such as melanoma, by DNMT1 demonstrated correlation with modulation of TME tolerogenicity by infiltration of CD4+ and CD8+ T cells. In different ovarian cancers, overexpression, and silencing of the *Ccl5* gene, respectively by demethylation and hypermethylation mediated by DNMT1, can modulate tumor infiltration by CD8+ T cells. In human ovarian cancer, overexpression of *Ccl5* leads to increased tumor immunoinfiltration. In ID8 ovarian tumors in mice, *Ccl5* silencing by hypermethylation is related to deficiency in both tumor-infiltrating lymphocyte recruitment (TILs) and Chemokine (C-X-C motif) ligand 9 (CXCL9) secretion by tumor-associated macrophages (TAMs). 2) Antigen presentation, necessary for the antitumor cytotoxic response, can be inhibited by EZH2-mediated histone methylation. In human melanoma, EZH2 inhibition and consequent upregulation of the HLA-I gene increased the recruitment and infiltration of CD8+PD-1+ T cells and CD20+ B cells into the TME. 3) Both DNA and histone methylations, respectively by DNMT and EZH2, result in partial or total downregulation of chemoattractant cytokine genes (Th1 Cks) *Cxcl9* and *Cxcl10*, decreasing recruitment of CD8+ T cells to the TME in mouse ovarian tumors.

Enhancing Immune-Oncology Strategies

Immuno-oncology is an emerging field in cancer treatment that aims to harness the power of the immune system to treat cancer cells.[13] One specific example was pointed in this article regarding the genes PD-1 and PD-L1, however there are several strategies that can be used to enhance the effectiveness of immune-oncology treatments, including the following (also shown on Figure 3):

(A) Targeting Immune Checkpoints: Cancer cells often use immune checkpoints to evade the immune system. Immune checkpoint inhibitors are a class of drugs that block these checkpoints, allowing the immune system to target and destroy cancer cells more effectively. Examples of these inhibitors are PD-1 and CTLA-4.[109]

(B) Adoptive Cell Therapy: Adoptive cell therapy is a type of immunotherapy that involves removing immune cells from the patient's body, genetically modifying them to target cancer cells, and then infusing them back into the patient. This strategy is designed to boost the number and activity of immune cells that can effectively target cancer cells.[110]

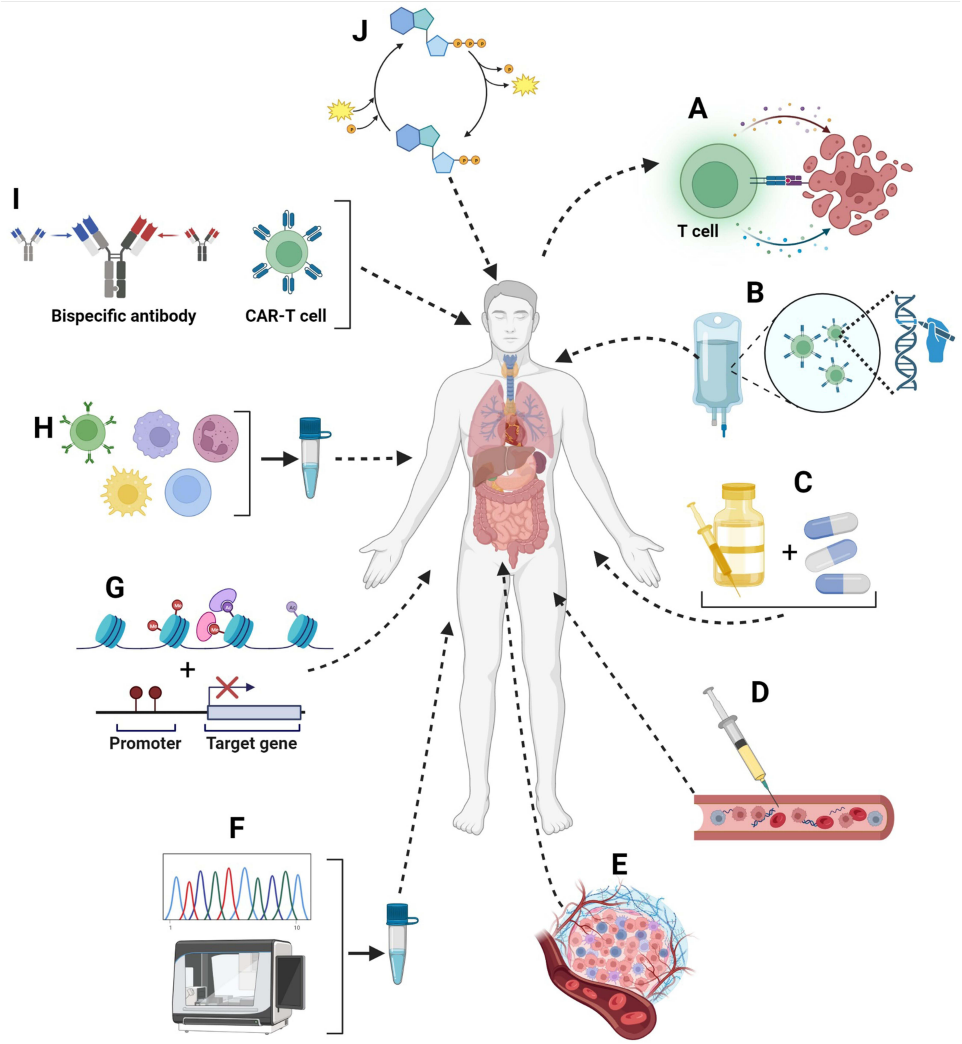

Figure 3 Examples of approaches available to increase the effectiveness of immuno-oncology therapies. (**A**) use of immune checkpoint inhibitors to target and destroy tumor cells; (**B**) anticancer immunotherapy based on the use of the patient's own genetically modified immune cells; (**C**) combination of two complementary anticancer therapies; (**D**) administration of anticancer vaccines in either a prophylactic or therapeutic approach; (**E**) use of oncolytic viruses, cytokines and colony stimulating factors to make the TME more conducive to immune cell infiltration; (**F**) genomic investigation of tumors for the personalized choice of anticancer immunological strategies; (**G**) prediction of antitumor responses through the detection and characterization of biomarkers; (**H**) use of immune cells to specifically target cancer cells; (**I**) use of CAR-T cells, T-cell receptor (TCR) gene therapy, and bispecific antibodies to activate and reprogram the immune system against cancer cells and (**J**) identification of targets in the specific metabolism of tumor cells to make them more recognizable by components of the immune system.

(C) Combination therapies: Combining different types of immunotherapies or combining immunotherapy with traditional chemotherapy or radiation therapy can enhance the body's overall anti-tumoral response. This can help overcome resistance to single agent therapies and improve the response rate for patients.[22]

(D) Cancer Vaccines: Cancer vaccines are a type of immunotherapy that can be used to help the immune system recognize and target cancer cells. These vaccines can be used in both a prophylactic or therapeutic setting. Several companies are evaluating the use of RNA therapies such as the one used to develop COVID19's vaccine against cancers.[111,112]

(E) Tumor Microenvironment Modulation: The tumor microenvironment plays a crucial role in cancer progression and response to therapy. Agents such as agents like oncolytic virus, cytokines, colony stimulating factors can be used to modulate the tumor microenvironment, making it more conducive to immune cell infiltration, activation and killing of cancer cells.[82,98]

(F) Personalized medicine: Advances in genomics and precision medicine have led to the development of targeted therapies that can be tailored to the specific genetic makeup of an individual's tumor. These therapies are designed to target specific mutations or other features of a cancer cell that make it more susceptible to the immune system. PD-1 and PD-L-1 targeted drugs are one example of these types of therapies.[20]

(G) Development of Biomarkers: Biomarkers are molecular signatures or characteristics of cancer that can be used to predict a patient's response to immunotherapy, allowing for more personalized treatment options.[67]

(H) Immune cell-based therapies: Usage of different subsets of immune cells as therapies has been proposed, such as T cells, natural killer cells, macrophages etc. These cells are either isolated from patient's blood or expanded ex-vivo and used as therapies to specifically target the cancer cells.[13]

(I) Reprogramming the Immune System: There are several strategies that are being developed to reprogram the immune system to recognize and target cancer cells. These include the use of CAR-T cells, T-cell receptor (TCR) gene therapy, and bispecific antibodies.[84,110]

(J) Immune-Metabolism: Cancer cells are known to have a unique metabolic profile which can be targeted to make them more vulnerable to immune cells. By understanding the metabolic pathways that cancer cells utilize, it may be possible to develop therapies that target these pathways and thereby make cancer cells more visible to the immune system.[111]

However, it is important to note that while these strategies show promise, they are still in the early stages of development and more research is needed to fully understand their potential and limitations. It's important to note that many of the strategies above are being tested in clinical trials[22,24,59] and not all of them have yet been approved for general usage. As such, it is important to work with a cancer specialist to understand the options available and which may be the best fit for a particular patient.

Concluding Remarks

The burgeoning understanding of epigenetic modifications' pivotal role in the genesis and modulation of various cancers stands as a captivating domain within modern medicine. Compounds like HDACIs, DNMTIs, and PD-1/PD-L1 blockers exhibit substantial potential for heightened efficacy against leukemia, lymphoma, and solid tumors. Numerous immunotherapy combination drugs, some already in the market, present promising results, propelling the need for precise comprehension of extensive epigenetic alterations in DNA and histones.

These modifications interplay intricately with environmental factors, shaping the genesis and progression of diverse tumors. Concurrently, the advent of immunotherapy drugs necessitates the identification of reliable biomarkers for predicting tumor development and prognosis. Personalized cancer therapies, typified by Nivolumab and Pembrolizumab targeting PD-1/PD-L1 axis overexpression, underscore the significance of biomarker-based treatments. Notably, these drugs prove ineffective in cases with diminished or absent biomarker expression, a phenomenon attributed to epigenetic regulation of these genes as discussed in this review article. Consequently, the synergy of epigenetic therapies and immunotherapy emerges as a promising avenue for enhancing cancer treatment efficiency.

Furthermore, delving into the epigenetic strategies of tumor evasion and mechanisms governing immune system defense cell activation can revolutionize anticancer immunotherapies. This expanding knowledge base, coupled with technological advancements, holds the potential to elevate the prospects of successful cure, even for aggressive tumor types previously deemed untreatable. Additionally, the pursuit of humanized and effective treatments promises to enhance the overall quality of life for cancer patients.

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Disclosure

The authors declare that there are no conflicts of interest in this work.

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