Epigenetic strategies synergize with PD-L1/PD-1 targeted cancer immunotherapies to enhance antitumor responses

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Abstract  Immunotherapy strategies targeting the programmed cell death ligand 1 (PD-L1)/programmed cell death 1 (PD-1) pathway in clinical treatments have achieved remarkable success in treating multiple types of cancer. However, owing to the heterogeneity of tumors and individual immune systems, PD-L1/PD-1 blockade still shows slow response rates in controlling malignancies in many patients. Accumulating evidence has shown that an effective response to anti-PD-L1/anti-PD-1 therapy requires establishing an integrated immune cycle. Damage in any step of the immune cycle is one of the most important causes of immunotherapy failure. Impairments in the immune cycle can be restored by epigenetic modification, including reprogramming the environment of tumor-associated immunity, eliciting an immune response by increasing the presentation of tumor antigens, and by regulating T cell trafficking and reactivation. Thus, a rational combination of PD-L1/PD-1 blockade and epigenetic agents may offer great potential to retrain the immune system and to improve clinical outcomes of checkpoint blockade therapy.

Abbreviations: 5-AzaC, 5-azacitidine; ACE1, angiotensin converting enzyme; ACP1, human red cell acid phosphatase; APC, antigen-presenting cell; BETi, bromodomain and extra-terminal motif inhibitors; CCL22 (MDC), macrophage-derived chemokine; CLL, chronic lymphocytic leukemia; CTA, cancer testis antigen; CTLA-4, cytotoxic T lymphocyte antigen 4; CTLs, cytotoxic T lymphocytes; CX3CL1, C-X3-C motif chemokine ligand 1; CXCL, CXC chemokine ligand; DC, dendritic cell; DNMT1, DNA methyltransferase 1; DNMTi, DNA methyltransferase inhibitors; EZH2, enhancer of zeste homolog 2; FDA, U. S. Food and Drug Administration; FOXP3, forkhead box P3; H3K27me3, tri-methylation of lysine 27 on histone H3; HDACi, histone deacetylase inhibitor; IDO, indoleamine 2,3-dioxygenase; IFN-γ, interferon-gamma; LAG-3, lymphocyte activation gene-3; MDSCs, myeloid-derived suppressor cells; MHC, major histocompatibility complex; OS, overall survival; PD-1, programmed cell death ligand 1; PD-L1, programmed cell death ligand 1; PRC2, polycomb repressive complex 2; TAA, tumor-associated antigen; TET2, ten-eleven translocation 2; TH-1, T helper type 1; TIL, tumor infiltrating lymphocytes; TIM-3, T cell immunoglobulin and mucin domain 3; Tregs, regulatory T cells; UHRF1, ubiquitin-like PHD and RING finger domain-containing 1

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1. Introduction

Due to a better understanding of fundamental principles of cancer biology and immunology, cancer immunotherapy has made significant achievements in the last several years, particularly in the immune checkpoint blockade field. Cancer immunotherapy is based on the stimulation and engineering of the immune system, such as the restoration of T lymphocytes, to combat cancer. Sequential steps are combined to create an integrated cancer immune cycle. One of the most promising ways to activate the immune system is the blockade of the immune checkpoints. Immune checkpoints are inhibitory pathways that are excessively hardwired into the immune system and are critical for maintaining self-tolerance and regulating the duration and magnitude of physiological immune responses to minimize incidental tissue damage. However, immune checkpoint molecules can mediate tumor immune escape, leading to malignant tumor progression. In the last decade, cancer immunotherapies targeting the immune checkpoint pathway, have achieved an unprecedented, long-lasting response rate in various cancers. This sustained response rate can be achieved primarily by blockade of the programmed cell death 1 (PD-1) and programmed cell death ligand 1 (PD-L1) pathway. Due to constant genetic changes in tumor cells, the varied conditions and complexity of the tumor immune microenvironment, most patients with advanced tumors have no response solely to immunotherapy. An objective response rate of 20%—40% is observed in the majority of solid tumors. Hence, combined therapies are urgently needed to improve the antitumor activity of anti-PD-L1/anti-PD-1 strategies. Encouraging outcomes from combinations with PD-L1/PD-1 blockade have been reported in a set of preclinical and clinical trials. These include combinations with other immunotherapy treatments, such as anti-CTLA-4 (cytotoxic T lymphocyte antigen 4) blockade, and co-therapy with radiation strategy, standard-of-care chemotherapy, and small-molecular inhibitors, especially epigenetic agents. 

Prerequisites for eliciting an immune response include the pre-existing antitumor T cells that are restricted by a particular immune checkpoint and a complete immune cycle. In most tumor patients, the antitumor response can maintain long-term disease control, but one-third of patients still have a recurrence. Nevertheless, there is evidence that changes in tumor antigen presentation and interferon-gamma (IFN-γ) signaling pathways play essential roles in primary and acquired resistance of PD-L1/PD-1 blockade, demonstrating a critical need for identifying resistant mechanisms.

**Figure 1**  The cancer immune cycle must be initiated and completed successfully to elicit an effective therapeutic immune response, and this cycle involves efficient (1) cancer-antigen presentation, (2) trafficking and infiltration of T cells to tumors, and (3) recognition and elimination of tumor cells by T cells. Many immune evasion mechanisms present at each of these steps can contribute to primary or acquired resistance to PD-L1/PD-1 immunotherapy. Potential epigenetic strategies can be used at each step to overcoming immunotherapy resistance.
combination therapies to overcome the resistance of immune checkpoint therapies. Recently, researchers have become increasingly aware of the role of the immune system in identifying tumors that have an effective clinical benefit in checkpoint blockade. Pre-existing immune responses can predict the response of PD-L1/PD-1 blockade by detecting gene expression of CTLA-4 and T helper type (TH) 1, as well as the absence of the C-X3-C motif chemokine ligand 1 (CX3CL1) in the baseline of tumor tissues. Therefore, an effective response to anti-PD-L1/anti-PD-1 therapies requires the establishing an intact immune cycle and identifying tumors by trafficking immune cells to the tumor microenvironment. First, cancer cells will synthesize and present tumor antigens, which are recognized by immune cells, and then the signals are presented to T lymphocytes. Then, the activated T cells infiltrate and traffic into the tumor tissues, targeting malignant cells. Finally, activated T cells eliminate these tumor cells by releasing factors such as IFN-γ and promoting the release of tumor antigens. We believe that every step of the immune cycle is critical to the function of immunotherapy, and every change in the immune cycle may improve the response rate of the PD-L1/PD-1 blockade.

Currently, with the advent of many small molecule inhibitors that target epigenetic regulatory enzymes, epigenetic reprogramming is becoming a viable and effective therapeutic route for chemotherapy and cancer chemoprevention. More importantly, each step of the immune cycle can be regulated by epigenetic therapies to improve antigen presentation, T cell trafficking and infiltration, and disruption of the immunosuppressive state. Epigenetic therapy, combined with immune checkpoint inhibitors, can restore immune recognition and tumor elimination, thus improving clinical response rates. Given the importance of the pre-existing immune cycle in the adoption of checkpoint inhibitors and the profound impact of epigenetics on the immune system, this review will examine how epigenetic modifications affect various aspects of the immune cycle and discuss how epigenetic modification therapy regulates immune responses in cancer patients treated with PD-L1/PD-1 blockade therapies. We will focus on epigenetic strategies in combination therapy in the following text.

Figure 2  Modeling of the interaction of PD-L1 and PD-1. The binding of PD-L1 to its corresponding receptor PD-1 triggers the apoptosis of T cells, leads to T cell exhaustion and results in immune evasion. During immunotherapy, PD-1/PD-L1 antibodies disrupt the interaction between PD-1 and PD-L1, enable T cell reactivation, proliferation, and target the tumor cell for destruction. PD-L1 is upregulated in response to some inflammatory signals (e.g., IFN-γ), which are produced by active T cells during anti-tumor immune responses.
2. Current PD-L1 targeted immunotherapy

PD-L1 (B7-H1, CD274) can be detected on the cell surface of multiple tumor types, as well as several types of endothelial cells, epithelial cells, and several lymphocytes, thus playing a role in maintaining peripheral tolerance\(^ {36}\). The binding of PD-L1 to its corresponding receptor PD-1 results in immune evasion via counteracting activation signals on T cells\(^ {20}\) (Fig. 2). Therefore, PD-L1 is a shield for tumor cells which protects them from T cell-mediated elimination. Furthermore, CD80 (B7-1) binds to PD-L1 and transmits negative signals in both humans and mouse models\(^ {31}\). A recent study also found that binding of CD80 to the PD-L1 ligand PD-L1 in cis form on primary activated dendritic cells restricts PD-1 function during the activation of T lymphocytes\(^ {22}\).

The potential biological and long-lasting response rates in many types of cancer patients suggest that the PD-L1/PD-1 blockade therapy is one of the greatest advances in the history of cancer therapy. Several clinical trials on various solid tumor types with anti-PD-1 therapy showed significant extensions in overall survival (OS), such as non-small-cell lung cancer\(^ {35}\), uterine cervical carcinoma\(^ {34}\), and melanoma\(^ {33}\). This remarkable outcome prompted the FDA (U. S. Food and Drug Administration) to approve six PD-L1/PD-1 blockade antibodies for various tumor indications: Keytruda (pembrolizumab), Opdivo (nivolumab), and most recently, Libtayo (cemiplimab) for PD-1 blockade; and Tecentriq (atezolizumab), Bavencio (avelumab) and Imfinzi (durvalumab) for anti-PD-L1 therapy. The PD-L1/PD-1 axis plays a crucial role in escaping the immune system in various advanced cancer types, the studies showing benefits of PD-L1/PD-1 blockade led to FDA approval of PD-L1/PD-1 inhibitors in more than 10 cancer indications\(^ {26}\).

In particular, for therapeutic uses of anti-PD-L1/anti-PD-1 agents, clinical response to monotherapy has been shown in patients with various tumors, including melanoma, Hodgkin’s lymphoma, non-small-cell lung cancer, and renal cancer\(^ {27-30}\). However, the main limitation of this therapy is the failure to elicit a response in most cancer patients. The response rate of this therapy varies across different tumor types, ranging from \(\sim 19\%\) in patients with head and neck cancer to \(\sim 70\%\) in patients with Hodgkin lymphoma, with an objective response rate of \(20\%\)–\(40\%\) in majority of solid tumors\(^ {6\sim7,11}\). These results indicate that pre-existing immunity mediated by T cells exists in patients who respond to PD-L1/PD-1 blockade therapy, and patients who do not respond are likely to have damages in each step of the immune cycle. However, the PD-L1/PD-1 axis is not the sole mechanism to restrain immune responses\(^ {13}\). In addition to negatively regulatory factors in the immune cycle, an individual’s immune heterogeneity also reflects the contribution of a range of factors. For instance, epigenetic downregulation of cytokines results in lower T lymphocyte infiltration, and increased expression of endothelin receptors causes higher cancer survival and angiogenesis\(^ {32}\). These immunosuppressive factors represent the obstacles that must be overcome for a cancer patient to be successfully treated with immunotherapy. Studies on the epigenetic regulation of the immune system reveal fundamental interactions between epigenetic regulation and immunoregulation. In this review, we will address the immune-cycle-related reasons for initial or acquired tolerance to PD-L1/PD-1 immunotherapy and suggest the possibility of epigenetics in elevated PD-L1/PD-1 immunotherapy.

3. Insights into resistance to anti-PD-L1 therapy

Many efforts have been made to obtain insights into the mechanisms of resistance to anti-PD-L1 or anti-PD-1 therapy. Primary resistance occurs in approximately \(40\%\)–\(65\%\) of patients with melanoma treated with anti-PD-1-based therapy\(^ {31-32}\), and \(43\%\) of responders develop acquired resistance by three years\(^ {25\sim38}\). Before proceeding to the next steps, each step in the cancer immune cycle must be initiated and completed successfully to elicit an effective therapeutic immune response with immunotherapy. This cycle involves efficient (1) cancer-antigen presentation and T cell activation, (2) trafficking and infiltration of T cells to tumors, and (3) recognition and elimination of tumor cells by T cells\(^ {31\sim33}\). In this regard, the reasons for failed anti-PD-L1/anti-PD-1-based therapies are primarily associated with disorders in T cell function within the tumor environment\(^ {24\sim25}\). More specifically, the reasons that have been linked to primary and acquired resistance are as followed: (1) insufficient antigen presentation and recognition\(^ {31,34\sim35}\); (2) absence of T cells in the tumor microenvironment\(^ {36-43}\); (3) upregulation of immunosuppressive markers [such as indoleamine 2,3-dioxygenase (IDO) and regulatory T cells (Tregs)], T cell immunoglobulin and mucin domain 3 (TIM-3), lymphocyte activation gene-3 (LAG-3)]\(^ {44\sim45}\); (4) insufficient T-cell activation\(^ {32\sim34}\), (5) decreased sensitivity to IFN-\(\gamma\) signaling.

Many advanced cancer patients do not respond to monotherapy of PD-L1 blockade\(^ {5}\). This problem requires researchers to identify efficient combinatorial therapies urgently. Recent studies indicate that epigenetic modulations can trigger an immune response, enhance trafficking and infiltration of T cells, and improve the sensitivity of anti-PD-L1/anti-PD-1 therapy. Based on these research outcomes, we seek to find workable epigenetic strategies to cooperate with anti-PD-L1/anti-PD-1 immunotherapy.

4. Epigenetic regulation of the tumor microenvironment

Epigenetic modulations refer to a large-scale of, reversible, and heritable changes in gene expression without changing DNA sequences\(^ {5}\). Recent studies have revealed that epigenetic modifications drive phenotypic changes in not only cancer cells, but also immune cells\(^ {5}\). Epigenetic modifications, including changes in histone modifications, DNA methylation, and noncoding RNAs\(^ {5}\), are often linked to cancer development, progression, and metastasis. Epigenetic dysregulation plays a vital role in the immunogenic deficiency of cancer cells and leads to the presence of more immunosuppressive immune and stromal cells\(^ {5}\). The accumulation of changes in epigenetic modifications during tumorigenesis might contribute to proteomic transcriptional regulation and profound changes in genetic stability for the promotion of tumor immune escape\(^ {5}\).

There is increasing evidence suggesting that epigenetic changes can alter the function and phenotype of immune cells, for cellular killing and functional adjustment. Epigenetic modification factors can activate many silent genes. Some of them are immune checkpoint regulators that trigger the immune response, while others turn them off, leading to immune evasion\(^ {5}\). It is feasible for pharmacological agents to affect the epigenetic regulation of immune checkpoints, and the second generation of “episomal modifiers” is under development and has shown potential immunomodulatory properties\(^ {60}\). Recent clinical trials have tested the combination of epigenetic agents...
and immunotherapy as a promising cancer treatment strategy. Furthermore, several studies have indicated the importance of tumor epigenomic data such as a loss of the IFN-γ signaling pathway, which is closely related to the resistance of anti-CTLA-4 treatment. Therefore, these data reveal the critical role of patients who choose to receive immunological checkpoint treatments (see Fig. 3).

4.1. Epigenetic regulation of cycle 1: cancer-antigen presentation

The ideal tumor-associated antigen (TAA) is essential for cancer cell function, which is expressed only on cancer cells and elicits an immune response in the host. Finding a specific TAA as a specific target is one of the most important goals of immunotherapy.

One of the most effective escape strategies adopted by cancer cells is damaging antigen presentation, which is an initial step in the immune cycle and plays an important role in tumor immunotherapy. Quantitative levels of costimulatory genes, as well as tumor antigens, and major histocompatibility complex (MHC) are essential for the determination of T cell priming in immune therapy. Available studies have indicated that antigen-specific T cell levels close to 1% CD8+ cells might be necessary to establish an effective antitumor response. However, current studies indicate that the majority of cancer cells have lower levels of antigen presentation or even have none, which is lower than the recognized limits of the immune system, to prime an immune response. Epigenetic regulation appears to represent the primary mechanism regulating TAAs expression in different tumor cells, and treatments with DNMT inhibitors can increase tumor immunogenicity and immune recognition by re-expression of TAAs.

Epigenetic events, especially DNA methylation, are thought to be the primary mechanism regulating the expression of cancer testis antigen (CTA). CTA represents a family of tumor-associated antigens expressed on tumors. Weber et al. obtained first-hand proof that DNA methylation plays a massive role in CTA expression. Moreover, the level of MHC class I is also down-regulated by reversible methylation. Uptregulation of MHC class I and MHC class II by DNA methyltransferases inhibitors (DNMTi) has appeared in many cancers. Decitabine (5-aza-2-deoxycytidine, DAC) is a potent inhibitor of DNA methylation. In vitro treatment of a chronic lymphocytic leukemia (CLL) cell line with DAC upregulated the expression of MHC class I and MHC class II. DAC treatment also upregulated the surface expression of MHC class I and exhibited an increase in IFN-γ release by tumor-specific cytotoxic T lymphocytes (CTLs). Treatment of RM-1 prostate cancer cells with another epigenetic modifier 5-azacitidine (5-AzaC) enhanced CTA expression. Moreover, 5-AzaC enhanced proinflammatory functions of dendritic cells (DC) by increasing MHC class I, MHC class II, CD80, CD86, CD205, and CD40. Many studies have shown that exposure to demethylating agents can lead to the expression of several CTAs in multiple tumor cell lines, including melanoma, lung cancer, colon cancer, and malignant glioma. The emerging study indicated that EZH2 (enhancer of zeste homolog 2) was activated or overexpressed by the mutation in partial melanoma and other tumors, resulting in the silencing of antigen presenting relevant genes and cancer suppressor genes.

The abovementioned studies indicate that epigenetic agents can effectively improve antigen presentation. On the one hand, these agents can enhance immunogenicity by increasing the expression of tumor-associated antigens; on the other hand, they can advance the proinflammatory functions of DCs to boost T-cell responses.

**Figure 3** The roles of epigenetic agents in multiple aspects of the immune cycle. Histone deacetylase inhibitors (HDACis) increase immune recognition by restoring the expression of various tumor-associated antigens (TAAs), major histocompatibility complex (MHC) molecules, and the generation of antigen-presenting cell (APC). Epigenetic agents may also enhance the migration of T cells to the tumor microenvironment by targeting the production of TH1 chemokine and generation of immunosuppressive cells, such as myeloid-derived suppressor cells (MDSCs). Targeting epigenetic regulators can provide promising and safe methods to restore T cell activation.
proliferation and effector T cell trafficking. The analysis of DNA methylation on a genome-wide scale showed that the maturation and differentiation of DC were related to hypomethylation at DNA enhancer regions, which represent binding domains for known transcription factors of DCs lineage specification, and along with dynamic changes in epigenetic modifier enzymes DNA methyltransferase 1 (DNMT1), DNMT3, and TET2 (ten-eleven translocation 2)\(^1\). Moreover, the expression of miRNAs, including miR-14a, miR-14b, and miR-22, inhibited the function of antigen presentation in DCs\(^2\,^3,^8\). Given that patients with primary resistance to PD-L1/PD-1 antibodies have a partial absence of activated immune cells, this inspires us to enhance the recognition and activation of immune cells by treatment with epigenetic agents, in order to further increase immune cells infiltration and the activity of PD-L1/PD-1 antibodies.

### 4.2. Epigenetic regulation of cycle 2: trafficking and infiltration of T cells

Tumors can be broadly classified into hot (T cell-infiltrated) and cold (non-T-cell-infiltrated) tumors\(^76\,^77\,^78\). Hot tumors are abundant in tumor infiltrating lymphocytes (TIL) and hold a primary immune response. However, cold tumors lack a liberal quantity of pre-existing TIL. Here, we discuss the epigenetic regulations that affect the migration of T cells to the tumor microenvironment. It is imperative to understand these epigenetic mechanisms in order to target them therapeutically. Through this summary, we hope to provide an effective epigenetic method to improve the response rate of PD-L1/PD-1 immunotherapy.

Previous studies have summarized the reasons for the inability of T cells to infiltrate tumors. These include vasculature induced by tumor cells, chemokines, and existing suppressive immune cells\(^8\). Other research suggests that epigenetic regulation is involved in all three aspects. It has been found that the polycomb repressive complex 2 (PRC2) component and the demethylase tri-methylation of lysine 27 on histone H3 (H3K27me3) inhibited the trafficking of effector T cells by downregulating the expression of CX3 chemokine ligand (CXCL) 9 and CXCL10. Besides, PRC2-mediated epigenetic silencing was correlated with the suppression of effector T-cell trafficking and improved outcomes of anti-PD-L1 therapy in the mouse model\(^8\). In addition, DNA methylation associated with EZH2-mediated DNMT1 and H3K27me3 may suppress the expression of the TH1-type chemokines CXCL9 and CXCL10 and the subsequent transport of effector T cells to the tumor microenvironment\(^8\). Administration of epigenetic agents can increase the infiltration of effector T cells to the tumor and enhance the clinical efficacy of PD-L1 checkpoint blockade\(^5\,^8\). Therefore, epigenetic silencing of TH1-type chemokines is an important mechanism of immune evasion. The formation of vascular trees is the result of complex interactions between genetic and epigenetic factors\(^8\).

The tumor microenvironment is often associated with immunosuppressive cells which have a negative impact on T cell activation, migration, and proliferation. These include MDSCs, immature DCs, macrophages, and Treg cells. Even the immune cells (granulocytes) are thought to participate\(^7\). The deletion of the epigenetic regulator ubiquitin-like PHD and RING finger domain-containing 1 (UHRF1) in CD4\(^+\) T cells demonstrated flawed proliferation and functional maturation of Treg cells and maintained gut immunological homeostasis\(^8\). Many Treg-specific epigenetic signature genes, such as CTLA4, IKZF4 (EOS), and TNFRSF18 (GITR), showed complete demethylation, which allowed forhead box P3\(^+\) (FOXP3\(^+\)) T cells to acquire Treg-specific gene expression, lineage stability, and specific immuno-suppressive activity\(^9\). On the other hand, M2 macrophages can recruit Treg cells through the production of CCL22 (MDC, macrophage-derived chemokine)\(^10\). Epigenetic modifications of both human red cell acid phosphatase (ACP1) promoter and angiotensin converting enzyme (ACE1) distal regulatory elements appear to enhance specific gene expression programs in M2 macrophages, including metabolic pathway remodeling\(^10\). Moreover, EZH2 plays a vital role in the differentiation of peripheral Tregs, which may also contribute to the efficacy of PD-1 immunotherapy\(^11\). This finding suggests that the effect of epigenetic modification on macrophages may also trigger an effect on T cell infiltration. In another study, immunosuppressive MDSCs were reduced by the DNA-demethylating agent 5-aza-C in the tumor microenvironment, thereby facilitating an antitumor immune response\(^9\). Epigenetic reprogramming is significantly associated with T cell trafficking and infiltration, as well as patient survival rate. Therefore, this is another effective means that can promote tumors with high T lymphocyte cells infiltration to improve the efficacy of current immunotherapies.

### 4.3. Epigenetic regulation of cycle 3: T cell immunity

In a normally-functioning immune system, T cells spot cancer cells and fill the site with cytotoxic T lymphocytes (CTL), thereby infiltrating and killing cancer cells. However, tumor cells can upregulate immune suppression signals through different immunization steps to block T cell activation and induce cell death, resulting in immune evasion and eventual metastasis. A growing body of research and clinical trials has shown that immunotherapeutic methods involving the inhibition of immunological checkpoint modulators can restore T cell activation and promote the antitumor activity of the immune system. Emerging evidence indicates that targeting epigenetic regulators can provide promising and safe methods to restore T cell activation\(^12\).

Tumor-specific CD8\(^+\) T cell dysfunction in solid tumors causes tumor progression. Recent studies suggest that T cell exhaustion is associated with epigenetic alterations\(^8\). It directs T cell differentiation to an arrested state, causing T cell exhaustion\(^13\). Also, miRNAs are known to participate. These include miR-214\(^9\), miR-126\(^9\), and miR-568\(^10\), which can promote the development of Tregs and improve their function, thus downregulating CTL activity. Recent research illustrates that epigenetic control in Tregs occurs through the regulation of FOXP3\(^13\). DNA hypomethylation is also necessary for Treg-specific gene expression and functional suppression\(^10\). The above studies suggest that epigenetic agents can facilitate the inhibition of the maturation of functional Tregs\(^13\).

Other new studies reveal that targeting epigenetic regulators can provide safe and effective methods to restore T-cell activation. T cells obtain a variety of inhibitory receptors such as LAG-3, PD-1, TIM-3, 2B4 (CD244), and CD106\(^5\), which can at least partially and synergistically mediate T cell exhaustion through nonredundant signaling pathways. Initial studies suggested that the PD-L1/PD-1 pathway mediates CD8\(^+\) T cell exhaustion, and PD-1 is considered as a marker of exhausted T cells\(^10\). Owing to the crucial influence of PD-1 and PD-L1 on CD8\(^+\) T cell dysfunction, we hope to identify transcriptional mechanisms involved in the regulation of gene expression encoding PD-1 and PD-L1.

It is worth noting that treatment with a demethylating agent, decitabine, results in dose-dependent upregulation of PD-1, its ligands (PD-L1 and PD-L2) and CTLA-4 mRNA expression\(^13\).
### Table 1  Current clinical trials.

| Clinical trial identifier | Status          | Phase     | Cancer type                                                                                           | Epigenetic drug (target)                                                                 | PD-1/PD-L1                        |
|---------------------------|-----------------|-----------|-------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------|-----------------------------------|
| NCT02936752               | Recruiting      | I         | Treating patients with myelodysplastic syndrome after deoxyribonucleic acid (DNA) methyltransferase inhibitor (DNMTi) therapy failure | Entinostat (HDAC1, HDAC2, Pembrolizumab HDAC3)                                              |                                   |
| NCT03250962               | Recruiting      | I/II      | Hodgkin lymphoma                                                                                      | Decitabine (DNMT1, DNMT3A, DNMT3B)                                                       | SHR-1210                          |
| NCT02961101               | Recruiting      | I/II      | Relapsed or refractory malignancies, including non-Hodgkin’s lymphoma, hepatocellular carcinoma, breast cancer, ovarian cancer or lung cancer or renal-cell cancer | Decitabine (DNMT1, DNMT3A, DNMT3B)                                                       | Anti-PD-1 antibody                |
| NCT03346642               | Recruiting      | I/II      | Primary mediastinal large B-cell lymphoma                                                             | Decitabine (DNMT1, DNMT3A, DNMT3B)                                                       | SHR-1210                          |
| NCT02892318               | Suspended       | I         | Acute myeloid leukemia                                                                                | Guadecitabine (DNMT1)                                                                     | Atezolizumab                      |
| NCT03308396               | Recruiting      | I/II      | Advanced kidney cancer, clear cell renal cell carcinoma                                                | Guadecitabine (DNMT1)                                                                     | Atezolizumab                      |
| NCT02508870               | Suspended       | I         | Myelodysplastic syndromes                                                                             | Azacitidine (DNMT1)                                                                       | Atezolizumab                      |
| NCT03161223               | Recruiting      | I/II      | Lymphoma                                                                                              | 5-Azacitidine (DNMT1)                                                                     | Durvalumab                        |
| NCT01928576               | Recruiting      | II        | Non-small-cell lung cancer                                                                           | Azacitidine (DNMT1), Entinostat (HDAC1, HDAC2, HDAC3)                                     | Nivolumab                         |
| NCT02890069               | Recruiting      | I         | Colorectal cancer, non-small-cell lung carcinoma, triple negative breast cancer, renal cell carcinoma  | Panobinostat (HDAC)                                                                       | PDR001                            |
| NCT02619253               | Recruiting      | I         | Renal cell carcinoma, urinary bladder neoplasms                                                       | Vorinostat (HDAC1, HDAC2, Pembrolizumab HDAC3, HDAC7, HDAC11)                             |                                   |
| NCT02512172               | Active, not recruiting | I       | Colorectal cancer                                                                                     | Romidepsin/CC-486 (HDAC1, HDAC2)                                                         | Pembrolizumab                     |
| NCT02453620               | Recruiting      | I         | Breast adenocarcinoma, HER2/Neu negative invasive breast carcinoma                                      | Entinostat (HDAC1, HDAC2, Ipilimumab/Nivolumab HDAC3)                                     |                                   |
| NCT02959437               | Active, not recruiting | I/II  | Solid tumors advanced malignancies metastatic cancer                                                    | INCB057643 (BET), Azacitidine (DNMT1)                                                     | Pembrolizumab                     |
| NCT03854474               | Recruiting      | I/II      | Advanced urothelial carcinoma, locally advanced urothelial carcinoma, metastatic bladder urothelial carcinoma | Tazemetostat (EZH2)                                                                      | Pembrolizumab                     |
| NCT02220842               | Active, not recruiting | I       | Lymphoma                                                                                              | Tazemetostat (EZH2)                                                                       | Atezolizumab                      |
| NCT03337698               | Recruiting      | I/II      | Non-small-cell lung cancer                                                                           | Tazemetostat (EZH2)                                                                       | Atezolizumab                      |
| NCT03019003               | Recruiting      | I/II      | Head and neck cancer                                                                                  | Azacitidine (DNMT1)                                                                       | Durvalumab                        |
| NCT03390296               | Recruiting      | II        | Acute myeloid leukemia                                                                                | Azacitidine (DNMT1)                                                                       | Avelumab                          |
| NCT02811497               | Recruiting      | II        | Microsatellite stable colorectal carcinoma, platinum resistant epithelial ovarian cancer type II, estrogen receptor positive and HER2 negative breast cancer | Azacitidine (DNMT1)                                                                       | Durvalumab                        |
| NCT02935361               | Recruiting      | I/II      | Chronic myelomonocytic leukemia, myelodysplastic syndrome, recurrent acute myeloid leukemia with myelodysplasia-related changes | Guadecitabine (DNMT1)                                                                     | Atezolizumab                      |
| NCT03179943               | Recruiting      | II        | Urothelial carcinoma                                                                                   | Guadecitabine (DNMT1)                                                                     | Atezolizumab                      |
| NCT03206047               | Suspended       | I/II      | Platinum-resistant ovarian carcinoma, recurrent fallopian tube carcinoma, recurrent ovarian carcinoma, recurrent primary peritoneal carcinoma | Guadecitabine (DNMT1)                                                                     | Atezolizumab                      |
| NCT03590054               | Recruiting      | I         | Stage III cutaneous melanoma, stage IV cutaneous melanoma, locally advanced melanoma, locally advanced solid neoplasm | Abexinostat (pan-HDAC inhibitor)                                                          | Pembrolizumab                     |
| NCT03812796               | Recruiting      | II        | GI cancer                                                                                              | Domatinostat (HDAC1, HDAC2, HDAC3)                                                        | Avelumab                          |
| NCT03982134               | Not yet recruiting | I       | Melanoma, non-small-cell lung cancer                                                                  | Panobinostat (HDAC)                                                                       | PDR001                            |
Another study investigated the connection between epigenetic modifications and the upregulation of immune checkpoint genes. The results indicated that TIM-3, PD-1, LAG-3, and CTLA-4 were significantly hypomethylated and upregulated in tumors compared with normal tissues in breast cancer patients. Two other preclinical studies have validated that HDAC inhibitors can upregulate the expression of PD-L1 and T cell chemokines. Bromodomain and extra-terminal motif inhibitors (BETi) can also increase the expression of PD-L1 through an MYC-dependent pathway. Other research has identified a novel mechanism of PD-L1 upregulation in non-small-cell lung cancer through epigenetic regulation. These results demonstrated that PD-L1 is upregulated by P53 via miR-34. Those results indicate a potential synergy between immune checkpoint inhibitors and epigenetic agents, as the former could upregulate the expression of immune checkpoints, which may improve the therapeutic effect and sensitivity of immune checkpoint inhibitors.

5. Combination strategies between epigenetic agents and PD-1/PD-L1 blockade

Epigenetic reprogramming has been demonstrated to play a vital role in protecting tumor cells from immune surveillance. All of these studies suggest that epigenetic reprogramming influences both cancer cells and immune cells, and suggest a potential combination of epigenetic agents and anti-PD-L1/anti-PD-1 immunotherapy. Of note, some clinical trials are designed to evaluate the effect of PD-L1/PD-1 antibodies combined with epigenetic agents among different cancer types, which are summarized in Table 1.

6. Conclusions

The impact of recent cancer immunotherapies has demonstrated the importance and ability of the immune system to fight malignancies, leading to the successful use of PD-L1/PD-1 blocking antibodies to combat many tumors during this process. Even though PD-L1/PD-1 blockade therapies have proven to have significant benefits in clinical trials, numerous patients with advanced cancer do not respond to anti-PD-L1/anti-PD-1 monotherapy. In addition, the development of primary or acquired resistance is a limitation of using these agents, and ongoing studies are pursuing strategies to overcome resistance and improve the efficacy of checkpoint treatment. This phenomenon calls for an urgent need to identify efficient combinatorial treatments.

We have presented three aspects of preclinical evidence to demonstrate how epigenetic treatment can optimize the outcome of patients with checkpoint treatment through several parts of the immune system involving tumor cells and host immune cells. Epigenetic agents can induce T cell attraction and reactivation by synergistically upregulating tumor antigen presentation, as well as by downregulating immune suppression signals. Although there are promising ongoing research programs to evaluate the clinical outcomes of epigenetic agents in combination with immunological checkpoint inhibitors for different cancers, other epigenetic mechanisms (such as lncRNA or miRNA) cannot yet be clinically targeted.

Recent studies have shown that epigenetic agents improve antitumor outcomes to a great extent through combination with PD-L1/PD-1 blockade therapy and shed new light on cancer immunotherapies. These epigenetic combination therapies may be optimally integrated to enhance the response rates of PD-L1/PD-1 blocking antibodies, which could save the lives of patients with uncontrolled malignancies in the near future.

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Author contributions

Xi Chen designed and wrote the paper. Xiaohui Pan, Wenzhen Zhang, Hongjie Guo, Shuyuan Cheng, and Qiaojun He revised the manuscript. Bo Yang and Ling Ding were responsible for the conception and design of the review.

Conflicts of interest

The authors claim that the researchers in this study have no conflict of interest.

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