Epigenetic engineering for optimal chimeric antigen receptor T cell therapy

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Abstract
Recent advancements in cancer immunotherapy, such as chimeric antigen receptor (CAR)-engineered T cell therapy and immune checkpoint therapy, have significantly improved the clinical outcomes of patients with several types of cancer. To broaden its applicability further and induce durable therapeutic efficacy, it is imperative to understand how antitumor T cells elicit cytotoxic functions, survive as memory T cells, or are impaired in their effector functions (exhausted) at the molecular level. T cell properties are regulated by their gene expression profiles, which are further controlled by epigenetic architectures, such as DNA methylation and histone modifications. Multiple studies have elucidated specific epigenetic genes associated with T-cell phenotypic changes. Conversely, exogenous modification of these key epigenetic factors can significantly alter T cell functions by extensively altering the transcription network, which can be applied in cancer immunotherapy by improving T cell persistence or augmenting effector functions. As CAR-T cell therapy involves a genetic engineering step during the preparation of the infusion products, it would be a feasible strategy to additionally modulate specific epigenetic genes in CAR-T cells to improve their quality. Here, we review recent studies investigating how individual epigenetic factors play a crucial role in T-cell biology. We further discuss future directions to integrate these findings for optimal cancer immunotherapy.

KEYWORDS
CAR-T cell, epigenetics, exhaustion, memory T cell, terminal differentiation

1 | INTRODUCTION

Chimeric antigen receptor (CAR)-engineered T-cell therapy has achieved remarkable success in hematological malignancies.1-3 However, its efficacy against solid tumors has been unsatisfactory.4 Terminal differentiation and impairment of effector functions (i.e., exhaustion) of the infused CAR-T cells hinder durable antitumor immune response, necessitating further modification of CAR-T cells to resist these dysfunctional mechanisms. One of the unique features of adoptive immunotherapy is that it includes an in vitro culture of T cells for expansion and genetic engineering, during which additional pharmacological or genetic modifications can be provided to enhance their antitumor functions.

The functional properties of CAR-T cells are dictated by their gene expression profiles, which are controlled by epigenetic mechanisms. Variability of epigenetic profiles in CAR-T cell products already exists at the time of infusion, and these molecular profiles are significantly associated with therapeutic efficacy.5-7 In addition,
CAR-T cells undergo further epigenetic and transcriptional changes following infusion, which significantly affects CAR-T cell effector functions and survival capacity.\(^3\,^9\)

DNA methylation is one of the most widely investigated epigenetic modifications. Methylation of CpG islands abundant in gene promoter regions has been established as a transcriptional repression mechanism.\(^10\) De novo DNA methylation is deposited at the fifth position of cytosine residues (5-methylcytosine, 5mC) by DNA-methyltransferase 3A (DNMT3A) and 3B (DNMT3B). DNA methylation decreases transcriptional activity by directly impairing the binding affinity of DNA to transcription factors or by recruiting repressive histone-modifying enzymes. Conversely, the ten-eleven translocation (TET) family regulates active DNA demethylation through the hydroxymethylation of 5mC (5hmC).

Histone modification is another well-investigated epigenetic change in which histone proteins acquire multiple posttranslational alterations, such as acetylation, methylation, phosphorylation, ubiquitination, and citrullination. Amino acid residues at specific positions of histones are modified by defined epigenetic enzymes, which are referred to as “writers” and “erasers.” Some epigenetic factors work as “readers,” which are transcriptional regulators that recognize regions with specific histone modifications. For example, histone acetylation of lysine residues is regulated by histone acetyltransferases (HATs) and deacetylases (HDACs). Acetylated histones are recognized by the bromodomain and extra-terminal domain (BET) family.\(^11\)

## 2 | EPIGENETIC CONTROL OF T CELL FUNCTIONS

### 2.1 | Memory T cell differentiation

Memory T cell formation principles have primarily been elucidated using mouse viral infection models.\(^12\) Naïve CD8\(^+\) T cells differentiate into effector T cells upon initial antigen stimulation. After pathogen clearance, most of the effector T cells undergo apoptosis, while a part of them acquire memory T cell properties and survive for a long time. As T cells are usually stimulated in vitro using anti-CD3 mAb in CAR-T cell generation, CAR-T cells acquire a memory T cell phenotype at the time of infusion. Memory T cells progressively differentiate during multiple rounds of cell division. The differentiation process is accompanied by gradual phenotypic changes, and several representative differentiation stages are referred to as stem cell-like memory T cells (T\(_{SCM}\)), CD45RA\(^-\)CCR7\(^-\)CD62L\(^-\)CD27\(^+\)CD28\(^+\)IL-7R\(^+\)CD95\(^+\)), central memory T cells (T\(_{CM}\), CD45RA\(^-\)CCR7\(^+\)CD62L\(^+\)), and effector memory T cells (T\(_{EM}\), CD45RA\(^-\)CCR7\(^-\)CD62L\(^+\)).\(^13\,^14\) Human T\(_{SCM}\), located at the apex of the differentiation hierarchy, can usually maintain themselves through self-renewal cell division for a decade-long period.\(^15\) However, CAR-T cells are terminally differentiated in a much shorter period because of massive expansion during in vitro culture and after antigen encounter in vivo in most cases (Figure 1). As CAR-T cell persistence and therapeutic efficacy are closely correlated, enhancing the self-renewal capacity of T cells is a rational intervention to elicit a durable antitumor response.\(^16\,^17\)

Multiple studies have identified key transcription factors that control effector differentiation memory formation, and progressive differentiation of memory T cells.\(^18\) Epigenetic profiles control the activity of these transcription factors by regulating their accessibility to cognate genomic regions. In fact, the epigenetic landscape is globally altered upon T cell phenotypic changes.\(^19\,^22\) As most epigenetic modifiers have nonredundant roles in T cell functions, modulation of single epigenetic enzymes could globally alter T cell differentiation status.

### 2.2 | T cell exhaustion

Chronic antigen stimulation of cytotoxic T cells impairs their proliferation and cytokine production capacity, which is frequently accompanied by upregulation of multiple immune checkpoint molecules, including programmed cell death 1 (PD1), TIM3, and LAG3. This state is referred to as T cell exhaustion. Because a detailed definition of T cell exhaustion has been reviewed elsewhere,\(^23\) we will discuss the epigenetic profiles of exhausted T cells. Exhausted T cells possess a chromatin landscape distinct from that of conventional memory and effector T cells.\(^24\) Importantly, these epigenetic profiles remain unchanged even after chronic antigen exposure resolution or immune checkpoint blockade therapy (ICT).\(^25\,^27\) These findings indicate that T cell exhaustion is fundamentally regulated by epigenetic mechanisms rather than by the expression of immunoinhibitory receptors. Recent studies have revealed the significance of several transcription/epigenetic factors, including TOX,\(^28\) NR4A,\(^29\) and DNMT3A,\(^27\) for the formation of exhaustion-related epigenetic profiles, which could be attractive targets to prevent or abrogate T cell exhaustion programs. Another important finding is that...
exhausted T cells contain a subset of T cells with an early memory phenotype, called precursor exhausted T cells (TPEX). Precursor exhausted T cells highly express the memory-related transcription factors TCF7, ID3, and BCL6, but not effector-associated factors such as B lymphocyte-induced maturation protein-1 (Blimp1) or ID2, compared to terminally exhausted T cells. They also possess surface marker phenotypes distinct from those of terminally exhausted cells: high expression of SLAMF6/Ly108 and CXCR5, and low expression of TIM3. As the TPEX population alone can regain effector functions upon ICT, at least transiently, it would be a reasonable strategy to expand or maintain this subset of exhausted T cells to augment the therapeutic efficacy of ICT (Figure 2).

3 | EPIGENETIC MODIFICATION TO ENHANCE CAR-T CELL EFFICACY

3.1 | DNA methylation

DNA-methyltransferase 3A plays a pivotal role in effector differentiation of naïve T cells. T cells upregulate the expression of DNMT3A after antigen stimulation, which then downregulates naïve-associated genes by de novo DNA methylation of their promoter regions. A part of the effector T cells become memory T cells through successful demethylation of some naïve-associated genes such as Sell, Ccr7, and Tcf7. Genetic KO of Dnmt3a in mouse CD8+ T cells promotes memory formation by enhancing the demethylation efficiency of these loci. In addition, the role of DNMT3A in exhausted T cells has been investigated. Chronic antigen stimulation causes progressive DNA methylation of genes expressed in early memory T cells, which contributes to the formation of gene expression profiles in terminally exhausted T cells. Although Dnmt3a-KO T cells did not prevent the upregulation of immune checkpoint molecules, they maintained early memory phenotypes and regained effector functions upon PD1 blockade, suggesting that inhibition of Dnmt3a supports the maintenance of a TPEX population under chronic antigen exposure. Based on these findings, the effect of DNMT3A inhibition on CAR-T cells has been explored. CRISPR/Cas9-mediated KO of DNMT3A in human CAR-T cells enhanced their proliferative capacity and maintained a TCM phenotype after repeated stimulation, which resulted in superior control of tumor progression in vivo.

Conversely, DNA demethylation through TET2 is associated with upregulation of effector-associated genes (e.g., Ifng, Tnf, and GzmB) following antigen exposure. Tet2 KO in murine CD8+ T cells promoted memory formation and enhanced pathogen control upon secondary antigen encounter. Fraietta et al. reported that a CAR-T cell clone in which the CAR transgene was inserted into TET2 gene locus and ablated with its expression showed substantial expansion in a patient with chronic lymphocytic leukemia and induced complete response. They further confirmed that TET2 inhibition conferred a proliferative advantage on repeatedly stimulated CAR-T cells. TET2 also appears to play an essential role in the immunosuppressive pressures of myeloid cells within the tumor microenvironment (TME). Tet2 KO, specifically in myeloid-lineage cells, endowed tumor-associated macrophages with pro-inflammatory gene expression profiles and promoted tumor infiltration of effector T cells. These studies suggest that pharmacological inhibition of TET2, although not developed at present, could enhance antitumor immunity by reversing immunosuppressive TME. Although DNMT3A and TET2 play reciprocal roles in DNA methylation, inhibiting both enzymes contributes to T cell longevity through different mechanisms. Further studies are required to elucidate how these factors coordinately regulate T cell properties.

3.2 | Repressive histone marks

As discussed above, terminal T cell differentiation is accompanied by decreased expression of memory-associated genes. As this is accomplished, at least in part, by the deposition of repressive histone marks, inhibition of the repressive epigenetic factors targeting these genes could promote the maintenance of long-lived memory T cells. We have recently identified that genetic KO of PR domain zinc finger protein 1 (PRDM1), encoding Blimp1, helped to maintain the functional properties of early memory T cells in human CAR-T cells. Although Blimp1 itself seems to lack histone-modifying enzymatic activity, it organizes the formation of repressive histone marks by interacting with several other epigenetic enzymes such as G9a (H3K9 methyltransferase), HDAC1/2 (histone deacetylase), and PRMT5 (protein arginine methyltransferase). In addition to its role as a master regulator for B cell terminal maturation, Blimp1 is closely associated with effector T cell functions. In CD8+ T cells, T

![FIGURE 2](image_url) Heterogeneity of exhausted T cells. Exhausted T cells have differentiation hierarchy as seen in memory T cells. Precursor exhausted T cells (TPEX) can be reinvigorated by immune checkpoint therapy (ICT). Epigenetic profiles of exhausted T cells cannot be reverted to those of memory and effector T cells by ICT.
cell receptor (TCR) stimulation and subsequent cytokine signaling induce the expression of Blimp1. Blimp1 promotes terminal T cell differentiation by negatively regulating memory-associated genes.

Blimp1 disruption in CAR-T cells significantly remodeled chromatin openness in approximately 7000 genomic regions, resulting in altered expression levels of more than 2000 genes. Blimp1-deficient CAR-T cells showed a less-differentiated memory phenotype, which resulted in better persistence after infusion. As expected, Blimp1 KO attenuated the effector functions of CAR-T cells, as indicated by reduced granzyme B and perforin production. Despite this disadvantage, Blimp1-KO CAR-T cells showed potent antitumor responses in vivo by acquiring a long-lived potential.

SUV39H1 is a histone methyltransferase that catalyzes H3K9me3 independent of the Blimp1 complex. During effector differentiation of CD8+ T cells, SUV39H1 contributes to the downregulation of naïve-associated genes by adding H3K9 trimethylation in their promoter regions. SUV39H1-KO CD8+ T cells showed enhanced memory formation and attenuated effector functions. In contrast, SUV39H1 also plays a role in repressing effector gene expression in tumor-infiltrating T-lymphocytes (TILs). In a mouse tumor model, the expression of SUV39H1 was upregulated in TILs. Treatment with the SUV39H1 inhibitor F5446 enhanced the effector functions of TILs through upregulation of effector genes. These studies suggest that SUV39H1 promotes the initial step of the naïve-to-effector transition; however, it suppresses effector T cell functions at the terminally differentiated stage.

Lysine-specific histone demethylase 1A (KDM1A), also known as lysine-specific histone demethylase 1 (LSD1), is a demethylase specific for mono- and dimethylated H3K4 and H3K9, thereby acting as both a transcriptional activator and repressor. Lysine-specific histone demethylase 1A promotes the self-renewal and proliferative capacity of cancer stem cells and is therefore considered a direct therapeutic target. Another interesting finding on KDM1A inhibition in tumor cells is that it induces double-stranded RNA stress and subsequent IFN pathway activation, which results in enhanced tumor immunogenicity through upregulation of HLA class I expression in tumor cells. A recent study has also elucidated the role of KDM1A in antitumor T cells. Liu et al. showed that KDM1A inhibition expanded exhausted T cells with a TPEX phenotype within the TILs, suggesting that KDM1A promoted terminal T cell differentiation. Inhibition of KDM1A prior to anti-PD1 immunotherapy augmented the antitumor response through the expansion of a less differentiated exhausted T cell population that can respond to ICT.

3.3 | Active histone marks

Histone lysine acetylation (H3K9ac or H3K27ac) and the trimethylated form of H3K4 (H3K4me3) are representative histone marks that promote gene expression. These histone modifications undergo genome-wide alteration upon memory formation and differentiation. The BET bromodomain protein family, including BRD2, BRD3, BRD4, and BRDT, contains bromodomain signatures that bind to acetylated lysine residues in histones and regulate gene expression as an epigenetic reader. We have previously reported that CAR-T cells treated with the BET bromodomain inhibitor JQ1 maintained an early memory phenotype during in vitro expansion and showed enhanced persistence in vivo. As one of the mechanisms, BRD4 positively regulates the expression of the effector gene BATF by binding to its promoter region. JQ1- or RNA interference-mediated downregulation of BATF results in increased expression of memory markers and interleukin-2 (IL-2) production. Although BATF is an essential transcription factor that induces potent effector functions in CD8+ T cells, its activity is not required during in vitro preparation of antitumor T cells. In more recent studies, Milner et al. investigated BRD4 target genes more comprehensively and showed that BRD4 transcriptionally regulates multiple genes necessary for effector T cell differentiation, including BATF, ID2, and ZEB2, further corroborating our findings. Another group has reported that JQ1 reinvigorates CAR-T cells from the exhausted state. JQ1 treatment promoted antigen-dependent proliferative capacity of CAR-T cells through maintaining surface expression levels of the CAR, which is frequently downregulated in repeatedly stimulated CAR-T cells. Intriguingly, JQ1 treatment also enhanced antitumor immunity by suppressing PD-L1 expression in tumor cells and tumor-associated macrophages. These findings suggest that BET bromodomain proteins play pleiotropic roles in T cell functions.

Acetylated histones are erased by HDACs, which are composed of 18 genes and grouped into classes I–IV. Multiple studies have elucidated the inhibitory effects of class I HDACs (HDAC1, 2, 3, and 8) on memory and effector T cell function. Tay et al. showed that HDAC3 inhibited the cytotoxic T cell response at an early time point during T cell activation. The HDAC3-specific inhibitor RGFP966 enhanced effector T cell functions through upregulation of the genes associated with cytotoxicity programs and effector differentiation. Conversely, HDAC3 inhibition negatively affected long-term T-cell persistence, which attenuated the recall T-cell response upon antigen rechallenge. Recent studies have shown that HDAC inhibitors can enhance the antitumor immune response by modulating several immune cell functions. Vorinostat and trichostatin A (class I/II inhibitor) decreased the infiltration of myeloid-derived suppressor cells into tumors, resulting in the activation of cytotoxic T cell functions. The HDAC8-specific inhibitor PCI-34051 induced the expression of T cell-trafficking chemokines in hepatic cellular carcinoma, resulting in the increased infiltration of CD8+ T cells within TME.

The histone methyltransferase DOT1L catalyzes mono-, di-, and trimethylation of H3K79. We previously reported that DOT1L inhibition in human T cells suppressed allogeneic T cell reactions. CD8+ T cells treated with the DOT1L inhibitor SGC0946 showed increased levels of the ERK phosphatase DUSP6, which shortened the duration of ERK phosphorylation triggered upon TCR stimulation. Interestingly, DOT1L inhibition did not compromise the high-avidity T-cell response mediated by genetically overexpressed CAR or TCR, suggesting its potential usefulness in selectively suppressing off-target T-cell reactions. Another study investigated the kinetics
of DOT1L-KO T cells in mice.\textsuperscript{64} T cell-specific Dot1l deletion resulted in the loss of naïve CD8\textsuperscript{+} T cell populations and promoted premature differentiation into a T\textsubscript{CM} phenotype, independent of antigen exposure. As Dot1l deletion also alters epigenetic profiles other than H3K79 methylation, it could regulate gene expression profiles in a complex manner by interacting with other epigenetic factors.

### 4 | CONCLUDING REMARKS

As discussed above, modification of vital epigenetic enzymes can reprogram the T cell differentiation status and effector functions, which can be used to potentiate antitumor T cell functions (Tables 1 and 2).\textsuperscript{65-68} Although epigenetic profiles affect the activity of transcription factors, accumulating evidence suggests that several transcriptional regulators can also modulate epigenetic profiles. For instance, the transcription factor TOX orchestrates the epigenetic landscape of exhausted T cells.\textsuperscript{28} Further investigation is warranted to understand the interactions between transcriptional regulators and epigenetic enzymes comprehensively.

From a therapeutic viewpoint, several strategies have been developed to enhance the efficacy of adoptive immunotherapy through epigenetic modifications. One strategy is the genetic modification of specific epigenetic genes in CAR-T cells during in vitro preparation, enabling stable remodeling of T cell functions (Figure 3). For clinical translation, further optimization of a genome-editing protocol is essential for efficient production of the cell products. In addition, as some of the candidate genes are associated with tumorigenesis (e.g., TET2, DNMT3A, and PRDM1), careful safety evaluation at the preclinical setting would be required. Another strategy is the pharmacological inhibition of specific epigenetic enzymes. This approach includes transient intervention during in vitro CAR-T cell preparation to improve the quality of infusion products or systemic administration to modulate the functions of infused CAR-T cells, tumor cells, and surrounding immunosuppressive cells. For rational modification, it is necessary to elucidate the effect of

| Gene       | Function                     | Effect on CD8\textsuperscript{+} T cell functions                              | Ref.         |
|------------|------------------------------|--------------------------------------------------------------------------------|--------------|
| DNMT3A     | DNA methylation              | Effector differentiation                                                      | [19, 33, 34] |
| TET2       | DNA hydroxymethylation       | Effector differentiation                                                      | [36, 37]    |
| MBD2       | Methylated DNA (reader)      | Memory T cell formation                                                       | [66]         |
| SUV39H1    | H3K9 methylation             | Effector differentiation                                                      | [46, 47]    |
| EZH2       | H3K27 methylation            | Effector differentiation                                                      | [67]         |
| DOT1L      | H3K79 methylation            | Naive phenotype maintenance                                                   | [64]         |
| PRMT1      | Arginine methylation         | Effector functions                                                            | [65]         |
| PRMT5      | Arginine methylation         | Memory T cell formation                                                       | [68]         |
| KDM1A      | Lysine demethylation         | Effector differentiation                                                      | [51]         |
| PRDM1      | Repressive histone modification (indirect) | Effector differentiation                                                                 | [42-44]     |
| HDAC3      | Histone deacetylation        | Suppress cytotoxic functions                                                  | [59]         |
| BRD4       | Acetylated histones (reader) | Effector differentiation                                                      | [54, 56, 57]|

| Ablated gene | Impact on CAR-T cell functions | Ref. |
|--------------|---------------------------------|------|
| DNMT3A       | ↑                               | ↑    | ↑    | →    | [35] |
| TET2         | ↑                               | ↑    | ↑    | →    | [16, 57] |
| PRDM1        | →                               | ↑    | ↑    | ↓    | [39] |

TABLE 1 Summary of epigenetic factors associated with cancer immunotherapy: Role of individual epigenetic factors for T cell functions

TABLE 2 Summary of epigenetic factors associated with cancer immunotherapy: Previously reported epigenetic gene modifications to improve the efficacy of chimeric antigen receptor T (CAR-T) cell therapy
individual factors on T cell functional properties. Another question is how multiple epigenetic factors coordinate T cell functions. Although most studies have investigated the effect of modifying a single factor, coordinated modulation could synergistically enhance T cell functions.

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**REFERENCES**

1. Maude SL, Laetsch TW, Buechner J, et al. Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. *N Engl J Med*. 2018;378(5):439-448.
2. Locke FL, Ghobadi A, Jacobson CA, et al. Long-term safety and activity of axicabtagene ciloleucel in refractory large B-cell lymphoma (ZUMA-1): a single-arm, multicentre, phase 1–2 trial. *Lancet Oncol*. 2019;20(1):31-42.
3. Munshi NC, Anderson LD, Shah N, et al. Idecabtagene vicleucel in relapsed and refractory multiple myeloma. *N Engl J Med*. 2021;384(8):705-716.
4. Shah NN, Fry TJ. Mechanisms of resistance to CAR T cell therapy. *Nat Rev Clin Oncol*. 2019;16(6):372-385.
5. Fraietta JA, Lacey SF, Orlando EJ, et al. Determinants of response and resistance to CD19 chimeric antigen receptor (CAR) T cell therapy of chronic lymphocytic leukemia. *Nat Med*. 2018;24(5):563-571.
6. Garcia-Prieto CA, Villanueva L, Bueno-Costa A, et al. Epigenetic profiling and response to CD19 chimeric antigen receptor T-cell therapy in B-cell malignancies. *J Natl Cancer Inst*. 2022;114(3):436-445.
7. Sheih A, Voillet V, Hanafi LA, et al. Clonal kinetics and single-cell transcriptional profiling of CAR-T cells in patients undergoing CD19 CAR-T immunotherapy. *Nat Commun*. 2020;11(1):219.
8. Good CR, Aznar MA, Kuramitsu S, et al. An NK-like CAR T cell transition in CAR T cell dysfunction. *Cell*. 2021;184(25):6081-6100.
9. Zebley CC, Brown C, Mi T, et al. CD19-CAR T cells undergo exhaustion DNA methylation programming in patients with acute lymphoblastic leukemia. *Cell Rep*. 2021;37(9):110079.
10. Wu X, Zhang Y. TET-mediated active DNA demethylation: mechanism, function and beyond. *Nat Rev Genet*. 2017;18(9):517-534.
11. Marks PA, Rifkind RA, Richon VM, Breslow R, Miller T, Kelly WK. Histone deacetylases and cancer: causes and therapies. *Nat Rev Cancer*. 2001;1(3):194-202.
12. Joshi NS, Cui W, Chandele A, et al. Inflammation directs memory precursor and short-lived effector CD8+ T cell fates via the graded expression of bet transcription factor. *Immunity*. 2007;27(2):281-295.
13. Sallusto F, Lenig D, Förster R, Lipp M, Lanzavecchia A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature*. 1999;401(6754):708-712.
14. Gattinoni L, Lugli E, Ji Y, et al. A human memory T cell subset with stem cell-like properties. *Nat Med*. 2011;17(10):1290-1297.
15. Akondy RS, Fitch M, Edupuganti S, et al. Origin and differentiation of human memory CD8 T cells after vaccination. *Nature*. 2017;552(7685):362-367.
16. Fraietta JA, Nobles CL, Sammons MA, et al. Disruption of TET2 promotes the therapeutic efficacy of CD19-targeted T cells. *Nat Biotechnol*. 2018;35(8):307-312.
17. Xu Y, Zhang M, Ramos CA, et al. Closely related T-memory stem cells correlate with in vivo expansion of CAR.CD19-T cells and are preserved by IL-7 and IL-15. *Blood*. 2014;123(24):3750-3759.
18. Kaech SM, Cui W. Transcriptional control of effector and memory CD8+ T cell differentiation. *Nat Rev Immunol*. 2012;12(11):749-761.
19. Scherer CD, Barwick VG, Youngblood BA, Ahmed R, Boss JM. Global DNA methylation remodeling accompanies CD8 T cell effector function. *J Immunol*. 2013;191(6):3419-3429.
20. Crompton JG, Narayanan M, Cuddapah S, et al. Lineage relationship of CD8 T cell subsets is revealed by progressive changes in the epigenetic landscape. *Cell Mol Immunol*. 2015;13(4):502-513.
21. Scott-Browne JP, López-Moyado IF, Trifari S, et al. Dynamic changes in chromatin accessibility occur in CD8+ T cells responding to viral infection. *Immunity*. 2016;45(6):1327-1340.
22. Yu B, Zhang K, Milner JJ, et al. Epigenetic landscapes reveal transcription factors that regulate CD8+ T cell differentiation. *Nat Immunol*. 2017;18(5):573-582.
23. Wherry EJ. T cell exhaustion. *Nat Immunol.* 2011;12(6):492-499.

24. Sen DR, Kaminski J, Barnitz RA, et al. The epigenetic landscape of T cell exhaustion. *Science.* 2016;354(6316):1165-1169.

25. Yates KB, Tonnerre P, Martin GE, et al. Epigenetic scars of CD8+ T cell exhaustion persist after cure of chronic infection in humans. *Nat Immunol.* 2021;22(8):1020-1029.

26. Pauken KE, Sammons MA, Odorizzi PM, et al. Epigenetic stability of exhausted T cells limits durability of reinvigoration by PD-1 blockade. *Science.* 2016;354(6316):1160-1165.

27. Ghoneim HE, Fan Y, Moustaki A, et al. De novo epigenetic programs inhibit PD-1 blockade-mediated T cell rejuvenation. *Cell.* 2017;170(1):142-157.

28. Khan O, Giles JR, McDonald S, et al. TOX transcriptionally and epigenetically programs CD8+ T cell exhaustion. *Nature.* 2019;571(7746):211-218.

29. Liu X, Wang Y, Lu H, et al. Genome-wide analysis identifies NR4A1 as a key mediator of T cell dysfunction. *Nature.* 2019;567(7749):525-529.

30. Kallies A, Zehn D, Utschneider DT. Precursor exhausted T cells: key to successful immunotherapy? *Nat Rev Immunol.* 2020;20(2):128-136.

31. Miller BC, Sen DR, AI Abosy R, et al. Subsets of exhausted CD8+ T cells differentially mediate tumor control and respond to checkpoint blockade. *Nat Immunol.* 2019;20(3):326-336.

32. Im SJ, Hashimoto M, Gernry MY, et al. Defining CD8+ T cells that provide the proliferative burst after PD-1 therapy. *Nature.* 2016;537(7620):417-421.

33. Ladle BH, Li KP, Phillips MJ, et al. De novo DNA methylation by DNA methyltransferase 3a controls early effector CD8+ T-cell fate decisions following activation. *Proc Natl Acad Sci USA.* 2016;113(38):10631-10636.

34. Youngblood B, Hale JS, Kissick HT, et al. Effectors CD8 T cells dedifferentiate into long-lived memory cells. *Nature.* 2017;552(7685):404-409.

35. Prinzing B, Zebley CC, Petersen CT, et al. Deleting DNMT3A in CAR T cells prevents exhaustion and enhances antitumor activity. *Sci Transl Med.* 2021;13(620):eaab0072.

36. Zebley CC, Abdelsamed HA, Ghoneim HE, et al. Proinflammatory cytokines promote TET2-mediated DNA demethylation during DCD T cell effector differentiation. *Cell Rep.* 2021;37(2):109796.

37. Carty SA, Gohil M, Banks LB, et al. The loss of TET2 promotes CD8+ T cell exhaustion. *Blood.* 2022;139(14):2156-2172.

38. Wang HF, Ning F, Liu ZC, et al. Histone deacetylase inhibitors deplete myeloid-derived suppressor cells induced by 4T1 mammary tumors in vivo and in vitro. *Cancer Immunol Immunother.* 2017;66(3):355-366.

39. Li X, Su X, Liu R, et al. HDAC inhibition potentiates antitumor activity of macrophages and enhances anti-PD-L1-mediated tumor suppression. *Oncogene.* 2021;40(10):1836-1850.

40. Yang W, Feng Y, Zhou J, et al. A selective HDAC8 inhibitor potentiates antitumor immunity and efficacy of immune checkpoint blockade in hepatocellular carcinoma. *Sci Transl Med.* 2021;13(588):eaaz6804.

41. Kagoya Y, Nakatsugawa M, Yamashita Y, et al. BET bromodomain inhibition enhances T cell persistence and function in adoptive immunotherapy models. *J Clin Invest.* 2021;131(16):e145459.

42. Zhang H, Bengsch F, Svoronos N, et al. BET bromodomain inhibition reverses chimeric antigen receptor extinction and reinvigorates exhausted T cells in chronic lymphocytic leukemia. *J Clin Invest.* 2021;131(16):e145459.

43. Araki Y, Wang Z, Zang C, et al. Genome-wide analysis of histone methylation reveals chromatin state-based regulation of gene transcription and function of memory CD8+ T cells. *Immunity.* 2009;30(6):912-925.

44. Belkina AC, Denis GV. BET domain co- regulators in obesity, inflammation and cancer. *Nat Rev Cancer.* 2012;12(7):465-477.

45. Kallies A, Zehn D, Utschneider DT. Precursor exhausted T cells: key to successful immunotherapy? *Nat Rev Immunol.* 2020;20(2):128-136.

46. Pace L, Goudot C, Zueva E, et al. The epigenetic control of stemness in CD8+ T cell fate commitment. *Science.* 2018;359(6372):177-186.

47. Lu C, Yang D, Klement JD, et al. Suv39H1 represses the expression of cytotoxic T-lymphocyte effector genes to promote colon tumor immune evasion. *Cancer Immunol Res.* 2019;7(3):414-427.

48. Maes T, Mascaró C, Tirapu I, et al. ORY-1001, a potent and selective covalent KDM1A inhibitor, for the treatment of acute leukemia. *Cancer Cell.* 2018;33(3):495-511.

49. Sheng W, LaFleur MW, Nguyen TH, et al. LSD1 ablation stimulates anti-tumor immunity and enables checkpoint blockade. *Cell.* 2018;174(3):549-563.

50. Sheng W, Liu Y, Chakrabarty D, Debo B, Shi Y. Simultaneous inhibition of LSD1 and TGFβ inhibits eradication of poorly immunogenic tumors with anti-PD-1 treatment. *Cancer Discov.* 2021;11(8):1970-1981.

51. Liu Y, Debo B, Li M, Shi Z, Sheng W, Shi Y. LSD1 inhibition sustains T cell invigoration with a durable response to PD-1 blockade. *Nat Commun.* 2021;12(1):6831.

52. Araki Y, Wang Z, Zang C, et al. Genome-wide analysis of histone methylation reveals chromatin state-based regulation of gene transcription and function of memory CD8+ T cells. *Immunity.* 2009;30(6):912-925.

53. Belkina AC, Denis GV. BET domain co-regulators in obesity, inflammation and cancer. *Nat Rev Cancer.* 2012;12(7):465-477.

54. Kogoya Y, Nakatsugawa M, Yamashita Y, et al. BET bromodomain inhibition enhances T cell persistence and function in adoptive immunotherapy models. *J Clin Invest.* 2016;126(9):3479-3494.

55. Kurachi M, Barnitz RA, Yosh N, et al. The transcription factor BATF operates as an essential differentiation checkpoint in early effector CD8+ T cells. *Nat Immunol.* 2014;15(4):373-383.

56. Milner JJ, Toma C, Quon S, et al. Bromodomain protein BRD4 directs and sustains CD8 T cell differentiation during infection. *J Exp Med.* 2021;218(8):e20202512.

57. Kong W, Dimitri A, Wang W, et al. BET bromodomain protein inhibition reverses chimeric antigen receptor extinction and reinvigorates exhausted T cells in chronic lymphocytic leukemia. *J Clin Invest.* 2021;131(16):e145459.
Gray SM, Amezquita RA, Guan T, Kleinsein SH, Kaech SM. Polycomb repressive complex 2-mediated chromatin repression guides effector CD8+ T cell terminal differentiation and loss of multipotency. *Immunity*. 2017;46(4):596-608.

Zheng Y, Chen Z, Zhou B, et al. PRMT5 deficiency enforces the transcriptional and epigenetic programs of KIrg1+ CD8+ terminal effector T cells and promotes cancer development. *J Immunol*. 2022;208(2):501-513.