REVIEW ARTICLE

Combining Epigenetic and Immunotherapy in Cancer: Molecular Mechanisms

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Abstract

BACKGROUND: Immunotherapy, particularly the idea of immune checkpoint blockage is currently draw much attention in cancer treatment. It has been approved as an adjuvant, however, it cannot be a single cancer treatment.

CONTENT: The discovery of the basic ligand-receptor interactions between immune and cancer cells inside the tumor microenvironment has led to the current interest in immunotherapy, specifically immune checkpoint inhibition. Different ligands produced by cancer cells interact with immune cells' surface receptors, activating inhibitory pathways, such programmed cell death protein 1 (PD-1)/ programmed cell death ligand 1 (PD-L1), that cause immune cells to become immunologically tolerant. On the other side, epigenetic modulators also play a critical role in enhancing the tumor microenvironment and regaining immunological recognition and immunogenicity. Some findings showed that such immune suppression can be reversed through various mechanisms involving antigens pathways, immune genetic, and epigenetic pathways. These findings have created a very encouraging foundation for research on the combination of epigenetic and immunotherapeutic drugs as cancer treatments.

SUMMARY: The effectiveness of this suggested paradigm can only be demonstrated by clinical studies. Epigenetic treatment might replace immune checkpoint therapy as a powerful new cancer care technique that is generally well tolerated and should be proven with adequate clinical trials.

KEYWORDS: epigenetics, immunotherapy, PTM, DNMT, HDAC, immune check point

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Introduction

In cancer, the complex networks to maintain gene expression homeostasis was deranged, allowing cells to grow without regard to the demands of the organism. Determining the subset of cellular regulatory mechanisms that are perturbed in human cancer has come a long way. One fundamental idea that has progressed the discipline is the understanding that many sets of cellular regulatory mechanisms are impacted and heritably impaired in almost all malignancies. (1) In the past, research has concentrated on the genetics of cancer, notably how mutational oncogene activation or inactivation of tumor-suppressor genes (TSGs) underlies the modifications to the pathways mentioned above. Since 1990s, many studies since have showed us that the evolution of cancer types in human also affected by the epigenetic shifting.(2)

Chromatin-disrupting genetic, metabolic, and environmental factors change cellular states and responses, predisposing people to a variety of prevalent disorders. Although chromatin and epigenetic abnormalities play a significant role in tumor potentiation, initiation, and development, cancer is often thought of as a hereditary illness.(3) Abnormalities in chromatin structure and/ or organization as well as aberrant DNA methylation

patterns and histone post-translational modifications (PTM) patterns are examples of epigenetic alterations. Disrupting the epigenetic machinery, which is now recognized to be disturbed in cancer, is a major factor in changes in the epigenome. These epigenomic alterations affect the expression patterns of genes that would otherwise be wild-type, and in some situations, they may even be the cause of those altered expression patterns. The knowledge about cancer epigenetics and the epigenome involve in cancer genesis and progressive give insight for cancer prevention and treatment strategies.(4)

Immunotherapy has transformed cancer care, but its effectiveness is still constrained in most clinical situations. Cancer affects the systemic immune system and alters them. The connections of many cell lineages throughout tissues control immunity. To better understand cancer immunology, it is necessary to evaluate the systemic immune landscape in addition to the tumor micro-environment (TME). Importantly, effective naturally occurring and medically generated antitumor immune responses depend on the peripheral immune system. Current research reveals that immunotherapy triggers new immune responses rather than reactivating already present ones.(5)

The fact that chromatin landscapes in cancer are significantly altered suggests that epigenetic modifiers may play a variety of functions in enhancing the effectiveness of cancer immunotherapy by combining epigenetic-targeting medicines with immune checkpoint inhibitors. Both in cancer and health, epigenetic modifiers especially the chromatin landscape play a role in lymphocyte proliferation and differentiation. The epigenetic regulators make up a very minor portion of the known landscape-shaping elements. To further understand the function of epigenetic regulators in cancer immunotherapy, data from animal models and ongoing clinical studies are required.

Immune checkpoint inhibitors have been generally praised for their effectiveness in cancer immunotherapy. However, a lot of cancer patients do not benefit from immune checkpoint treatment, and others experience relapses as a result of developed tumor resistance. Recent research suggests that epigenetics might enhance cancer immunotherapy. Thus, targeting epigenetics to alter immune cell development and function, and reverse the cancer immune escape mechanism may be helpful. These are focused on the epigenetic regulators of histone acetylation, methylation, and DNA methylation as well as inhibitors of the immunological checkpoints cytotoxic T-lymphocyte associated protein 4 (CTLA4) and programmed cell death protein 1 (PD-1).(6)

Systemic Immunity in Cancer

Inflammation is known to be involved in a lot of illness including cancer, where chronic inflammation become one of cancer's hallmark since it initiates tumorigenesis as well as supports the tumour growth. Inflammation also known to be closely related with immune system, and that's how tumour progression, inflammation and global immune system are linked each other.(7)

In cancer, immune system was altered in many ways. The prolonged inflammation in early carcinogenesis increases neutrophils and monocytes in order to kill the cancer cells. On the other hand, neutrophils can also support the tumour growth by releasing toxic substance that increase DNA damage and increase angiogenesis. Extensive disruption of this hematopoiesis system further increases immature neutrophils and monocytes. Finally there is an accumulation of of immature immunosuppressive neutrophils, monocytes and macrophages.(8)

Besides disrupted hematopoiesis system, some studies showed the alteration of dendritic cells in cancer as well. Dendritic cells are the critical regulator for cluster of differentiation (CD)8^+ and $CD4^+$ T cell priming, differentiation and also proliferation. The maturation of dendritic cells was inhibited by vascular endothelial growth factor (VEGF) induced by tumour cells, even in pancreatic cancer, the dendritic cell goes to apoptosis mediated by interleukin (IL)-6.(9) Hence, the $CD4^+$ T cells and regulatory T (Treg) cells were suppressed.

Another suppressive immune cell in tumour progression is B cells. While the number of total B cells is not reduced, but activity was inhibited by CTLA4 and PD-1. Then, the tumour will infiltrate B cells to secrete pro-tumour factors and lymphotoxins to promote angiogenesis.(10) Natural killer (NK) cells are another important antitumour immunity that can kill tuour cells fast and directly. The phenotypes of NK-cells also altered in cancer, and have decreased expression of activating receptors, since NK cells as all antibodies were produced by B cells. Thus, the NK cells ability to kill and degranulate tumour cells was impaired.(11)

Last but not least, the mutation acquired by tumour cells disable the immune check points such as PD-1 and CTLA4, and can therefore continue unimpeded into S-phase, through G2-phase as well as into mitosis and then bring their chromosomal DNA damage. Altogether, these immune disruption supports tumor growths and progressive.(12)

Non-genetic Therapy Resistance in Cancer

Possible customized cancer treatments are now being closer, utilizing particular chemicals required for tumor development and/or maintenance to destroy cancer cells. Over the past two decades, a variety of targeted medicines, including monoclonal antibodies and small-molecule inhibitors that aims to disrupt critical cancer-promoting pathways or block immunological checkpoints, have developed quickly.(13,14) Patient outcomes have been transformed by these medications, many of which are now considered standard of care, either alone or in conjunction with other treatments.

Unfortunately, most cancer patients continue to have therapeutic resistance, which is most frequently shown as local or distant disease recurrence. This is especially true for individuals who had advanced or metastatic cancer at the time of therapy. All treatment approaches commonly leave behind leftover cancer cells, known as minimum residual disease (MRD), which act as a reservoir from which cancer recurrence inexorably develops. The main factor frequently cited for therapeutic resistance is genetic evolution, in which one or more cancer cells possess or acquire a particular genetic change, such as a mutation, gene amplification, gene deletion, or chromosomal translocation, that gives them a clonal advantage to evade the effects of therapy. It is still difficult to pinpoint the molecular processes causing non-genetic resistance, and it is unclear how common genetic and non-genetic mechanisms of resistance are in cancer. In spite of current best efforts, therapeutic resistance remains a formidable adversary in the fight for curative cancer therapy. Notably, there is a strong correlation between the non-genetic processes utilized to resist therapy and the genetic makeup of the tumor.

Cancer treatment resistance can be roughly divided into two categories: primary (intrinsic) such as re-existing genetic mutation (Figure 1A), or during the therapy as the cell's mechanism for survival (Figure 1B), and secondary (acquired) on the primed cell (Figure 1C) or later as the result of rewiring mechanism (Figure 1D). The absence of an objective clinical response after therapy indicates primary resistance. Secondary resistance, in contrast, denotes the tumor returning locally or distantly following a therapeutic response.(15) Although these phrases are common terms,

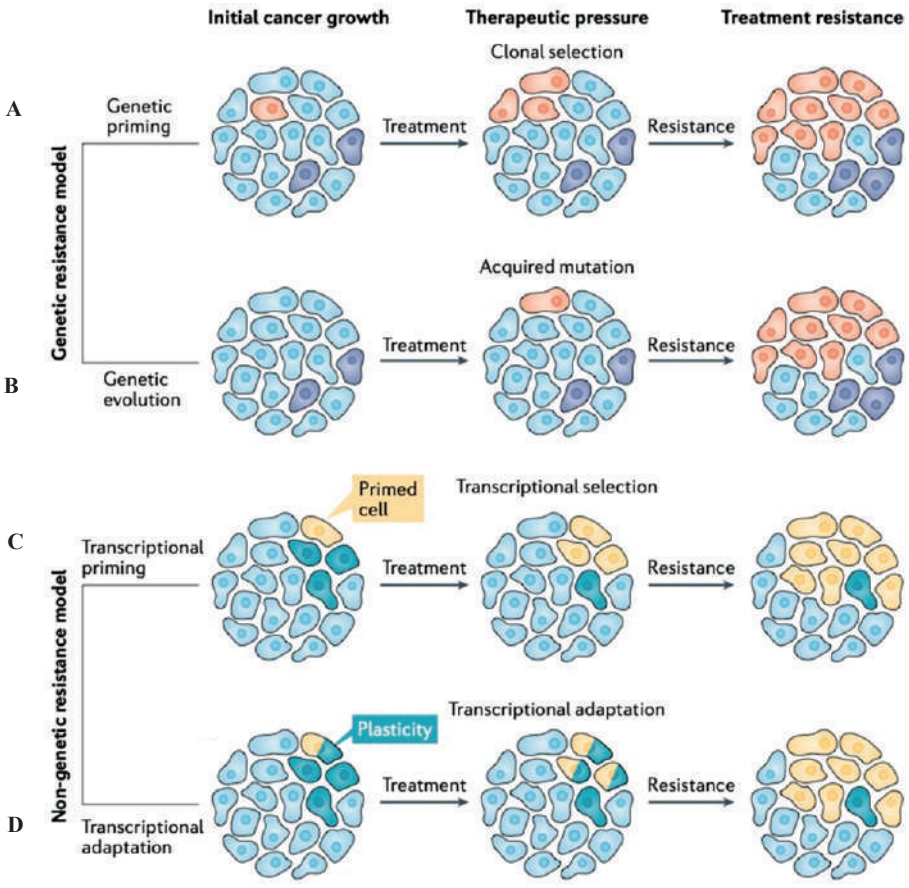

Figure 1. Models of genetic and non-genetic therapy resistance. A: re-existing genetic mutation resistance; B: resistance during the therapy as the cell's mechanism for survival; C: resistance on the primed cell; D: resistance that occur later as the result of rewiring mechanism.(15) (Adapted with permission from Springer Nature).

it's vital to understand that the differentiation depends entirely on the method of answer evaluation. A complicated and quickly developing field, response assessment in cancer comprises a number of modalities, including clinical and pathological evaluation, several imaging techniques, and an expanding array of genetic diagnostics.

There is growing proof that medication resistance cannot be explained by a single genetic factor alone. More and more evidence supports the idea that a single cancer genome may generate many phenotypic states, and that cancer cells can transition between these states without genetic modifications. It's crucial to note that these non-genetic reprogramming processes are seen after therapy exposure, and these adaptive responses are linked to higher treatment resistance. For example, chemotherapy triggers a phenotype-switching process known as the epithelial-mesenchymal transition (EMT) in a variety of epithelial malignancies, including breast, gastric, lung, and colon cancers.(16,17) Chemoresistance has long been linked to this reprogramming step. Similar to this, it is widely known that melanoma cells may alternate between a proliferative and invasive (mesenchymal-like) cell state, with the latter being innately resistant to mitogen-activated protein kinase (MAPK) inhibitors.(18,19) Targeted treatment consistently induces a change in the total cell population towards the undifferentiated cell state, which aids in the development of drug tolerance and/or resistance.(19-21) Notably, this undifferentiated program also seems to be a sign of resistance to PD-1 inhibitors.(22) Similar to how activation of alternative immune checkpoints causes resistance in non-small cell lung cancer (NSCLC) treated with PD-1 checkpoint inhibitors (23), our finding shows that non-genetic adaptive responses happen in response to the majority, if not all, treatment modalities.

Tumor cells that show treatment resistance usually have characteristics of cancer stem cells (CSCs) with a higher tumor-initiating ability.(24-26) In contrast, tumor cells with more differentiation are typically more responsive to cancer treatments. Tissue-specific stem cells have the innate potential to be pliable on the cellular level and to react quickly to pressure or external stimuli.(19,27) Normal stem cells, and hence CSCs, are endowed with greater xenobiotic tolerance due to their lifetime in tissues. It is therefore easy to assume that therapeutic resistance is only the result of passive Darwinian selection. An alternative explanation is that tumor recurrence might be brought on by surviving cells that do not naturally have a higher capacity to start tumors but through the process of adaptive reprogramming, take on the phenotypic characteristics of CSCs, leading to the continuous replenishment of the CSC pool over the course of treatment. In the second case, the cancer cells were triggered intrinsically and extrinsically by the cancer treatment itself, converting the cells non-genetically to a stem-like state and having the capability to initiate the tumor again. We postulate that the dedifferentiated or stem-like condition is a significant factor in tumor recurrence and that Lamarckian induction is at work in many distinct forms of cancer.

The interaction of genomic and non-genomic evolution in cancer is comparable to Darwin's and Lamarck's evolutionary ideas. Darwin believed in the theory of "survival of the fittest" according to which all living things have intrinsic distinctions that make certain species more likely to survive than others. All tumors in the setting of cancer exhibit high cell-to-cell and spatial variability within the tumor itself. This embodies the idea that some cancer cells, typically as a result of particular genetic mutations, have a clear survival advantage and can proliferate over time. Contrarily, Lamarck proposed that organisms may alter as they go along in order to live and transmit these changes to their progeny. This idea is comparable to the idea that cancer cells might use an epigenetic and transcriptional adaptive mechanism to resist cancer treatments. Due to the complexity of cancer medication resistance, both Darwinian and Lamarckian concepts are in operation. Invoking a paradigm of either exclusive genetic or non-genetic evolution is rife with perilous presumptions, and there is currently an expanding amount of data to support the non-exclusive nature of both processes. The pervasive interaction between genetic mutations and the changing non-genetic environment demonstrates the need of viewing these processes as a whole rather than separately.

Immune checkpoint therapy and epithelial cancers are not the only conditions in which cellular plasticity is used to circumvent antitumor immunity. According to a study, mouse melanoma can develop resistance to T cell treatment by dedifferentiating due to inflammation, which is similar to the drug-tolerant neural crest stem cell (NCSC) condition discussed previously in this perspective.(28) Together, the data show that strong therapeutic pressure causes cellular plasticity in solid malignancies and haematological tumors, which not only leads to lineage shift but also makes it easier to evade the immune system. It is essential to comprehend the molecular processes that oncogenic drivers employ to facilitate lineage flipping in order to create treatment approaches that limit this plasticity and stop acquired resistance through this mechanism.

The Mechanisms of Cancer Epigenetics

DNA in our cells is packaged as chromatin, a dynamic structure made up of nucleosomes as the basic building blocks. Chromatin includes the genetic material found in eukaryotic cells and serves as the framework for the packing of our complete genome. The histone octamer, which has two of each histone H2A, H2B, H3, and H4, is surrounded by 147 base pairs of DNA. In a basic manner, chromatin is subdivided into two main regions: heterochromatin, which is extremely compacted, slow to replicate, and mostly includes dormant genes; and euchromatin, which is relatively open and contains the majority of active genes. Many studies showed that nucleosome components are prone to covalent modification and can affect chromatin.(29,30)

Histones are small proteins as the central component of the nucleosomal subunit which built by a high amount of basic amino acids. Each nucleosome consists of a 147-base-pair piece of DNA wrapped in sequence around four histone protein core, known as H3, H4, H2A, and H2B. The distinctive side chain, or tail, of each of the mainly globular histone proteins is heavily packed with basic lysine and arginine residues. The extensive covalent PTMs that the histone tails are susceptible to work together to control the chromatin state. PTMs are covalent processes involving proteolytic cleavage or group adding (acetyl, phosphoryl, glycosyl and methyl) that change the chromatin structure or function. Some PTMs can change the charge density between histones and DNA, affecting the architecture of chromatin and underlying transcriptional processes.

Using the ChIP-chip, a chromatin immunoprecipitation with DNA microarray analysis, that connected to chromatin immunoprecipitation technology, changes in the patterns of histone PTMs both at the global level across the genome, and at individual gene loci have been widely related to cancer.(31,32) These discoveries follow the finding of linked abnormal DNA methylation.(4) Sequencing initiatives, together with subsequent PTM mapping work, revealed many of the enzymes responsible for adding "writers" and erasing "erasers" such epigenetic markers. Such enzyme mutations end up being some of the most commonly mutated targets in malignancies.(33) The epigenetic factors offer a different way of silencing the tumor suppressor genes, or activating the oncogenes.(33) Genetic mutations frequently develop in the genes that encode the enzymes that add, delete, and interpret the covalent histone modifications. Genomic investigations have conclusively shown that dysregulation of chromatin modifiers serves as a driver in many forms of cancer.(34) Intriguingly, several chromatin modifiers have been linked to increased and reduced levels of functioning in cancer, indicating that they may serve as both TSG and oncogenes.

While certain genetic or epigenetic stimuli cause epigenetic restrictions that have an oncogenic impact, other stimuli lower the slope in Waddington's epigenetic landscape. Some gene pathways can activate the oncogenes and others do the opposite. This may be sampled by premalignant or malignant cells when their chromatin is permissive or "plastic." Adapted chromatin will grow a new improved clone, further detail described in Figure 2. It is helpful to compare the genetic instability caused by carcinogens or deficiencies in DNA repair when thinking about the epigenetic plasticity paradigm. Increased mutation frequency causes "driver" events, such as mutations that activate oncogenes, as well as "passenger" events that do not affect the fitness of tumor cells in that genetic framework. Thus, in the context of epigenetic plasticity, drivers are the results of chromatin or transcriptional modifications, while genes that fail in expression or a consequential gene will be the passengers. In contrast to the catastrophic genetic errors linked to "chromothripsis", epigenetic changes can develop singly over time or alternatively as many simultaneous disruptions.(35,36) Heritable epigenetic changes, somehow, can be selected and used as the hallmarks to predict cancer.

Since non-coding RNA (ncRNA), microRNAs (miRNAs), and other sections that play crucial roles in genome regulation are now recognized as part of epigenetic control, it is not just canonical coding genes that are affected.(34,37-40) As mentioned above, analysis of thousands of solid and liquid tumors has shown an unexpectedly large number of mutations in the genes that regulate the operation of the epigenome.(2,33,34,41-43) By first discussing changes in DNA methylation and cell metabolism associated with cancer, the idea of an improperly regulated chromatin language is introduced. There is a lot of evidence that different metabolic processes produce metabolites that act as a cofactor, including acetyl-CoA, S-adenosylmethionine, and lactate. These cofactors attach particular chemical tags to chromatin writer enzymes. While certain chromatin erasers require cofactors such as α-ketoglutarate and Nicotinamide adenine dinucleotide (NAD^+).(44,45) Changes in chromatin state, a defining feature of cancer, can result from altered cell metabolism and result in the dysregulation of gene expression.(44,46-48) Additionally, it is becoming more and more evident that a flexible collection of reader domains develops as a "toolkit" enabling cells to perceive the hither chromatin PTMs including lysine crotonylation (Kcr) or

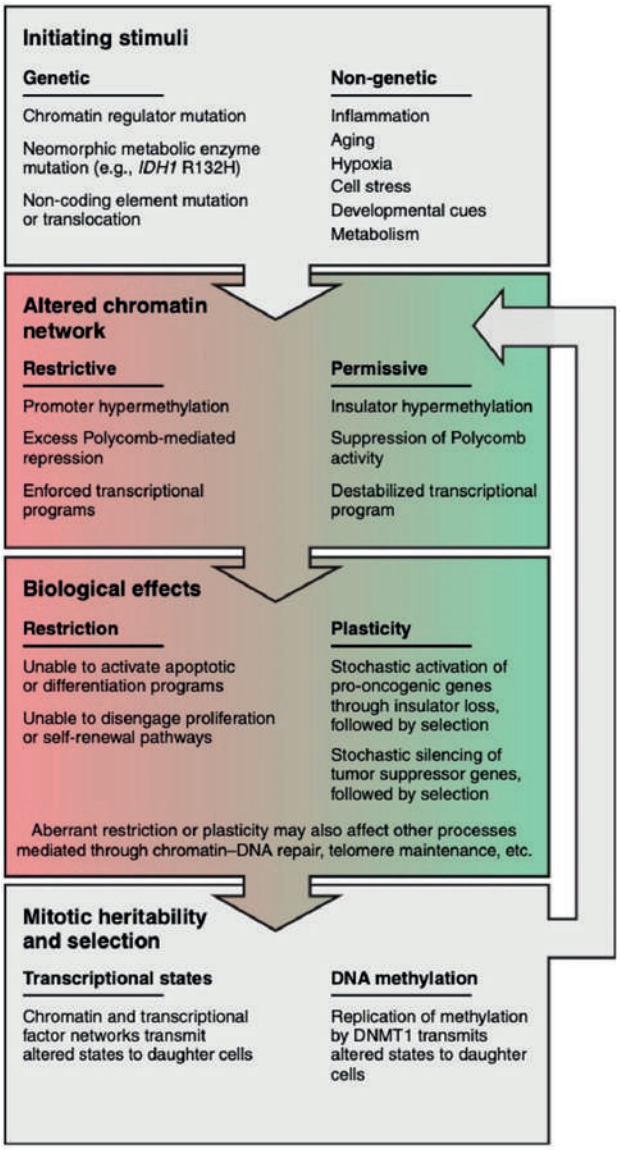

Figure 2. Chromatin homeostasis is disrupted in cancer to be overly restrictive or permissive to activate oncogenes.(3) (Adapted with permission from The American Association for the Advancement of Science).

benzoylation.(49-51) Therefore, in addition to the fact that chromatin writers, readers, or erasers are commonly mutated in malignancies, an excess or shortage of metabolites and/or their abuse can also contribute to the misregulation of chromatin modification, significantly influencing the pathogenesis of cancer.(44,46-48)

The detection of recurring oncogenic somatic mutations of histones, commonly known as oncohistones (52), spanning cancer types such as glioma (53), sarcoma (54), and lymphoma was accidentally found from The Cancer Genome Atlas data (55). Histone mutations modify epigenomic patterning, altering DNA-templated activities including gene transcription and DNA damage repair, as demonstrated by research on the most prevalent oncohistones. The functional inhibition of homologous histone writers that oncohistones bind to, which results in the alteration of epigenetic and transcriptome states, is a recurring motif of classic H3 tail oncohistones. H3 oncohistones have been thoroughly discussed elsewhere.(52-56) Therefore, one major method by which tumors change the normal process of chromatin-based gene and genome control in order to benefit from growth is represented by recurring oncohistone and chromatin remodeler mutations.

Cancer frequently harbors mutations in epigenetic writers, readers, and erasers as well as in the proteins that make up chromatin-remodeling complexes, and very few, if any, malignancies do not also include mutations in one of these important chromatin rheostat proteins. More than 20% of malignancies are thought to have mutations in the mammalian SWI/SNF chromatin-remodeling complex.(57) Only one mutational landscape including epigenetic regulators usually is found among more than 100 chromatin-modifying complexes, indicating that epigenetic dysregulation plays a causal role in the onset and progression of cancer, together with the amazing discovery that oncohistones can arrest the differentiation and develop malignancy.(58) Figure 3A described some important writers, erasers and readers of histones methylation, but is not close to other modifications, while Figure 3B describes a misregulated language of chromatin modification underlies oncogenesis. The oncohistone and chromatin remodeller mutations also take part to alter numerous fundamental aspects of chromatin. If we imagine the chromatin remodellers as the "paper", cellular metabolites and oncometabolites as the "ink" and "erasers" while the histones and DNA are the "language". Collectively, the altered mechanisms were "binding" and induce the chromatin language misinterpreting.(59)

Immune checkpoint inhibitors (ICIs), such as anti-CTLA4, anti-PD-1, and anti-programmed death-ligand 1 (PD-L1) modify the patient's natural immune system. Additionally, leukemia patients have found success with the injection of enlarged autologous tumour-specific T cells or chimeric antigen receptor T cells. However, immunotherapy showed different results in most cancer patients.(60,61) Immunotherapy is usually applied to advanced cancer patients, so the response rate in less advanced illnesses is still not entirely understood. It is necessary to have a greater understanding of the immunological interactions between tumors and their hosts throughout the body in order to make further advancements toward immunotherapeutic techniques that are more generally effective.

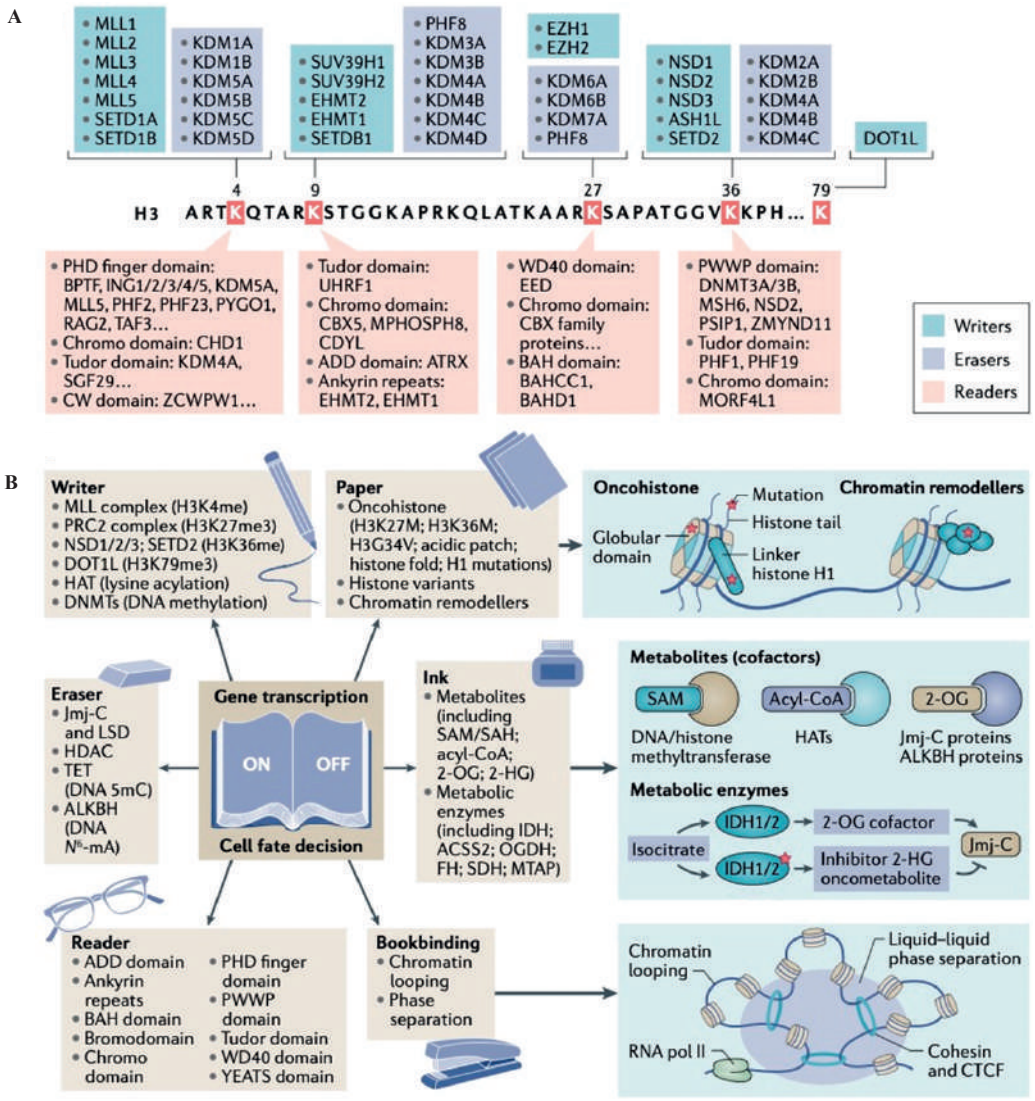

Figure 3. The misregulated language of chromatin modification in cancer. A: overview of certain key writers, erasers and readers of histone H3 methylations at K4, K9, K27, K36 and K79; B: a misregulated language of chromatin modification underlies oncogenesis.(59) (Adapted with permission from Springer Nature).

Major haematopoiesis disruption is a common feature of many human malignancies and cancer-causing mice models.(5) The most obvious sign of this disturbance is an increase in immature neutrophils and monocytes in the periphery of hosts with tumor burdens. These cells subsequently go to the TME and contribute to local immunosuppression. As a result of the mobilization of hematopoietic stem and progenitor cells for proliferation and differentiation into the monocytic and granulocytic lineages, immature immunosuppressive neutrophils (also known as polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs)), monocytes, and macrophages are accumulated in the periphery and within tumors.(62-66)

However, this expansion also frequently co-occurs with changes to many other peripheral immune lineages, and many cancer studies now highlight the rise in immature and immunosuppressive myeloid populations. Interestingly, many of the alterations were reversed by tumor resection surgery or cytokine blocking therapies, suggesting the flexibility in the peripheral reconfiguration of the immune macroenvironment in cancer. A dramatic restructurization of the global immune system across immune cell lineages was found as a result of tumor development as mirrored in the spleen in mouse models (Figure 4).(12)

Another crucial element of antitumor immunity is NK cells, which have the ability to both directly destroy tumor cells and modify the antitumorigenic behavior of other immune cells.(67) Additionally, the phenotypes of peripheral NK cells from breast cancer patients are altered, where the expression of activating receptors is decreased

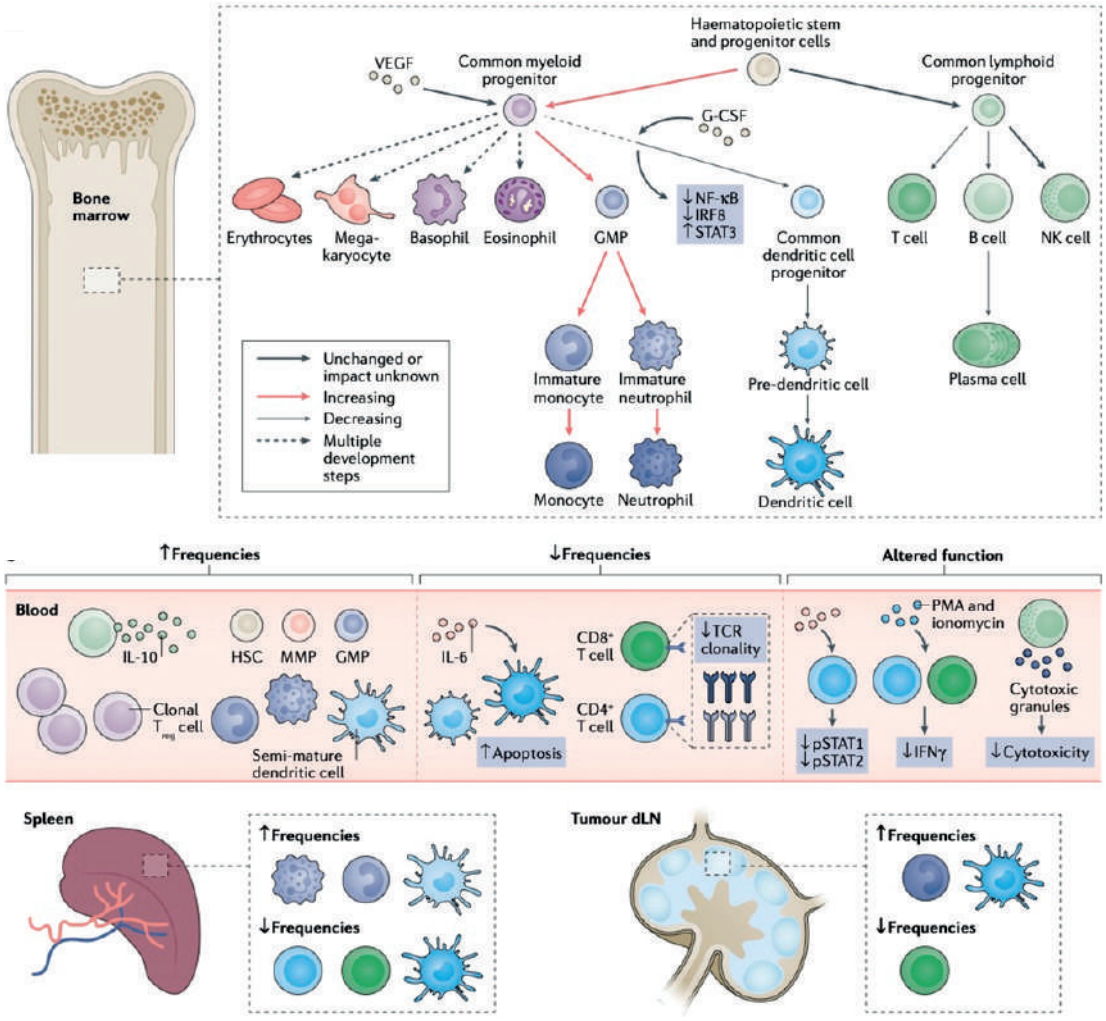

Figure 4. Systemic perturbations to immune organization by the tumour burden.(12) (Adapted with permission from Springer Nature).

while the inhibitory receptor NKG2A is increased, as well as diminished ability to degranulate *in vitro* and directly kill target cells.(68) Peripheral NK cells from patients with gastrointestinal stromal tumors had lower NKp30 activating receptor expression levels and poor degranulation in response to NKp30 cross-linking. Contrarily, IL-2 stimulation or incubation with dendritic cells increased the production of interferon (IFN) by NK cells from patients with gastrointestinal stromal tumors, and the latter event indicated a better response to imatinib mesylate therapy.(69)

The immunological organization is consistently compromised across a range of tumor forms. It is an interesting question why systemic immunological alterations are fairly substantial in some circumstances but subtle in others.(5) With the development of immunotherapy, therapeutic approaches are moving toward the use of antitumor immunity booster agents which can release tumor antigens to activate the adaptive immune system, or even destruct the tumor stroma.(70-72) Many cancer immunotherapy agents have been approved by the US Food and Drug Administration (FDA) including seven ICIs for treating 19 different cancer types, chimeric antigen receptor T cells, bispecific T cell engager (BiTE) treatments, and cancer vaccines.

The prevalent theory of cancer immunotherapy effectiveness has focused on the idea of reactivating cytotoxic effectors inside the TME, however awareness of the essentially systemic nature of efficient antitumor immunity is developing. Recent research has shown that systemic immune processes are necessary for ICIs, including inhibition of the PD-1 and PD-L1 axis, to provide effective antitumor responses. Additionally, the microbiome is showing promise as a strong immune system regulator with implications for antitumor immune responses.

Inhibiting the PD-1–PD-L1 axis has been shown to have effects beyond preventing local immunosuppressive signals in the tumor, and current research has identified critical peripheral immune cells that regulate responses in these

conditions. This is probably because ICI is only effective in hosts with intact PD-1 and PD-L1 expression, while cancer cells are not always dependent on PD-L1 expression.(73-75) The bulk of cells that express PD-L1 outside of tumor cells are antigen-presenting cells (APCs), such as macrophages and, at even greater levels, classical dendritic cells (cDCs). Clinically full responses to anti-PD-L1 and anti-CTLA4 treatment are correlated with intratumoural macrophage and cDC PD-L1 expression levels in patients with melanoma or ovarian cancer. Furthermore, a number of studies have recently shown that dendritic cells play a crucial role in the effectiveness of PD-L1 inhibition.(76-79)

Although specific immunological traits in the TME have been found to be related to prognosis in diverse circumstances, we still need an adequate systemic immune biomarker to be able to predict outcomes (80), and to support patient treatment decisions.

Targeting Epigenetic Modifications in Cancer Therapy

Epigenetic modification involves DNA methylation and acetylation, histone modification, chromatin remodeling, and noncoding RNA. Three roles, namely "writer", "reader", and "eraser", can be assigned to the components involved in various alteration patterns. Enzymes that add or remove chemical groups to or from DNA or histones, respectively, are referred to as "writers" and "erasers". Proteins known as "readers" can detect altered histones or DNA. The epigenome works in concert with other regulatory elements, including transcription factors and noncoding RNAs, to control the expression or repression of the genome in order to coordinate numerous biological activities. Cellular signaling networks and external stimuli can also affect epigenetics. These effects are both transient and pervasive, given the significance of epigenetics in affecting cell functioning.(81)

It is now generally acknowledged that cancer is both a hereditary illness and a disease of dysregulated epigenetic changes. Genetic alteration affects the DNA sequence including point mutations, gene deletions, and gene shifting, while epigenetic modification does not modify the DNA sequence.(82) Although the cells retain the same genetic information, the epigenetic modifications in gene expression are typically formed during cellular differentiation and are inheritable through several cell division cycles.(83) There are more options to improve the illness phenotype since epigenetic markings are reversible.(84) Recent developments in epigenetics have confirmed that epigenetic changes are the primary causes and origins of various cancer types.(85) Three basic epigenetic processes function in cancer cells and share a similar route with incorrect chromatin activation or repression, which in turn activates or inhibits a variety of cancer-related cell signaling pathways. These epigenomic changes include chromatin remodeling complex mutations, post-translational alterations of histone proteins, and methylation of cytosine bases in DNA, also known as DNA methylation.(86) Since its discovery four decades ago, DNA methylation has received the greatest attention among s malignant cell lesions and continues to be a significant marker for the majority of cancer types.

DNA methylation becomes the target for anti-cancer therapy to restore the normal epigenetic landscape and genes involved in the disease, which is considerably highlighted as a result of the epigenetic silencing of cancer-related genes by DNA methylation. The "readers" such as methyl-CpG binding domain proteins; "writers" such as DNA methyltransferases (DNMTs), histone acetyltransferases (HATs), and histone methyltransferases (HMTs); and "erasers" such as DNA-demethylating enzymes, histone deacetylase (HDACs), and histone lysine demethylase (KDMs); perform this epigenetic process as described in Figure 5. A potential family of anticancer medicines known as DNMT inhibitors (DNMTIs) alters the epigenome by reversing DNA hypermethylation patterns, reactivating the transcription of tumor suppressor genes that had been silenced earlier, which is TSGs. These DNMT-targeting medications fall into two categories: proteosomal degradation and non-nucleoside analog classes. The first one bound with DNA during its replication, induced by nucleoside analog inhibitors. While non-nucleoside analog was bound directly to the catalytic region of DNMTs so it can inactivate enzyme with no need of covalent enzyme trapping. Some examples of approved treatments are DNMTIs, 5-azacytidine (azacytidine, 5-aza-CR (AZA)) and 2′-deoxy-5-azacytidine (decitabine, 5-aza-CdR (DAC)) for the treatment of hematologic malignancies (87), and are now generating significant interest as priming agents in the management of solid tumors (88). In addition to these well-established treatments, a large group of DNMT-targeting medications is presently through clinical trial stages or pre-clinical research for a variety of solid cancers, including blood-related malignancies.

Cellular absorption, intracellular metabolism, and incorporation into nucleic acids are the three key stages that make up these AZN medications' molecular activity. Decitabine is absorbed into freshly generated DNA, whereas

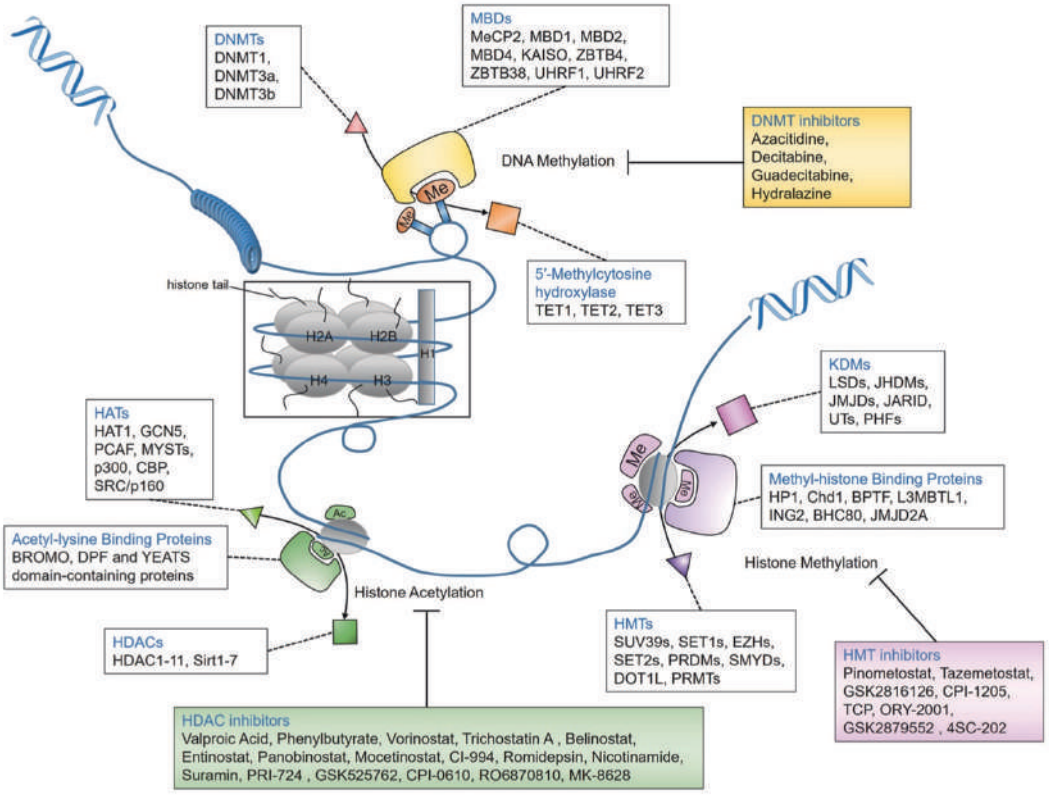

Figure 5. Epigenetic regulation of DNA methylation, histone acetylation, and histone methylation.(81) (Adapted with permission from Springer Nature).

azacytidine, after undergoing a multi-step conversion process by the enzyme ribonucleotide reductase, is incorporated into RNA to an extent of 80-90% and only 10-20% into DNA. (89) Azacytosines replace cytosine after being incorporated into DNA, creating azacytosine-guanine dinucleotides that are recognized as a natural substrate by DNMTs (DNMT1 at low dosages and DNMT3A/3B only at high levels). Due to the irreversible trapping of DNMTs caused by the covalent connection formed between azacytosine-containing DNA and these enzymes, loss of methylation marks during replication, and finally the reactivation of silenced TSGs are the end outcomes.(89) Additionally, covalent DNMT-azacytosine activates DNA damage ATM/ATR response pathways and induces apoptosis by stopping the G2 cycle.(90) Azacytidine's partial effectiveness is also a result of RNA-dependent (cell-cycle independent) effects since it is mostly absorbed into RNA. When azacytidine is incorporated into RNA, it prevents tRNA from being methylated at DNMT2 target sites (91), and it also impairs rRNA processing, which slows protein synthesis and causes apoptosis (92). Additionally, azacytide also prevents DNA synthesis and repair by blocking the ribonucleotide reductase thus it cannot be convert into deoxyribonucleotides.(93)

DNA methylation is a common and highly conserved epigenetic alteration of DNA in many species, involved in many biological processes together with 5-mC loss. (94) Gene silencing is the result of DNA methylation For instance, DNA demethylation is crucial for primordial germ cells (PGCs) to acquire the capacity for pluripotency. (95,96) The ten-eleven translocation enzymes (TET) protein family functions to remove the methyl group from 5-mC, convert it into 5-hydroxymethylcytosine (5-hmC), known as demethylation.(97) As a rather stable intermediate substrate, 5-hmC is less likely than 5-mC to be subsequently oxidized by TET proteins.(98) However, excessive expression of TET1 and TET2 alone can result in a 5-mC global reduction.(99) TET proteins can stepwise oxidize 5-hmC to produce 5-formylcytosine (5-fC) and 5-carboxylcytosine (5-caC), respectively.(100) Thymine-DNA glycosylase (TDG) has the ability to remove these two molecules and eventually repair them to unmodified C.(101) Through replication-dependent dilution of 5-mC, DNA demethylation or restoration of the unmodified cytosine can also take place passively. Oncogenesis is hypothesized to be correlated with abnormal DNA demethylation. Leukemia and TET proteins were first linked.

There is still controversy over the hypothesis that TET enzyme mutations may contribute to a DNA hypermethylation phenotype in cancer.(102) The upstream isocitrate dehydrogenase (IDH) enzymes, IDH1 and IDH2, were also

involved in TET-mediated DNA demethylation and altered the cells. Normally, these enzymes generate α-ketoglutarate, a necessary cofactor for the TET hydroxylases.(11,103,104) However, IDH1/2 mutations significantly enhance the production of the aberrant metabolite 2-hydroxyglutarate, which is produced from -ketoglutarate.(105) In this situation, DNA hypermethylation is more likely to occur, as is the case with leukemias and brain tumors.(33,106,107) The necessity of ongoing demethylation to maintain the proper amount of cellular 5mC is highlighted by the fact that TET and IDH mutations in cancer are mutually exclusive.(108) IDH mutations in the hematopoietic system are significant because they seem to be the source of cancer because they prevent cells from responding to differentiation signals, which skews lineage choice.(109,110) Importantly, the investigational medicine appears to restore a component of cellular differentiation responses in association with IDH mutations, offering therapeutic hope for treating these types of malignancies.(109,110)

The balance between the activities of acetyltransferases and deacetylases controls the amount of acetylation. Lysine acetyltransferases (KATs), which are found in both the nucleus and the cytoplasm and contain a large number of non-histone substrates, were formerly known as HATs due to their activities toward numerous histone substrates. Similar to lysine deacetylases (KDACs), deacetylases also have a variety of substrates, however they are more commonly referred to as HDACs than KDACs. There are several top-notch articles about HDAC families and their roles.(111-113)

Acetylation has long been linked to active gene transcription and the opening of chromatin. The ability of RNA polymerase and other agents to reach gene promoters can be hindered by both individual nucleosomes and higher-order chromatin folding. As the acetyl group is added, the positive charge of the lysine is neutralized, weakening the interactions between the negatively charged DNA backbone and the histones as well as the links between nearby nucleosomes, resulting in more flexible chromatin configurations. Additionally, the binding of regulatory proteins involved in certain phases of the transcription process can be facilitated by acetylation at particular lysine residues on particular histones.(103)

The earliest and, until recently, only acetyl lysine-binding domains reported were bromodomains.(104,105) Most of these domains have a strong affinity for acetylated lysines while only weakly binding non-acetylated lysines, allowing them to 'read' the acetylation status of histones or other proteins. These domains have undergone extensive evolutionary conservation.(105) As a result, bromodomains operate as links in the connections between histone proteins and other proteins. There are several branches of the bromodomain family, and each has unique structural features that give it selectivity for certain acetylation stages or proteins.(114) Even though the sequences of these families vary greatly, bromodomains have a conserved binding site that is surrounded by a loop region connecting four -helices and may attach to acetylated lysines. Specificity for substrate binding is provided by variances in sequence, variations in the length of the loop region, and post-translational modifications on the residues nearby the acetylated lysine.(114,115) It's interesting to note that many KATs have bromodomains of their own or form stable complexes with proteins that have them, raising the prospect of feed-forward loops in which these proteins strengthen their connections with chromatin or other proteins to promote even more acetylation. Among these KATs are GCN5, PCAF, and CBP/p300.(116)

Several epigenetic mark-removing enzymes have been linked to cancer, largely due to their overexpression in various tumor types or because of a mechanistic connection. HDACs are the most developed category, and inhibitors have been authorized for use in clinical trials around the globe since 2009. These first-generation HDAC inhibitors are difficult to combine because, like DNMT inhibitors, their action is restricted to hematological malignancies and they have high toxicity.(117-120) The goal of second-generation HDAC inhibitor discovery has been to increase selectivity across HDAC family members since the toxicity is most likely caused by widespread action across HDAC isoforms.(121-131)

There is increased interest in combining HDAC inhibitors with immunotherapies due to their immunomodulatory properties, which include upregulating major histocompatibility complex (MHC) class I genes, tumor antigens, and PD-1 ligands; inducing immunogenic cell death (ICD) hallmarks in tumor cells; and reducing Treg cells.(132-134) The first of these trials to be finished examined effects of high doses of IL-2 and the class I selective HDAC inhibitor entinostat on metastatic renal cell cancer.(135) The combination increased median progression-free survival and overall response rate (ORR). Interaction with entinostat lowered Treg cells as anticipated from preclinical research, and this drop was related to a response, offering justification to use these drugs with immunotherapy.(136) Theoretically, the idea of epigenetic treatment for cancer has a solid foundation, and clinical efficacies are beginning to show, suggesting considerable potential.(4)

Epigenetics and Immunotherapy: The Current Status

Recent studies have produced ground-breaking understandings of the ligand-receptor interactions between immune and cancer cells inside the TME are at least partially responsible for the current clinical success of immunotherapy in cancer patients, particularly immune checkpoint blockage. Different ligands produced by cancer cells interact with immune cells' cell surface receptors, activating inhibitory pathways that cause immune cells to become immunologically tolerant or inactive. The co-inhibitory receptors PD-L1 or programmed death ligand 2 (PD-L2) on cancer cells, for instance, bind to the important T cell surface receptor PD-1, preventing the generation of cytokines and eventually leading to T cell malfunction or apoptosis.(137-139) These immunological checkpoints regulate or regulate the host's immune response to infections under typical circumstances. Immune checkpoints can, however, be misregulated or taken over as a defense mechanism in the setting of malignancy.

Both the adaptive and innate immune systems have a role in the detection of cancerous cells. NK cells are largely used by the innate immune system to destroy cancerous cells. Atypical cells producing stress-induced ligands, such as MHC class I-related chain A (MICA and B (MICB) and ULB16-binding proteins (ULBPs), are recognized by NK cells' activating surface receptors, such as NKGD2.(140-142) NK cells destroy tumor cells to undergo apoptosis, first by expressing NK-cell ligands to activate death receptors such as Fas or TNF-related-apoptosis inducing ligand (TRAIL) receptor on the target cells, and then release cytotoxic granules such as granzymes and perforin.

DCs and macrophages are additional innate immune system cells that function mainly as expert APCs and activate the antigen-specific adaptive immune system. Growth factors and immunosuppressive cytokines from the tumor and stroma can rewire the activity of macrophages, turning them into tumor-associated macrophages (TAMs) with an immunosuppressive M2 phenotype.(143,144) By generating growth factors, such as epidermal growth factor (EGF) and fibroblast growth factor (FGF)-2; angiogenic factors, such as VEGF and matrix metallopeptidase (MMP)-9; and inflammatory cytokines, such as tumor necrosis factor (TNF)-α and IL-1; M2 or repair-type cells encourage the genesis, development, and spread of tumors.(143) Naive $CD4^+$ T cells and $CD8^+$ T cells develop into several antigen-specific T cell subsets upon APC-mediated activation. For instance, activation of naïve $CD8^+$ T cells results in effector cytotoxic T lymphocytes, whereas stimulation of $CD4^+$ T cells results in T helper cells (such as Th1, Th2, and Th17 cells), T follicular helper (TFH) cells, and Treg cells.

The antigen processing and presentation machinery, which is made up of several distinct molecules, is assembled into the peptide-human leukocyte antigen (HLA) complex over the course of several steps including the interaction between co-stimulatory molecules with peptide-HLA class I molecule complex on APC.(145,146) As a result, cytokine synthesis and cellular proliferation are brought on by peptide-mediated Treg cells activation, which also triggers an intracellular signaling cascade.(147)

A key factor underlying the emergence and spread of cancer is epigenetic dysregulation.(148,149) The term "epigenetic regulation" refers to heritable alterations to DNA that affect gene expression and chromatin structure without altering the nucleotide sequence that underlies such changes.(149,150) DNA methylation and PTMs are examples of these epigenetic alterations or marks.(150,151) Interdependent epigenetic marks activate and deactivate genes in response to external inputs. Chromatin primarily occurs in two interchangeable modes with respect to transcriptional regulation: heterochromatin (closed form) and euchromatin (open form). These conditions were controlled by epigenetic mechanisms and from accessing and/or functioning at target genes; this condition is typically linked to transcriptional silence. In contrast, an open chromatin state allows for active transcription and is accessible to the transcriptional machinery.(152)

A range of processes, including PTM of histone proteins, DNA methylation, adenosine triphosphate (ATP)-dependent chromatin remodelling complexes, histone variant exchange, and the action of non-coding RNAs, are used by chromatin remodelling to control the transcriptional state of a gene, such as miRNAs. Acetylation, methylation, phosphorylation, and ubiquitylation are the most frequent histone modifications; however, numerous additional alterations have been discovered.(153) In order to control gene transcription, epigenetic changes to DNA and histone proteins dynamically sculpt the chromatin environment.

The focus of most current epigenetic therapeutics is on two functional groups of epigenetic regulators: those that target the "writers," or enzymes that create epigenetic marks such as DNMT, and those that target the "erasers," or enzymes that erase epigenetic marks for example HDAC. By causing differentiation, apoptosis, growth inhibition, cell cycle arrest, and cell death, DNMT and HDAC inhibitors demonstrate anti-tumor properties. By

directly integrating into the DNA and trapping DNMTs for proteosomal destruction, DNMTis limit the activity of DNA methyltransferases, which add methyl groups to DNA. This reactivates gene transcription. Passive hypomethylation of DNA occurs in daughter cells following cell division as a result of the loss of DNMT, which is dependent on DNA replication. Similar to HDAC inhibitors, HDACs remove acetyl tags from tagged histones to promote global histone acetylation. HDAC inhibitors prevent this process. The global nuclear architecture may be altered by these inhibitors, at least in part, to restart gene expression. A relaxed chromatin configuration can be caused by a decrease in DNA methylation and/or an increase in histone acetylation, allowing transcriptional activators to access the chromatin and resume gene production. In both immune and cancer cells, epigenetic medicines that target these enzymes can restore and occasionally overexpress genes that have been epigenetically silenced.(145,146,154) In general, the reexpression of epigenetically suppressed tumor suppressor genes and cell cycle regulators is increased when DNMT and HDAC inhibitors are combined.(155)

Epigenetic control can either be direct or indirect.(156) The lower expression in cancer is considered to be caused by epigenetic dysregulation of antigen processing machinery (APM) components. Both DNMT inhibitors and HDAC inhibitors were higher in many types of tumour, and induce some APM pathways factors such as transporter associated with antigen processing (TAP)-1, TAP-2, Le Mans prototype (LMP)2, LMP7, tapasin, as well as MHC molecules.(157-159) In addition to APM components, it is well known that exposure to epigenetic agents can increase the surface expression of a number of co-stimulatory molecules on tumor cells, including CD40, CD80, CD86, and intercellular adhesion molecule (ICAM)-1, as well as stress-induced ligands and death receptors.(159-165) They become more susceptible to immune-mediated cell lysis in particular as a result of these immuno-modulatory processes. Additionally, epigenetic medications have been demonstrated to increase the immunological checkpoints CTLA4, PD-1, PD-L1, and PD-L2 on tumor cells and tumor-infiltrating lymphocytes (TILs), sensitizing cancer cells to immune checkpoint treatment and perhaps facilitating immune escape.(166-168) Moreover, positive clinical outcomes from anti-PD-1/PD-L1 treatment have been associated with high cancer cell and TIL PD-L1 expression.(169,170)

Drug resistance is still a problem in cancer treatment, and epigenetically targeted medications are no exception. Just getting started is the molecular knowledge of resistance to epigenetic treatment.(171,172) The idea of combination therapy is to combat treatment resistance by combining two or more medications that target several cancer cell dependencies. Additionally, when their potential adverse effects can be reduced, combination therapy should enhance treatment regardless of medication resistance. As was already established, a notable example is how lysine-specific demethylase (LSD)1 inhibitor makes non-APL AML cells more susceptible to the effects of all-trans-retinoic acid (ATRA).(173) Moreover, it is demonstrated that telomeric silencing 1-like (DOT1L) inhibitor and bromodomain-containing protein (BRD) inhibitor are synergistic in the treatment of mixed-lineage leukemia (MLL)-rearranged leukemia, probably as a result of their functional cooperation at the highly transcribed super-enhancer genes.(174,175)

Epigenetic Modulation in Cancer Immunotherapy

During apoptotic cancer cell death, ICD stimulates the innate and adaptive components of the immune system. In terms of cancer immunotherapy, the process of ICD causes dying cancer cells to become more adjuvant and antigenic, which in turn encourages the establishment of the therapeutically desired antitumor immunity. Cancer ICD necessitates the manifestation of many immunomodulation "hallmarks," such as calreticulin cell-surface translocation, type I interferon production, and release of high-mobility group box-1 and ATP, which together activate an immune response to cancer cells. It is interesting to note that various linkages to ICD hallmarks have been found in recent papers looking into the utilization of epigenetic modifying medicines as anticancer treatments. The epigenetic process involves averting TME-associated immunoevasion and altering the immunogenic characteristics of cancer cells.

The immunosuppressive properties of the TME can be overcome by the immunogenic response triggered by ICD. These result in the restoration of the three signals. The first signal is the APC presentation after cancer cell death and phagocytosis, the second signal is the co-stimulation from recruited and matured APCs, and the third signal is where both cancer cells and APCs induce cytokines release such as IFNs. As a result, when ICD is successfully induced, antitumor T cells are activated, which can destroy cancer cells and stop the illness from coming back. In order to enhance the effectiveness of existing cancer immunotherapies, it is crucial to comprehend how epigenetic alterations contribute to ICD.

ICD develops when a therapeutic intervention prompts the development of a particular combination of "hallmarks" following cancer cell death. These characteristics are a collection of premortem stress reactions that encourage the cancer cell's generation of "danger signals" as it dies. Immune cells can then identify these danger signals and activate antitumor T cells as a result. Diverse damage-associated molecular patterns (DAMPs), which eventually lead to the formation of T cell immunity, are important ICD markers. It is becoming more and more obvious that the majority of the ICD characteristics are controlled by epigenetic pathways, either directly or indirectly. In addition, several therapeutic epigenetic modulators that are now being researched are being acknowledged for their effects on dendritic cell activation, antigen absorption, and T cell activation (*e.g.*, HDAC inhibitors).(147,176) Thus, it is possible to harness the anticancer effects of ICD by using the epigenome's intrinsic or therapeutically changed activity.(132)

Since a few decades ago, cancer immunotherapy has been used to treat cancer by passively transferring antibodies or lymphocytes that specifically target cancer cells or by utilizing vaccines and/or cytokines to stimulate immune responses. But the full potential of the immune system to combat cancer was not realized until the development of antibodies that interfered with immunological checkpoints on lymphocytes.(177) Following these outcomes, melanoma patients receiving therapy with monoclonal antibodies directed against the PD-1 checkpoint on T-cells saw even more striking outcomes. Initial phase I/II trials for the two anti-PD-1 antibodies, nivolumab and pembrolizumab, revealed response rates of about 30%.(178,179) Backbone of care for melanoma patients is anti-PD-1, which was proven to be more effective than conventional chemotherapy or ipilimumab in later phase III trials.(180,181) Studies on anti-CTLA4 and anti-PD-1 treatment in combination showed even better response rates.(182)

One of the several checkpoint immunotherapy limitations is ineffective therapy for many people. Even with the combination, the best response rate is around 60%, whereas responses to single agent therapy are even lower.(179,182,183) Another thing is relapse patients after the therapy. This was around 30% at 2 years in the phase 1 pembrolizumab study and 50% after 2 years in the phase 1 Nivolumab study.(178,179) Surprisingly, the survival curve plateaued at about year three following anti-CTLA4 therapy, and recurrence was quite infrequent.(184)

Some research was conducted into the causes of non-response or recurrence to checkpoint immunotherapy. (185) Tumors with mesenchymal transition-related gene signatures, inflammatory phenotypes, chemotactic genes for macrophages, high angiogenesis/VEGF, and beta-catenin signaling have been linked to non-response to checkpoint blocking.(186-188) On the other hand, acquired resistance has been connected to the selection of cancer cells with Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway alterations.(189) Recent evidence of immune escape includes the choice of tumor variants that do not express immunodominant antigens. (190) There are also unproven alternatives, such as the expression of different immunological checkpoints.(191)

Both the biology of tumors and the growth and differentiation of immune cells involve epigenetic processes. Therefore, focusing on epigenetics has enormous potential to support immunotherapy.(6) The tumor may consist of tens of millions of cells when a patient is initially diagnosed with cancer. These cell populations have already changed, leading to the possibility of a very diverse tumor. Individual cells in solid tumors or leukemias as well as geographically separate sections of solid tumors have been shown to exhibit this type of intratumoral heterogeneity (ITH).(192) Profiling ITH offers a significant chance to reconstruct the evolution of the tumor and trace its origins from the initial tumor-initiating events to the following stepwise development of malignant clones.(193,194) Despite our greater understanding, most cancer treatments still fail to have long-lasting effects, which is frequently related to ITH. Most clinical studies still do not evaluate ITH, which is significant since it misses an opportunity to test ITH's prognostic usefulness in a controlled environment.

DNA methylation, histone post-translational changes, and chromatin remodeling, all of which are crucial for genome organization, gene expression, and cell function, are epigenetic pathways that may cause ITH.(195) The existence of neoantigens in treated cells may result from the activation of many cancer genes and repetitive sequences, boosting the visibility of treated cells to host immune surveillance. (196) In agreement with this, it has been demonstrated that endogenous retroviruses (ERVs) expressed, for example in clear cell renal cell carcinomas (ccRCCs) encode peptides that cause T and B cell immunoreactivity.(197) Additionally, the activation of truncated virally generated long terminal repeats (LTRs) anchored in introns may result in the creation of neo-antigens.(198) Viral infection in common will induce innate immune responses such as cytokine release including IFN I and II. Cancer cells can evade a viral mimicry as a result of metabolic alteration and epigenetic mechanism, and induce the same way as an exogenous virus attack, then

activate the ERVs and possibly other transposable elements like Alu elements and long interspersed elements (LINEs).(199,200)

Overall, the viral mimicry response leads to a decline in the fitness of cancer cells and draws cytotoxic T lymphocytes (CTLs) to the TME. Due to the fact that the tumor cells were not in the S phase at the time of exposure, which is a need for incorporation into DNA to produce passive demethylation, this bystander effect may be crucial in the death of tumor cells that were not directly impacted by the DNMT inhibitor's activity.(201) This characteristic is also advantageous since it protects non-cycling normal cells, which make up the bulk of a person's body mass, from the negative consequences associated with off-target suppression of DNA methylation. Additionally, invading immune cells have anomalies in epigenetic regulation that can be treated with epigenetic treatments.(202) CTLs are a good example since they may burn out from constant stimulation inside the TME. Aberrant DNA methylation at genes linked to T cell effector activities, such as IFN, characterizes this fatigued state.(203) Anti-PD-1 and anti-PD-L1 are two common immune checkpoint blockade medications, although they don't always entirely convert worn-out CTLs back into effector cells.(202,204,205) But DNMT inhibitor can be effective by delaying the development of fatigue and reprogramming worn-out CTLs into effector phenotypes.(203) Together, these new findings suggest that DNMT inhibitor can boost antitumor immune responses by acting on both cancer cells and immune cells, complementing immunotherapies. Notably, it has now been demonstrated that inhibiting other epigenetic modifiers, such as SET domain, bifurcated 1 (SETDB1), LSD1, and cyclin-dependent kinase 9 (CDK9), also induce viral mimicry responses and work in concert with PD-1 blockade in mouse models.(206-208)

ERV-independent pathways via which epigenetic treatment may alter the immunological milieu in a way that works in concert with immune checkpoint inhibition and the antitumor effects produced by ERV-dependent processes. Given the well-established functions of epigenetic regulation in regulating their destiny and function, epigenetic treatment may potentially impact the activity of T cells already present within tumours in addition to changing the composition of the immune milieu in these ways.(209)

Additionally, epigenetic inhibitors can alter PDL1 expression both *in vitro* and *in vivo*, as described in Figure 6, while more research is needed to determine the clinical consequences.(210) For instance, bromodomain and extra-terminal motif (BET) inhibitor JQ1 increases the activity of cytotoxic T cells *in vivo*, decreases PD-L1 expression, and inhibits tumor growth.(210,211) Contrarily, DNMT inhibitors increase PD-L1 expression in leukemia and NSCLC cell lines.(167,168) Additionally, HDAC inhibitors increase PD-L1 expression in melanoma, and it has been demonstrated that combining an HDAC inhibitor with PD-1 inhibition increases the antitumor effect *in vivo*.(212)

Adoptive T cell therapy and immune checkpoint blockade therapy may both benefit from the use of epigenetic inhibitors.(213) Treatment with an enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2) inhibitor, namely GSK126, greatly increased the effectiveness of both adoptive T cell treatments and checkpoint inhibitors *in vivo*.(214) Additionally, treating patient tumor samples with azacitidine or an HDAC inhibitor (valproate) improved the ability of patient-derived T cells to destroy tumors by focusing on the tumor antigen melanoma-associated anti-gen C2 (MAGEC2).(215) Clinical studies have thus been started to examine the interaction between immune checkpoint blockade and DNMT, HDAC, or EZH2 inhibitors. In one clinical trial, individuals with relapsed or resistant Hodgkin lymphoma who received azacitidine before immune checkpoint inhibitors (pembrolizumab or nivolumab) had a greater rate of full remission.(216)

Epigenetic changes are becoming more widely acknowledged as potentially being a significant contributor to cancer treatment resistance. As a result, epigenetic inhibitors have been created to prevent the development of drug-resistant cells or to destroy them immediately. For instance, it was discovered that certain cells developed reversible medication resistance and that drug resistance development happens more frequently than genetic changes.(217) These "drug-tolerant persisters" have developed an epigenetic mechanism of resistance, as evidenced by their enhanced KDM5A levels and strongly suppressed chromatin state. A KDM5A inhibitor, namely CPI-455, or an HDAC inhibitor, namely trichostatin A, may be able to prevent their development.(210,217,218)

Combining Epigenetic and Immunotherapy in Cancer

Elegant foundational findings of ligand receptor interactions that regulate the immunological activity of T cells against malignant cells have led to an increase in the use of immunotherapy.(137,219-222) These fundamental developments and their translational uses are a crucial part of a paradigm known as tumor "immune evasion".

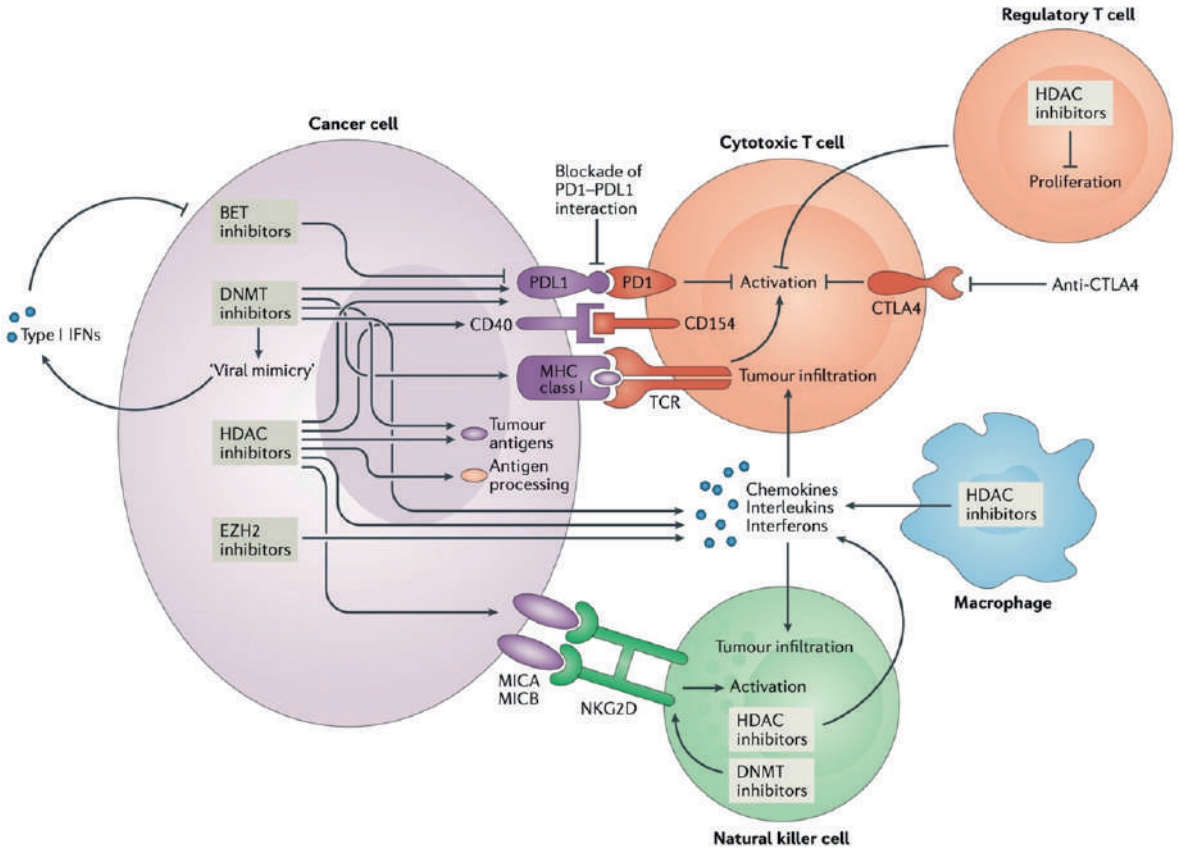

Figure 6. Epigenetic inhibitors in immunotherapy. These inhibitors are able to alter PD-L1 expression both *in vitro* and *in vivo*.(210) (Adapted with permission from Macmillan Publishers).

(223,224) The idea of attacking cancer immunologically was completely revived by the discovery and molecular analysis of the tolerant state. This means optimizing the immune signaling in both cells, tumor and host, to maximize the tumor elimination.(224)

Most types of cancer showed beneficial results from immune checkpoint therapy, except melanoma.(178,223) This naturally prompts the issue of whether combining immunotherapy with other treatments might significantly increase clinical response and efficacy across a wider range of cancer subtypes. Such ideas are, in fact, developing. First off, combining immune checkpoint targeting medicines in clinical trials that give patients anti-PD-1 and anti-CTLA4 while requiring specific care for toxicities shows significant potential for melanoma.(225) Second, tactics that combine conventional chemotherapy with methods of targeted therapy may be taken into consideration. In this context, we address the intriguing prospect that epigenetic treatment might effectively sensitize patients to immune checkpoint therapy, as suggested by a signal observed by our team in the clinic and a growing body of preclinical data.(146)

Aberrant transcriptional programs that support cancer initiation and progression are driven by epigenome dysregulation. Epigenomic changes may also influence tumor immunogenicity and immune cells engaged in antitumor responses, even though faulty gene regulation frequently affects oncogenic and tumour-suppressor networks. The development and use of cancer immunotherapies, epigenetic treatments, and their combinations might all be significantly impacted by this.(226)

Genes encoding epigenetic regulators are frequently found to be non-oncogene dependent in tumors, according to extensive DNA sequencing of cancer genomes and functional genomics screens, which have revealed that chromatin regulators are a nexus for oncogenic transcription programs.(227-231) The development of epigenetic therapies as anticancer agents has been emphasized over the past two decades based on their direct effects on cancer cells.(30,58,232)

Combining immunotherapies with DNMT inhibition to stimulate more robust antitumor immune responses and overcome adaptive immunological resistance linked to immune checkpoints may be a workable approach. To this purpose, multiple clinical trials are presently evaluating the combination of DNMT inhibitors with cancer checkpoint inhibitors.(233) HDAC inhibitors' immunomodulatory

properties offer a justification for using them in conjunction with immunotherapies. Different HDAC inhibitors can improve the effectiveness of adoptive T cell immunotherapy, and immune checkpoint blockade.(212,234-240) Numerous mechanisms, including enhanced tumour-infiltrating lymphocyte infiltration, cytokine generation, and T cell activation, have been linked to the effectiveness of this combo therapy.(241)

Cytosine methylation, especially in the long terminal repeat (LTR) sections, is the main method used to silence the production of newly transposed ERVs.(242,243) Although silencing is required to maintain their transcriptional quiescence, some ERVs activate at particular developmental stages. Another indication of the significance of DNA methylation in the repression of ERVs is the intriguing fact that a subgroup of evolutionarily young ERVs avoids demethylation and subsequently activation in development. (244) Cytosine methylation is essential for controlling a significant number of ERVs, therefore reactivation by DNMT inhibitor is quick and efficient after exposure to DNMT inhibitor. Despite the fact that DNA methylation is the key initial mechanism (245,246), 5-methylcytosine tends to deaminate to thymine, causing the progressive loss of CpG sites and declining the ERV function. As a result, the LTR can no longer be effectively silenced by DNA methylation, at which point histone changes play a greater role in their suppression.(247) This may trigger an innate immune reaction in response to the perceived retrovirus infection, activating type I and type III interferon.(199,200) Numerous ICD-related processes are controlled by epigenetic changes, as previously mentioned. Interestingly, studies that may not have been specifically assessing ICD induction have seen the onset of certain ICD characteristics following treatment with epigenetic modifying medications. In order to stimulate more potent antitumor T cell immunity, the combination of epigenetic modifiers with immunotherapies presents an appealing option. In actuality, this idea is already in use. Azacitidine and Romidepsin combined with IFN induce genuine ICD in colorectal cancer cells. (248) Additionally, Decitabine treatment induces a viral or altered-self mimicry state in these cells, which results in the expression of the ICD hallmarks via the retinoic acid inducible gene-I (RIG-I) pathway. In models of pancreatic cancer, acute promyelocytic leukemia, and melanoma, this route has been demonstrated to elicit ICD.(199) This idea has most recently been demonstrated to be crucial in neutrophil-based anticancer action, where apoptotic cancer cells produce epigenetically controlled cytokines including chemokine C-X-C motif ligand (CXCL)1, CXCL10, and chemokine ligand (CCL)2, which stimulate neutrophils to phagocytose dying cancer cells in response to nucleic acids.(249)

Compared to conventional cancer therapies, such as chemotherapy and radiation, immunotherapy has a number of significant benefits, including the ability to be applied worldwide to many cancer subtypes and to induce precise and long-lasting immune responses through immunological memory. Future immunotherapy for cancer patients has a lot of potentials since epigenetic medicines can selectively prepare epithelial cancer cells for host immune responses. In fact, several immunotherapeutic and epigenetic medication regimens have previously been utilized or are the subject of extensive research in a variety of tumor mouse models, such as colon, breast, and melanoma.(250)

Conclusion

Immune checkpoint inhibitors inspire the creation of numerous combination strategies as a powerful new method for personalised cancer therapeutics. Complex interactions occur between immune cells and tumors throughout the growth of tumors, and epigenetic alterations are a major contributor to several pathogenic changes that allow the immune system to escape. Despite its potencies, not all patients in responsive groups see clinical improvement. Finding combination methods for the right patient groups is therefore essential. Additionally, methods for treating individuals who have gained immunological resistance are required. Finally, it is important to pay attention to the discovery of other epigenetic regulators that might enhance the immune response to immunotherapy.

Authors Contribution

AM drafted, wrote, and edited the manuscript. AW proposed the manuscript topic, supervised, and edited the manuscript. All authors had agree with the final manuscript.

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