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Epigenetic reprogramming holds promise in enhancing anti-tumor efficacy of CAR T cell therapy

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ABSTRACT

Chimeric antigen receptor (CAR) T cell therapy has emerged as a pivotal treatment modality for advanced hematological malignancies. However, clinical evidence suggests that CAR T cell therapy has a low response rate, poor efficacy for solid tumor, and a high complication rate. Recent research highlighted the crucial role of epigenetics in tumor immunity, particularly in modulating the fate and function of T cells. The epigenetic landscapes among T cell subpopulations show substantial differences, which in turn have a profound impact on the effector function and persistence of T cells. Epigenetic reprogramming holds promise for enhancing the persistence of CAR T cells, augmenting T cell infiltration, and ameliorating the immunosuppressive microenvironment while impeding immune evasion. In addition, biomarkers derived from the epigenetics serve as indicators to predict patient prognosis. In recent years, a growing number of clinical trials have been initiated to explore the combination of epigenetic drugs with CAR T cell therapy, highlighting the therapeutic promise of this synergistic approach in improving efficacy and overcome therapeutic resistance. However, the non-specificity of epigenetic drugs, side effects of epigenetic gene editing, poor efficacy in solid tumors, and instability of epigenetic biomarkers for predicting prognosis remain areas for further exploration. In this review, we explored the characterization of epigenetic modification landscapes across CAR T cell subpopulations, discussed how epigenetic reprogramming addresses challenges associated with CAR T cell therapy, and provided insights into the limitations of combining epigenetic strategies with CAR T cell therapy.

1. Introduction

For decades, surgery, radiotherapy, and chemotherapy have been the primary modalities used to treat malignant tumors. Chimeric antigen receptor (CAR) T cell therapy has recently emerged as an important immunotherapeutic approach, marking a considerable milestone in the treatment of malignant tumors. It has been approved by the Food and Drug Administration (FDA) for several hematological malignancies

Abbreviations: AP-1, activator protein 1; BATF, basic leucine zipper ATF-like transcription factor; BET, bromodomain and extra-terminal; BRD4, bromodomain-containing protein 4; CAR, chimeric antigen receptor; CCL4, C-C motif ligand 4; CCR6, CC-chemokine receptor 6; CECR2, cat eye syndrome chromosome region candidate 2; CR, complete response; CRS, cytokine release syndrome; DNMT1, DNA methyltransferase 1; EphA2, ephrin type-A receptor 2; FDA, Food and Drug Administration; Gzmb, granzyme B; H3K27me, methylation of histone 3 lysine 27; H3K27me3, trimethylation of histone 3 lysine 27; H3K4ac, acetylation at histone 3 lysine 4; H3K4me3, trimethylation at histone 3 lysine 4; H3K9me3, trimethylation of histone 3 lysine 9; HDAC8, histone deacetylase 8; IDH2, isocitrate dehydrogenase 2; IFNG, interferon-gamma; IRF4, interferon regulatory factor 4; ITAMs, immunoreceptor tyrosine-based activation motifs; KAT6A, lysine acetyltransferase 6 A; KDM1A, histone lysine demethylase 1 A; MDSCs, myeloid-derived suppressor cells; MUC1, mucin 1; NFAT, nuclear factor of activated T cells; Osr2, odd-skipped related 2; PRDM1, PR domain zinc finger protein 1; RORC, RAR-related orphan receptor C; STAT4, transcription 4; SWI/SNF, SWItch/sucrose non-fermentable; TAMs, tumor-associated macrophages; T_{CM}, central memory T cells; T_{EFF}, effector T-lymphocyte; T_{EM}, effector memory T cells; TET2, tet methylcytosine dioxygenase 2; T_{EX}, exhausted T-lymphocyte; Th1, T helper 1; T_M, memory T-lymphocyte; TME, tumor microenvironment; T_N, Naïve T-lymphocyte; TOX, thymocyte selection-associated high mobility group box protein; Tregs, regulatory T cells; TRIM24, tripartite motif-containing 24; TRUCKS, T cells redirected for universal cytokine-mediated killing; T_{SCM}, stem cell memory T cells.

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(Abramson et al., 2020; Berdeja et al., 2021; Maude et al., 2018; Munshi et al., 2021; Neelapu et al., 2024; Schuster et al., 2019; Wang et al., 2020). However, it is less effective against solid tumors compared to hematologic malignancies(Wagner et al., 2020). A remarkable proportion of patients with solid tumors do not respond to or relapse after treatment(Ghilardi et al., 2021; Shah et al., 2021). Also, clinical trials have shown that the use of CAR T cells can lead to toxicities associated with the induction of a robust immune response, the most common of which are cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome(Morris et al., 2022; Schubert et al., 2021). Although these complications are generally well-managed by experienced physicians, they still cause worry for CAR T cell therapy. Thus, while CAR T cell therapy has great potential to improve the clinical prognosis of advanced hematologic malignancies, it still faces significant challenges in terms of efficacy and safety.

Recently, more studies have focused on the epigenetics of tumors (Chen et al., 2021; Huang et al., 2018). Epigenetics means changes in gene activity that are heritable without changing the DNA sequence. Epigenetic modifications include DNA methylation, histone modification, chromatin accessibility, non-coding RNA, and RNA modification (Dawson and Kouzarides, 2012). Emerging evidence shows that epigenetics plays an important role in controlling how immune cells work (Henning et al., 2018) and regulating the immunosuppressive tumor microenvironment (TME)(Niu et al., 2024). Therefore, targeting epigenetic modifications and subsequent transcriptional changes, a process known as epigenetic reprogramming, has great potential to improve the therapeutic performance of CAR T cells(Akbari et al., 2021). Epigenetic reprogramming covers all stages of CAR T cell therapy: before treatment, during CAR T cell manufacturing, and after CAR T cell infusion. For example, the use of epigenetic drug decitabine after CAR T cells infusion has shown promising outcomes in preclinical trials and is currently being tested in clinical trials (NCT04553393)(Wang et al., 2021a). In addition, a strategy for manipulating tumor epigenetic landscape using CAGM regimen containing chidamide, azacitidine, obinutuzumab and mitoxantrone liposome prior to CAR T cell therapy is currently undergoing clinical trials (NCT05823701). Moreover, a growing number of phase I and II clinical trials are now being carried out to evaluate the combined effects of epigenetic drugs and CAR T cell therapy. These trials show the translational potential of this combination strategy.

Recent progress in immunotherapy has led to more attention on the epigenetic regulation of CAR T cells. Ahn et al.(Ahn et al., 2024) explored key transcription factors and epigenetic regulators involved in the differentiation, maintenance, and function of exhausted CAR T cells in solid tumors. They also reviewed ways to improve CAR T cell effectiveness by reversing T cell exhaustion. Qin et al.(Qin et al., 2025) highlighted the role of transcriptional and epigenetic regulators in CAR T cell function and differentiation, and discussed possible targeting strategies. In this review, we aim to provide a comprehensive overview of the landscape of epigenetic modifications in CAR T cell subsets and by outlining how the challenges in CAR T cell therapy can be addressed through epigenetic strategies. Meanwhile, we summarize ongoing and completed clinical trials investigating the combination of epigenetic drugs with CAR T cell therapy. Finally, we highlight the unresolved challenges in combining epigenetic modifications with CAR T cell therapy and explore possible directions.

2. Fundamentals of CAR T cell therapy

CAR T cells refer to T lymphocytes that have been genetically engineered to express the chimeric antigen receptor CAR. This receptor is typically made up of three major components: extracellular, transmembrane, and intracellular signaling domains(June and Sadelain, 2018; Qu et al., 2022a). The extracellular domain of the CAR is responsible for antigen recognition. Unlike traditional T cells, the targeting domains of CAR are derived from antigen-binding fragments of antibodies and therefore do not require major histocompatibility complex molecules for antigen recognition(Singh and McGuirk, 2020).

CAR T cells are classified into four generations, distinguished by the varying composition of their intracellular structural domains. First-generation CAR T cells include only a CD3ζ chain that contains immunoreceptor tyrosine-based activation motifs (ITAMs), which are inefficient in T cell activation and have short life spans *in vivo*. Second- and third-generation CAR T cells are developed based on the first-generation. They add one or two costimulatory domains, usually CD28 or 4-1BB, in the intracellular structural domains to improve the activation efficiency of T cells(Imai et al., 2004; Maher et al., 2002). The current CAR T cells used in clinical practice are mainly second-generation CAR T cells containing CD28 or 4-1BB signaling domains (Shimabukuro-Vornhagen et al., 2022). Third-generation CAR T cells have been tested in clinical trials but are not yet approved by the FDA (Del Bufalo et al., 2023). Fourth-generation CAR T cells, also called T cells redirected for universal cytokine-mediated killing (TRUCKS), build on the second-generation by incorporating constitutive or inducible expression of cytokines, such as IL-12(Tokarew et al., 2019). This modification facilitates the recruitment of additional immune cells and enhances CAR T cell adaptation to the TME(Tokarew et al., 2019). Table 1 summarizes the characteristics of CAR T cell generation.

Notably, despite the fact that most of the literature reports limited durability and anti-tumor efficacy of first-generation CAR T cells, a recent clinical trial reported more than 18 years of complete remission in neuroblastoma treated with first-generation CAR T cells(Li et al., 2025). This finding implies that under specific immunological or cellular conditions, first-generation CAR T cells can achieve durable tumor control even in the absence of costimulatory domains. This challenges the widely held belief that first-generation CAR T cells are obsolete. Although the precise epigenetic or transcriptional program underlying this long-term response remains undefined, this observation highlights the need for more in-depth analysis of CAR T cell subsets with durable persistence.

3. Epigenetic landscape of CAR T cell subpopulations

Patient-derived T cells are collected and isolated as the first step in CAR T cell therapy. Some trials specifically isolated central memory T or naïve T cells for subsequent manufacturing, while the majority utilized the isolated bulk T cells directly for the production process(López-Cantillo et al., 2022; Meyran et al., 2021). Isolated T cells are activated through the TCR (CD3) and co-stimulated by a selected ligand (often CD28), in the presence of cytokines such as IL-2, IL-15, and IL-4(Bai et al., 2024; Brown et al., 2024; Enblad et al., 2018). Following activation, T cells are modified through genetic engineering to express the CAR capable of recognizing tumor-associated target antigens. CAR T cells that have undergone *in vitro* culture and expansion comprise a mixed population that includes both memory and effector subpopulations(Zhang et al., 2022b). The expanded CAR T cells are infused back into the patient. After infusion into the body, some T cells will gradually move toward exhaustion under repeated chronic antigenic stimulation.

The anti-tumor ability of CAR T cells largely depends on their subpopulation characteristics. T cells with a less differentiated phenotype, like memory T cells, are believed to have greater proliferative capacity and persistence(Fraietta et al., 2018). Epigenetic mechanisms play an important role in determining T cell fate(Chan et al., 2021; Pace et al., 2018). Understanding the epigenetic state of various differentiated T cell subpopulations and their dynamics provides novel insights into sensitizing CAR T cell therapy through epigenetic programming (Fig. 1).

3.1. Naïve T-lymphocyte (T_N)

T_N is characterized by high cell surface expression of CD45RA and lack of CD95, which has high proliferative potential and can

Table 1
Summary of CAR T cell generations.

	Intracellular domain	Advantages	Limitations	References
First-generation	Contains only the ITAM segment of CD3; does not contain costimulatory signaling domain	Opens new directions in tumor treatment	Lacks persistence and a proliferation capacity because of the lack of costimulatory signals	(Thistlethwaite et al., 2017)
Second-generation	Contains a costimulatory signaling domain in the endodomain region, such as CD28 or 4-1BB	Defines the basic structure of CAR, which simultaneously satisfies the two characteristics of highly specific recognition of tumors and efficient activation of T cell; strong anti-tumor efficacy; is a mainstream product that is either on the market or in clinical trials	High potency but short duration of T cell killing with CD28 and limited potency but long duration with 4-1BB; poor outcome in solid tumors; has a certain rate of complications, such as CRS and neurotoxicity	(Fujiwara et al., 2021; Roselli et al., 2021)
Third-generation	Contains two costimulatory signaling domains in the endodomain region, such as CD27, CD28, 4-1BB, OX40, TLR2, and ICOS	Different combinations of costimulatory domains have different impact on various properties, including metabolic pathways, proliferation, and development of memory T cells; has superior expansion and longer persistence than the second-generation	Increased tonic signaling; increased rate of CRS; some studies show that its clinical performance is not superior to that of second-generation	(Derigs et al., 2024; Guedan et al., 2018; Ramello et al., 2019; Ramos et al., 2018)
Fourth-generation	Based on the second-generation CAR; a constitutively or inducibly expressed chemokine, antibodies, enzymes, and other secretory proteins were added	Increased T cell infiltration in tumor tissue; stronger T cell proliferation and release of killing agents; enhanced killing of solid tumors; is a research hotspot	The theoretical risk of severe adverse events, including neurotoxicity and CRS	(Lanitis et al., 2021; Tang et al., 2023)

Abbreviations: CAR, chimeric antigen receptor; ITAM, immunoreceptor tyrosine-based activation motif; CRS, cytokine release syndrome.

differentiate into effector or memory phenotypes upon antigenic stimulation(van den Broek et al., 2018). The epigenetic landscape of T_N is characterized by demethylated and highly expressed naïve-associated genes, including *CD62L, Bcl-2, CD127, Ccr7*, and T cell factor 7 (*Tcf7*) (Youngblood et al., 2017). Furthermore, high methylation and closed chromatin of effector genes, such as granzyme B (*Gzmb*) and interferon-gamma (*IFNG*), and memory genes, such as CC-chemokine receptor 6 (*CCR6*) and RAR-related orphan receptor C (*RORC*), are also observed (Schmidl et al., 2018; Schmidl et al., 2011; Steinfelder et al., 2011). When T_N is stimulated by different external environmental signals, such as cytokines, hormones, and growth factors, it experiences dynamic shifts in its epigenetic landscape, especially trimethylation of histone 3 lysine 4 (H3K4me3) and trimethylation of histone 3 lysine 27 (H3K27me3)(Wei et al., 2009). Such epigenetic reconfigurations endow T_N with phenotypic plasticity, shaping its functional property and determining its ultimate cellular fate. When the T_N is activated, changes in epigenetic remodeling of gene expression occur, promoting the transformation of memory or effector T cell(Schmidl et al., 2018).

In summary, T_N has a tightly packed chromatin structure and low gene expression, but it has high plasticity. This epigenetic feature endows T_N with the latent capability for activation, poised to respond to antigenic stimulation.

3.2. *Effector T-lymphocyte (T_{EFF})*

This subpopulation of T cells has potent effector functions but a short lifespan and poor self-renewal capacity(McLellan et al., 2019). At the epigenetic level, T cells induce large-scale effector-associated epigenetic remodeling upon antigenic stimulation. This includes changes in DNA methylation across many gene regions, including silencing of naïve-associated genes such as *Tcf7* and *Ccr7*, and demethylation at effector genes such as *IFNG* and *Gzmb*(Scharer et al., 2013). Among these, enzymes involved in methylation play important roles in driving and maintaining effector phenotypes. The early fate of effector CD8+ T cells is regulated by DNA methyltransferase 3 A (DNMT3A), an enzyme responsible for *de novo* DNA methylation(Ladle et al., 2016). The deletion of DNMT3A activates the genes that will otherwise be silenced in effector T cells, thereby suppressing T_{EFF} phenotypes(Ladle et al., 2016). The histone methyltransferase enhancer of zeste homolog 2 (EZH2), which is the catalytic component of polycomb inhibitory complex 2, can maintain effector features and prevent exhaustion by influencing T cell metabolism(Koss et al., 2020; Li et al., 2020). It is essential for development of T_{EFF}. Besides enzymes involved in epigenetic modifications, some transcription factors, such as forkhead box protein 1 (FOXP1) and kruppel-like factor 2 (KLF2), can also affect the differentiation of T_{EFF} by controlling gene transcription and chromatin accessibility(Zhu et al., 2024). FOXP1 helps maintain stem-like properties and reduces the formation of T_{EFF} subset in CAR T cells. On the other hand, KLF2 promotes T_{EFF} subset differentiation and inhibits CAR T cell exhaustion.

Collectively, T_{EFF} demonstrates an active state characterized by an open chromatin configuration, with the upregulation of numerous cytokine and cytotoxic molecule genes. These cells can rapidly counter tumors but have a short lifespan and may progress toward exhaustion under persistent antigenic stimulation.

3.3. *Memory T-lymphocyte (T_M)*

Memory CAR T cells can be divided into several subtypes, including stem cell memory T cells (T_{SCM}), central memory T cells (T_{CM}), and effector memory T cells (T_{EM}), which are sequentially more differentiated and are accompanied by a decrease in proliferative and differentiation potentials(Chan et al., 2021). Memory phenotypes have greater proliferative capacity and *in vivo* persistence than effector phenotypes. The infusion of a higher proportion of T_M during CAR T cell therapy has been demonstrated to enhance both the strength and persistence of the anti-tumor response(Wang et al., 2023).

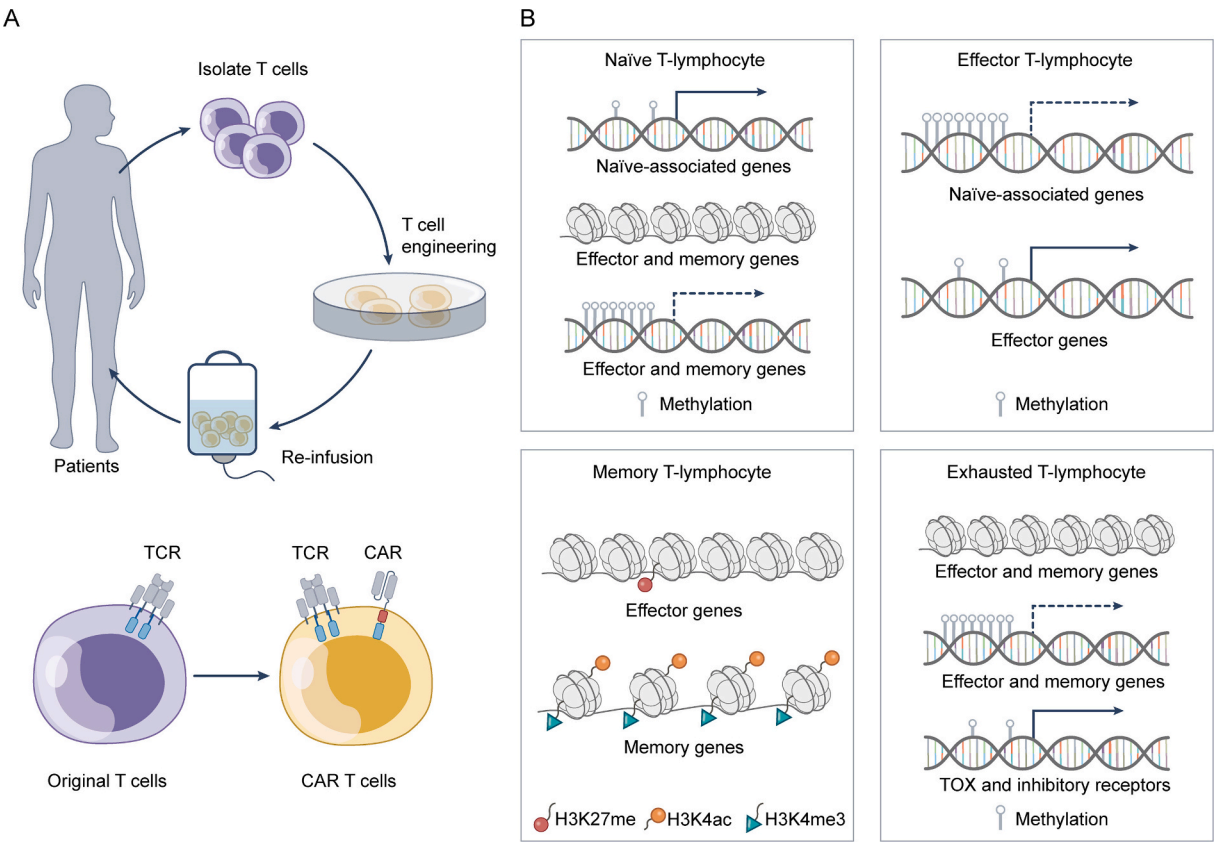

Fig. 1. Production process of CAR T cells and epigenetic landscape of different CAR T cell subpopulations.
A. CAR T cell production process. T cells are first isolated from the patient, then engineered *in vitro* to express CAR targeting specific antigen, and finally expanded and infused back into the patient. B. Epigenetic landscape of different CAR T cell subpopulations. Naïve T-lymphocytes have considerable demethylation of naïve-associated genes. The effector and memory genes of naïve T cells are highly methylated and have closed chromatin. Effector T-lymphocytes are characterized by hypermethylation of naïve-associated genes and demethylation of effector genes. The motifs encoding effector genes in memory T-lymphocytes have decreased chromatin accessibility owing to a decrease in methylation of histone 3 lysine 27 (H3K27me), whereas the histones of the memory genes undergo H3K4ac and H3K4me3, with increased chromatin accessibility. High methylation and closed chromatin of effector and memory genes, as well as substantial demethylation of TOX and inhibitory receptors, were observed in exhausted T-lymphocytes.
Abbreviations: CAR, chimeric antigen receptor; TCR, T cell receptor; H3K27me, methylation of histone 3 lysine 27; H3K4ac, acetylation of histone 3 lysine 4; H3K4me3, trimethylation of histone 3 lysine 4; TOX, thymocyte selection-associated high mobility group box protein.

Reduced H3K27me in *IFNG* and *Gzmb* chromatin characterizes T_M(Zediak et al., 2011). Histone tails located within the promoter regions of memory genes typically undergo acetylation of histone 3 lysine 4 (H3K4ac) and H3K4me3, a change that drives T cell differentiation into a memory phenotype(Zebley et al., 2020). In addition, FOXO1 is a key mediator of memory CAR T cell differentiation, inducing chromatin remodeling that leads to increased accessibility of gene loci such as *IL7R* and *KLF3*, while decreasing the accessibility of exhaustion-associated gene loci like thymocyte selection-associated high mobility group box protein (*TOX*) and *FASLG*(Doan et al., 2024). This results in the induction of memory-like gene expression programs. In contrast, another study found that the ribonuclease Regnase-1 is involved in the epigenetic repression of memory-related programs(Zheng et al., 2021). Regnase-1 deficiency causes significant changes in the DNA methylation landscape, promoting the formation of memory-like CAR T cells and increasing their lifespan and function.

To encapsulate, T_M harbors relatively stable epigenetic modifications and an open chromatin state, expressing genes associated with durable survival. These epigenetic marks contribute to their long-term persistence.

3.4. Exhausted T-lymphocyte (T_{EX})

The exhausted CAR T cells develop from effector CD8+ T cells and memory-like precursor cells(Jiang et al., 2022). T_{EX} consists of two subsets: the precursors of exhausted T cells and terminally exhausted T cells; and the former can differentiate into the latter(Brummelman et al., 2018). Exhaustion of CAR T cells is thought to be a unique epigenetic state, and this epigenetic state is remarkably stable and cannot be reversed by immune checkpoint blockade or withdrawal of antigen stimulation(Belk et al., 2022; Gennert et al., 2021; Henning et al., 2018; Sen et al., 2016). Chromatin accessibility and three-dimensional conformation undergo extensive changes during the onset of CAR T cell exhaustion, long before changes in gene expression occur(Gennert et al., 2021).

The epigenetic profile of T_{EX} can be summarized as considerable demethylation of motifs of the exhaustion marker, *TOX*, and various inhibitory receptors, including *PD-1*, *CTLA-4*, *TIM3*, and *LAG3*(Zebley et al., 2021b), decreased mitochondrial biosynthesis mediated by the chromatin remodeling complex SWItch/sucrose non-fermentable (SWI/SNF) and histone acetylases(Bengsch et al., 2016), decreased accessibility of effector-associated chromatin(Wherry, 2011), and silencing of T_{CM} and T_{SCM}-associated genes in response to methylated programs of DNA and histones(Ghoneim et al., 2017).

Although the specific epigenetic mechanisms that maintain the T_{EX} phenotype have not yet been elucidated, studies have identified multiple epigenetic-modifying enzymes that play a role. Exhausted T cells undergo *de novo* DNA methylation, and TOX is a key epigenetic regulator

involved in T cell exhaustion and is required for the generation of T_{EX}(Khan et al., 2019; Seo et al., 2019). TOX contributes to opening the chromatin structure and promotes continued upregulation of various inhibitory receptors by binding to and recruiting multiple epigenetic remodeling proteins(Khan et al., 2019; Scott et al., 2019). It is noteworthy, however, that although the *TOX*-knockout T cells did not show signs of exhaustion, their effector function was still impaired(Scott et al., 2019). This means that TOX is necessary to protect T cells from overstimulation and activation-induced cell death under conditions of chronic antigen exposure(Scott et al., 2019). Together, these findings suggest that effector function and exhaustion are closely connected, which raises questions about whether deleting *TOX* is a good approach for improving CAR T cell therapy. Nuclear factor of activated T cells (NFAT) proteins work with activator protein 1 (AP-1) transcription factors to form NFAT: AP-1 complexes that are essential for inducing T cell activation(Macián et al., 2000). A functional deficiency of AP-1 contributes to CAR T cell exhaustion by altering chromatin accessibility(Gennert et al., 2021; Lynn et al., 2019). Additionally, partnerless NFAT in the absence of AP-1 can drive a different transcriptional program that leads to T cell exhaustion(Martinez et al., 2015). In this case, NFAT lowers TCR signaling and upregulates the expression of inhibitory receptors by directly binding to their gene promoters(Martinez et al., 2015). NATF also induce downstream transcription factors like NR4A and TOX, which further reinforce the epigenetic and transcriptional landscape of T cell exhaustion(Chen et al., 2019; Khan et al., 2019; Seo et al., 2019). Collectively, these findings suggest that the balance between NFAT and AP-1 is a determinant of T cell fate, and that sustained antigenic stimulation disrupts this balance, initiating exhaustion-related epigenetic programs. Another study reported a marked increase in chromatin accessibility of the basic leucine zipper ATF-like transcription factor (*BATF*) and interferon regulatory factor 4 (*IRF4*) in terminal T_{EX}, which are considered key regulators of CAR T cell exhaustion (Jiang et al., 2022). Knockdown of *BATF* or *IRF4* can prevent CAR T cells from become exhausted in hematological malignancies(Jiang et al., 2022). However, Seo et al. found that the overexpression of *BATF* in CAR T cells promoted anti-tumor activity, reduced exhaustion, and supported the formation of long-lived memory T cells in solid tumor models(Seo et al., 2021). These results suggest that *BATF* has dual roles in CD8+ T cells, contributing to either memory formation or exhaustion depending on the signaling context within different tumor microenvironments.

In conclusion, T_{EX} exhibits a chromatin structure with the dysregulation of multiple transcription factors and high expression of inhibitory receptors. This leads to a diminished effector function in T_{EX}, characterized by reduced cytokine production and decreased cytotoxicity.

3.5. The potential epigenetic landscape of different CAR T engineering strategies

CAR T cell technology has evolved across multiple axes, including costimulatory signaling domains, transgene delivery methods, and modular structural constructs. While the genetic design and functional performance of these modifications have been extensively studied, their potential to reshape the epigenetic landscape of T cells remains poorly understood. Nevertheless, emerging insights suggest that various aspects of CAR design and manufacturing may influence transcriptional programs, memory phenotypes, and long-term function, possibly through epigenetic mechanisms. Based on current transcriptomic and phenotypic observations, we summarize three major dimensions of CAR T engineering that may affect the epigenetic landscape.

3.5.1. Intracellular signaling domains: CD28 vs. 4-1BB

Clinical studies have shown that CD28-based CAR T cells experience faster expansion but shorter duration compared to 4-1BB-based CAR T cells(van der Stegen et al., 2015). The epigenetic mechanisms responsible for these differences are unknown. Single-cell transcriptomic analyses have revealed that the signaling dynamics, phenotype, cell fate, and persistence of CAR T cells are influenced by their costimulatory domains(Boroughs et al., 2020). Compared to CD28-based CAR T cells, 4-1BB-based CAR T cells exhibit significantly higher expression of HLA class II genes (such as *HLA-DR*), *ENPP2*, and IL-21 axis–related genes (such as *IL21*, *IL21R*, and *IL12RB2*) upon activation, along with lower expression of *PDCD1* and *LGMN*(Boroughs et al., 2020). Furthermore, after CAR stimulation, CD4+ CAR T cells with the 4-1BB domain show higher expression of Th1-related genes, while those with the CD28 domain show higher expression of early Th2-related genes(Boroughs et al., 2020). 4-1BB-based CAR T cells also display a central memory phenotype in CD8+ subsets and upregulation of fatty acid metabolism-related genes(Boroughs et al., 2020). These transcriptional differences in gene expression may result from epigenetic changes caused by different costimulatory signals, although the exact mechanisms are still unclear. In addition, CD28 has been shown to enhance T cell responses by promoting chromatin remodeling and CpG demethylation at the *IL2* gene, which help increase its transcriptional accessibility(Murayama et al., 2006; Thomas et al., 2005). Similar epigenetic changes have also been observed at other cytokine loci, including IL-4 and IFN-γ(Grogan et al., 2001). These findings support that CD28 and 4-1BB may lead to distinct epigenetic patterns in CAR T cells.

3.5.2. Gene delivery methods: Viral vs. non-viral transduction

CAR T cells are commonly produced by either lentiviral transduction or transposon-based electroporation methods, such as the PiggyBac system. Compared to CAR T cells made using PiggyBac, those generated through lentiviral transduction often show a stronger memory-like phenotype(Niu et al., 2023). This may be due to the fact that that lentiviral vectors tend to integrate into parts of the genome that are more actively transcribed(Niu et al., 2023). Transcriptome analysis further revealed clear differences between the two types of CAR T cells. CAR T cells made using PiggyBac expressed higher levels of several cytokines, chemokines, and their receptors, including *CXCL13*, *IFNG*, and *IL9*(Niu et al., 2023). This suggests that the two CAR T platforms may be involved in distinct anti-tumor immune pathways.

The above differences are probably related to the gene delivery approach. Different methods may lead to different genomic integration patterns and changes in chromatin structure, which can affect how genes are regulated through epigenetic mechanisms. Supporting this idea, a previous study demonstrated that lentiviral transduction induces methylation changes in approximately 900 genes in human CD34+ hematopoietic stem cells, with most changes showing increased methylation at CpG site(Yamagata et al., 2012). These changes were particularly enriched in the chromosome 6p22.2–22.1 region, which encompasses histone genes(Yamagata et al., 2012). Such findings suggest that lentiviral transduction may broadly affect gene expression and cellular differentiation through genome-wide alterations in the epigenetic landscape.

3.5.3. Structural innovations: Logic-gated and redirectable platforms

While CAR T cells are often categorized into four generations, some researches contend that this classification may no longer capture the full scope of innovation. As engineering and manufacturing techniques advance, next-generation CAR T cells, such as logic-gated, scFv-redirectable, and biotin-redirectable CAR T cells, are being actively explored (Borrok et al., 2022; McCue et al., 2022; Verma et al., 2023; Young et al., 2022). Although epigenetic remodeling plays a critical role in the function and persistence of conventional CAR T cells, studies specifically characterizing the epigenetic landscapes of next-generation CAR T cells remain lacking. It is plausible that the distinct antigen-sensing mechanisms and activation thresholds inherent to these platforms may give rise to different patterns of exhaustion dynamics, and memory fate decisions, ultimately shaping their functional durability. For instance, in logic-gated CAR T cells, such as those employing AND, OR, or NOT gates, the incorporation of more complex and precise signal integration mechanisms may reduce tonic signaling and help mitigate exhaustion

(Hyrenius-Wittsten et al., 2021). In scFv-redirectable or biotin-redirectable CAR T systems, the ability to finely tune the frequency and intensity of antigen stimulation may foster the development of long-lived memory and non-exhausted phenotypes(Viaud et al., 2018). While these platforms differ from conventional CAR T designs, it is conceivable that their long-term epigenetic trajectories may eventually converge on exhaustion-related pathways, including transcriptional programs governed by regulators such as TOX. As these engineered platforms continue to evolve, future studies elucidating how their unique designs impact T cell epigenetics will be essential for advancing and optimizing next-generation CAR T therapies.

Collectively, epigenetics is closely related to the fate and function of CAR T cells. In various T cell subsets, a unifying theme in epigenetic regulation is the shaping of phenotypic characteristics and functions through epigenetic modifications. This includes increased chromatin accessibility and transcription at loci associated with the phenotype, while gene modules related to other subset functions are suppressed by epigenetic modifications. These epigenetic modifications are plastic and dynamic, enabling the enhancement of CAR T cell therapy through epigenetic reprogramming.

4. Address challenges in CAR T cell therapy through epigenetic strategies

4.1. Enhancing T cell persistence and effector function

The persistence and effector function of CAR T cells are critical for maintaining clinical responses in patients, and their efficacy and persistence are largely dependent on the state and phenotype of the T cells(Yang et al., 2023). Analysis of the dynamics of the CAR T cell phenotype after its introduction into the human body revealed that T_M significantly correlated with disease remission, whereas T_{EX} led to treatment relapse or non-response(Li et al., 2021). Therefore, the key to improving T cell persistence and effector function is to prevent or inhibit exhaustion phenotypes and promote the formation of memory phenotypes (Fig. 2).

With the increased understanding of epigenetic biology, genetic engineering strategies have been developed to promote T cell differentiation toward the T_M and inhibit T_{EX} formation. DNA methylation plays a critical role in the regulation of T cell phenotypes. For instance, the insertional disruption of tet methylcytosine dioxygenase 2 (*TET2*) *via* lentiviral transduction, an enzyme responsible for oxidizing 5-methylcytosine during active DNA demethylation, promoted the differentiation of CD19 CAR T cells into a T_M phenotype in a chronic lymphocytic leukemia patient(Fraietta et al., 2018). Knockout of *DNMT3A* involved in

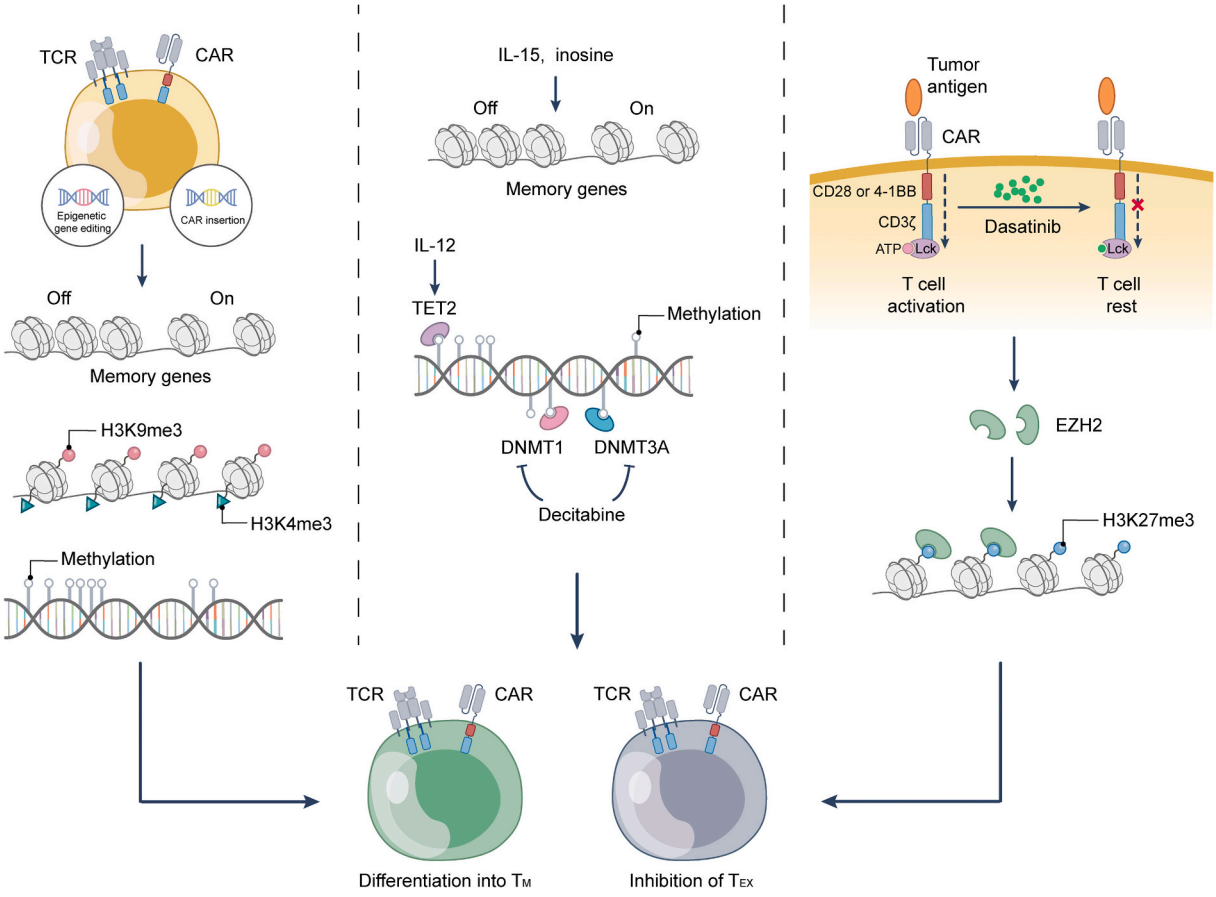

Fig. 2. Strategies to enhance the persistence and effector function of CAR T cells.
Epigenetic editing through genetic engineering can promote memory T cell differentiation and inhibit T cell exhaustion by altering the chromatin accessibility, histone modifications, or DNA methylation levels of memory genes. In addition, the use of epigenetic drugs, cytokines, or cellular metabolites can alter T cell phenotypes. Finally, drug-gated CAR T cells enable transient rest of CAR signaling, which enhances persistence and effector function by increasing EZH2 and subsequently H3K27me3.
Abbreviations: CAR, chimeric antigen receptor; TCR, T cell receptor; H3K9me3, trimethylation of histone 3 lysine 9; H3K4me3, trimethylation of histone 3 lysine 4; H3K27me3, trimethylation of histone 3 lysine 27; EZH2, enhancer of zeste homolog 2; T_M, memory T-lymphocyte; T_{EX}, exhausted T-lymphocyte; DNMT1, DNA-methyltransferase 1; DNMT3 A, DNA-methyltransferase 3 A; TET2, tet methylcytosine dioxygenase 2; Lck, lymphocyte specific protein tyrosine kinase.

DNA methylation in HER2, IL13Rα2, ephrin type-A receptor 2 (EphA2), and CD19 CAR T cells maintained stemness, proliferative capacity and effector function of T cells in mouse models of glioma, osteosarcoma, and leukemia(Prinzing et al., 2021). In addition, the ablation of certain genes can regulate T cell differentiation trajectory by affecting histone modifications. For example, knockout of isocitrate dehydrogenase 2 (*IDH2*) in CAR T cells targeting BCMA, HER2, and CD19 increased TCA metabolic perturbation and elevated H3K4me3 levels(Jaccard et al., 2023). This modification induced the formation of T_M and enhanced anti-tumor efficacy in mouse models of melanoma, leukemia and multiple myeloma(Jaccard et al., 2023). Editing *SUV39H1*, which encodes a histone lysine methyltransferase, in 41BB-based and CD28-based CAR T cell contributed to differentiation toward T_M by reducing trimethylation of histone 3 lysine 9 (H3K9me3) levels in mouse models of leukemia and solid tumors(Jain et al., 2023b; Lopez-Cobo et al., 2023). Finally, deficiencies in certain genes can increase chromatin accessibility of memory-associated genes. Knockout of the PR domain zinc finger protein 1 (*PRDM1*) or *EGR2* in T cells expressing CD19, mesothelin, and PSMA CARs increased memory-like T cell-associated chromatin accessibility in mouse models of leukemia, pancreatic cancer, and prostate adenocarcinoma, leading to the expansion of less differentiated T_M(Jung et al., 2023a; Jung et al., 2022; Yoshikawa et al., 2022).

In addition to inducing epigenetic remodeling through genetic editing, the pretreatment of CAR T cells with epigenetic drugs, cytokines, or cellular metabolites can also epigenetically remodel CAR T cells and alter their phenotypes. Decitabine is a clinically approved DNA methyltransferase inhibitor. Pretreatment with decitabine downregulates DNA methyltransferase 1 (DNMT1) and DNMT3A in CD123 CAR T cells, in which methylation at the CpG sites of *TIM3* and *PD-1* completely disappeared, thereby promoting T cell differentiation into T_M, inhibiting T_{EX} formation, and enhancing anti-tumor efficacy in leukemia mouse models(Wang et al., 2021a; You et al., 2020). Supplementation with cytokines during *in vitro* expansion helped generate CAR T cells with superior anti-tumor capability(Kagoya, 2023). IL-12 is a key proinflammatory cytokine involved in shaping T cell responses (Trinchieri, 2003). In CD4+ T cells, IL-12 promoted differentiation toward the T helper 1 (Th1) lineage by activating the transcription 4 (STAT4)–T-bet signaling axis(Thieu et al., 2008). In parallel, STAT4 enhanced histone acetylation at the *IFNG* promoter region, thereby upregulating IFN-γ production and enhancing effector functions in CD4+ T cells(Morinobu et al., 2004). Emerging evidence also indicated that IL-12 modulated the effector functions of CD8+ T cells. Specifically, IL-12 signaling facilitated TET2-dependent DNA demethylation at the *IFNG* locus in human CD8+ T cells, leading to increased IFN-γ production and augmented effector functions *in vitro*(Zebley et al., 2021a). Pretreatment of HER2 and Lewis-Y CAR T cells with IL-15 *in vitro* enhanced the accessibility of memory-associated genes (*Id3, S1pr1, Lef, Il7r*, and *Foxo1*) and increased anti-tumor response of CAR T cells in mouse models of breast and colon cancer(Giuffrida et al., 2020). The addition of inosine, a key intermediate in purine metabolism, to CD19, HER2, and GD2 CAR T cells cultured *in vitro* increased the chromatin accessibility of genes associated with memory differentiation(Klysz et al., 2024). This effect was mediated through the catalysis of hypusination on the translation elongation factor EIF5A, consequently enhancing the *in vivo* anti-leukemic and anti-osteosarcomatic efficacy of these engineered T cells in murine models(Klysz et al., 2024).

The FDA-approved tyrosine kinase inhibitor dasatinib was utilized to gate CAR T cells expressing GD2 CAR, CD19 CAR, or HA CAR, and temporarily induce them into a function-off state(Mestermann et al., 2019; Weber et al., 2021). Compared to CAR T cells with continuous CAR signaling, drug-gated CAR T cells expand in the CAR-off state. When the CAR signaling is turned on, these cells demonstrate superior cytotoxicity against leukemia and osteosarcoma both *in vitro* and *in vivo* (Weber et al., 2021). Transient cessation of CAR signaling induces epigenetic remodeling, in which EZH2 upregulates the H3K27me3, causing changes in the accessibility of exhaustion-associated genes, including *TBX21*, *NFATC1*, and AP-1 family transcription factors, preventing CAR T cells from progressing toward T_{EX} and instead shifting to a memory phenotype(Weber et al., 2021).

4.2. Increasing T cell residency in solid tumor tissues

CAR T cells are able to directly recognize malignant cells in the bloodstream in hematologic cancers. In solid tumors, however, they must first navigate through adjacent lymph nodes or blood vessels to access the tumor site(Zhang et al., 2023). Subsequently, even if T cells reach the tumor surface, the dense extracellular matrix and secretion of aberrant chemokines form a second barrier to T cell infiltration(Scholler et al., 2022).

One potential pathway for enhancing CAR T cell residency within solid tumor tissues is the induction of chemokine secretion that facilitates T cell recruitment. Histone deacetylase 8 (HDAC8) inhibitors increase CD8+ T cell infiltration into hepatocellular carcinomas by altering the enhancer landscape of tumor cells, which produces the chemokine C—C motif ligand 4 (CCL4) that recruits T cells(Yang et al., 2021). Another strategy to enhance CAR T cell retention in solid tumors involves modulating the transcriptional and epigenetic profile of T cells to promote a tissue-resident memory phenotype. Although TGF-β is widely recognized as an immunosuppressive cytokine that contributes to CAR T cell dysfunction in solid tumors, recent studies highlight that its effect is context-dependent. On one hand, transient and controlled *in vitro* exposure to TGF-β during the manufacturing of PSMA- and mesothelin-targeted CAR T cell has been shown to epigenetically reprogram T cells toward a resident memory phenotype(Jung et al., 2023b). This phenotype is characterized by increased expression of CD103, CD39, and stemness-associated markers such as TCF1 and IL7R (Jung et al., 2023b). These CAR T cells accumulate efficiently in tumor tissues and are highly resistant to tumor-imposed dysfunction, resulting in enhanced persistence and anti-tumor activity in mouse models of pancreatic ductal adenocarcinoma and prostate cancer, despite displaying some transcriptional features of exhaustion(Jung et al., 2023b). On the other hand, persistent exposure to TGF-β within the TME, as seen in many solid tumors such as prostate cancer, can lead to CAR T cell exhaustion and impaired function. This rationale has led to strategies to suppress TGF-β signaling, aiming to enhance the efficacy of CAR T cells against solid tumors(Kloss et al., 2018; Li et al., 2024; Liang et al., 2024; Stüber et al., 2020; Tang et al., 2020). Among these, PSMA CAR T cells co-expressing a dominant-negative TGF-β receptor showed feasibility and general safety in a phase I clinical trial for prostate cancer (NCT03089203)(Narayan et al., 2022). These seemingly opposing effects of TGF-β may be explained by differences in exposure context and T cell subsets. While chronic exposure to TGF-β in the TME typically promotes immunosuppression and functional exhaustion, short-term *in vitro* exposure appears to induce favorable epigenetic remodeling without triggering full exhaustion. Moreover, TGF-β may have different effects on different T cell subsets. It can help reprogram CD8+ T cells into a resident memory phenotype, but it may suppress terminally differentiated effector cells. These results show that it is important to carefully control when, how much, and under what conditions TGF-β is used in CAR T cell therapy. Because TGF-β can play many roles in CAR T therapy, more studies are needed to better understand how to use it in different types of tumors.

4.3. Overcoming the immunosuppressive microenvironment

During tumor growth, a microenvironment often forms that supports tumor survival and blocks anti-tumor immune responses. This has become another major challenge for CAR T cells after they reach the tumor tissue. The immunosuppressive TME creates many obstacles for CAR T cells. These include a variety of immunosuppressive cells, such as regulatory T cells (Tregs), tumor-associated macrophages (TAMs), and myeloid-derived suppressor cells (MDSCs), as well as highly expressed

immunosuppressive genes(Murad et al., 2021).

Several studies have focused on reducing the recruitment, activation, and differentiation of suppressive immune cells in the TME *via* epigenetic reprogramming. Knockdown of *BATF* affects the chromatin accessibility of *Ctla4, Tnfrsf4, Tnfrsf9*, and *Ccr8* in Tregs, thereby inhibiting Treg activation in animal models of non–small cell lung cancer(Itahashi et al., 2022). Low doses of DNA methyltransferase and HDAC inhibitors, 5-azacytidine and entinostat, inhibit the protumorigenic effects of MDSCs by downregulating the chemokine receptors, CCR2 and CXCR2, and promoting the differentiation of MDSCs into a more interstitial macrophage-like phenotype(Lu et al., 2020). Lysine acetyltransferase 6 A (KAT6A) inhibitor, which inhibits the binding of oncogenic chromatin-modifying factor tripartite motif-containing 24 (TRIM24) to chromatin, can suppress the recruitment of MDSCs(Yu et al., 2021). Cat eye syndrome chromosome region candidate 2 (CECR2) is the most important upregulated epigenetic factor for the increased abundance of M2 macrophages, causing immune inertia by increasing chromatin accessibility, thus promoting the polarization of M2 macrophages(Zhang et al., 2022a). CECR2 inhibition substantially abrogated the recruitment and polarization of TAMs and suppressed breast cancer metastasis in a mouse model(Zhang et al., 2022a). These studies support that epigenetic reprogramming may increase tumor sensitivity to CAR T cell therapy by inhibiting tumor-promoting immune cells in the TME. However, to the best of our knowledge, no previous study has directly validated this conclusion. Considering that CAR T cell therapy can shape the TME by inducing immunogenic cell death(Hou et al., 2021), the above findings require further validation in CAR T mouse models or CAR T trials.

Other advances include the use of epigenetic strategies to reduce immunosuppressive gene expression and overcome immune resistance. For example, treatment with EGFR CAR T cell therapy in glioblastoma activated an enhancer program that drove the upregulation of multiple immunosuppressive genes, such as *PD-L1*, *PD-L2*, *HVEM*, *GAL9*, *IL6*, *IL8*, *CSF2*, *BIRC3*, *IL1B*, and *IDO1*, which led to therapeutic resistance(Xia et al., 2021a). In contrast, the bromodomain-containing protein 4 (BRD4) inhibitor JQ1 combined with EGFR CAR T cells substantially reduced the expression of these immunosuppressive genes, thereby inhibiting the proliferation and metastasis of glioblastoma in mice(Xia et al., 2021a). Similarly, immunosuppressive genes such as *PD-L1*, *PD-L2*, *HVEM*, *IL6*, *IL8*, *CSF1*, *CXCL2*, *IDO1*, and *IL1B*, were substantially suppressed by the combination of the CDK7 inhibitor, THZ1, and EGFR CAR T cells in tumor models of triple-negative breast cancer(Xia et al., 2021b).

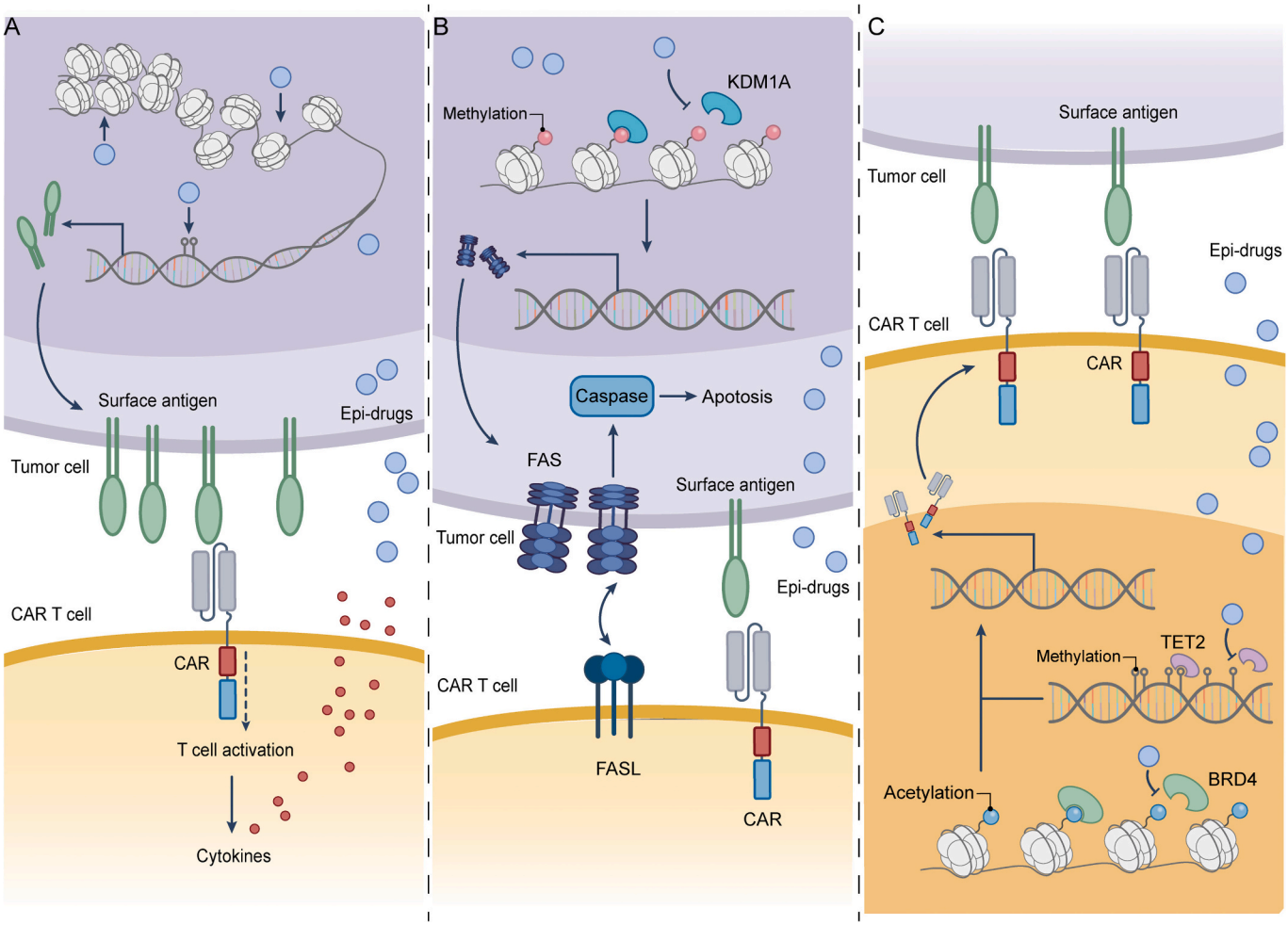

Fig. 3. Epigenetic strategies for inhibiting immune escape.
A. Epigenetic drugs upregulate the expression of surface antigens in tumors through altering chromatin accessibility, histone modification, and DNA methylation. B. Epigenetic drugs upregulate the expression of FAS on the surface of tumor cells by inhibiting the demethylation of histones by KDM1A. Subsequently, FAS binds to FASL on the surface of CAR T cells to induce tumor cell apoptosis. C. Epigenetic drugs upregulate CAR expression by inhibiting TET2 and BRD4.
Abbreviations: CAR, chimeric antigen receptor; Epi-drugs, epigenetics drugs; KDM1A, histone lysine demethylase 1A; TET2, tet methylcytosine dioxygenase 2; BRD4, bromodomain-containing protein 4; FASL, FAS ligands.

4.4. Suppressing immune escape

Immune escape often occurs during CAR T cell therapy and is a major cause of treatment resistance(Hirschhorn et al., 2023). A key reason for immune escape is the heterogeneous expression of target antigens (Kailayangiri et al., 2019). This phenomenon is more typical in solid tumors, which show antigenic heterogeneity between different patients with the same tumor, or even between primary and metastatic foci in the same patient(Guzman et al., 2023), increasing the difficulty of targeting. Additionally, tumors silence the expression of antigenic proteins upon stimulation by immune cells, thereby causing target antigen loss. For example, under the selective pressure of CD19-specific CAR T cells, CD19+ leukemia cells can internalize CD19 from the cell surface, thereby impairing CAR recognition and facilitating immune escape(Im et al., 2022). Likewise, another study found that B7-H4 levels were remarkably reduced in breast cancer cells that were resistant to B7-H4 CAR T cell therapy(Zhou et al., 2023). Meanwhile, dynamic down-regulation of B7-H4 was associated with increased H3K27me3 levels in breast cancer cells, which exhibited stronger stem cell-like features (Zhou et al., 2023).

Epigenetic modifications upregulate the expression of tumor antigens (Fig. 3A). An epigenetic drug, bryostatin, upregulated CD22 expression on the surface of leukemia cells, thereby increasing the killing of B-cell acute lymphoblastic leukemia by CD22 CAR T cells in human cellular models(Wang et al., 2022b). Decitabine and chidamide upregulated CD70 expression in a mouse model of acute myeloid leukemia treated with CD70 CAR T cells(Cheng et al., 2023). In addition, epigenetic strategies, in combination with CAR T cell therapy, have shown promising results in solid tumors. Histone methyltransferase EZH2 inhibitors promoted disialoganglioside GD2 antigen expression in mouse models of Ewing sarcoma and lung cancer, thereby enhancing the anti-tumor efficacy of GD2 CAR T cells(Kailayangiri et al., 2019; Reppel et al., 2022). Decitabine treatment substantially increased mucin 1 (MUC1) antigen expression in the pancreatic cancer mouse model and enhanced its sensitivity to MUC1 CAR T cells(Anurathapan et al., 2014). Moreover, B7-H3 expression was elevated in most solid tumors, and treatment with the HDAC inhibitor, vorinostat, further upregulated B7-H3 expression, which in turn enhanced the therapeutic efficacy of B7-H3 CAR T cells in several solid tumor models, including triple-negative breast cancer, head and neck squamous cell carcinoma, non-small cell lung cancer, and cutaneous melanoma(Lei et al., 2021).

In addition to tumor surface antigens, the upregulation of FAS expression on the surface of tumor cells is also a target for epigenetic regulation, which could suppress immune escape through FAS-mediated bystander killing (Fig. 3B). Histone lysine demethylase 1 A (KDM1A) regulates histone lysine demethylation. Inhibition of KDM1A increases FAS expression in antigen-deficient or even negative human neuroblastoma cell lines, and mediates tumor cell apoptosis by interacting with FAS ligands on LICAM CAR T cells(Sulejmani et al., 2021).

Beyond targeting tumor cells, the characteristics of CAR T cells themselves warrant attention. After CAR T cell infusion, both the level and duration of surface CAR expression play important roles in determining treatment efficacy. A decrease or loss of this expression can significantly reduce treatment durability and may cause relapse or resistance. The use of epigenetic reprogramming to promote the surface expression of CAR molecules in CAR T cells has been explored (Fig. 3C). BRD4 binds to the CD19 CAR vector-specific *EF1α* promoter, reducing the expression of CAR(Kong et al., 2021). Treatment with JQ1, a BRD4 inhibitor, reduces TET2 levels and lead to epigenetic remodeling(Kong et al., 2021). As a result, CD19 CAR expression is increased, and the efficacy against human chronic lymphocytic leukemia is improved *in vitro*(Kong et al., 2021). This strategy provides a new epigenetic approach for CAR T cells to treat tumors with low or negative target antigen expression.

4.5. Predicting the prognosis of CAR T cell therapy

One key problem in CAR T cell therapy is the lack of reliable methods to predict treatment efficacy and patient outcomes. CAR T cell therapy is often not the first-line treatment for tumors. Most patients who receive this therapy have advanced, metastatic, or recurrent tumors. If it is possible to predict prior to the treatment whether patients can benefit from treatment, unnecessary treatments can be avoided.

Epigenetics provide valuable information for predicting the prognosis of CAR T cell therapy. A recent study on B-cell malignancies established an epigenetic signature of T cells associated with complete response (CR), known as EPICART, based on methylation site difference observed between CAR-transduced and untransduced T cells(Garcia-Prieto et al., 2022). EPICART positivity correlates with low progression-free and overall survival, which could help identify patients likely to benefit from CD19 CAR T cell therapy(Garcia-Prieto et al., 2022). In terms of T cell clustering, EPICART negativity was associated with effector memory T cells, whereas EPICART positivity was associated with naïve or early memory phenotype T cells; these results were consistent with the clinical outcomes(Garcia-Prieto et al., 2022). Similarly, another study found that the methylation landscape of host T cells prior to CD19 CAR T cell infusion affects treatment outcomes(Zhao et al., 2023). The methylation sites in T cells of the patients with diffuse large B-cell lymphoma in the non-CR group were remarkably different from those in the CR group, and these differentially methylated sites were mainly associated with T cell immune activation and cytotoxicity (Zhao et al., 2023). In pediatric acute lymphoblastic leukemia, a stem cell-like epigenome of tumor cells was identified as a predictor of primarily resistance or sensitivity to CD19 CAR T cell therapy(Masih et al., 2023). Patients with primary non-response exhibited increased accessibility of genes in regions associated with the characteristics of hematopoietic stem cells and multilineage progenitors(Masih et al., 2023). Evidence from these studies implies that the epigenetic state could help identify patients who can benefit from CAR T cell therapy.

In summary, the role of CAR T cell exhaustion in its insufficient efficacy has been established, and this state of exhaustion is associated with specific epigenetic changes. Epigenetic reprogramming can regulate the gene expression of CAR T cells, thereby affecting their differentiation subtypes and anti-tumor functions. From this perspective, one of the most promising epigenetic strategies is to enhance the persistence and anti-tumor activity of CAR T cells by preventing or reversing exhaustion phenotype. Specifically, the combination of gene editing or epigenetic drugs with CAR T cell therapy has shown good efficacy and feasibility in animal experiments and has entered clinical trials. The newly proposed strategy of using dasatinib gating to induce intermittent “rest” in CAR T cells, which prevents exhaustion by temporarily inhibiting CAR signaling, holds considerable potential. These strategies have shown significant preclinical and clinical promise and are expected to enhance the efficacy and safety of CAR T cell therapy.

5. Current barriers to combining CAR T cell therapy with epigenetic strategies

As previously discussed, targeting epigenetic factors represents a promising strategy to improve the therapeutic performance of CAR T cells. The combination of the two can help address some challenges in CAR T cell therapy, showing good prospects for clinical transformation, but there are still critical obstacles that raise new concerns, including non-specificity of epigenetic drugs, clonal expansion of CAR T cells caused by epigenetic gene editing, limited therapeutic efficacy in most solid tumors, and instability of epigenetic biomarkers for predicting prognosis.

5.1. Non-specificity of epigenetic drugs

Epigenetic modulation strategies mainly consist of pretreatment of

CAR T cells prior to infusion and the combined application of CAR T cells and epigenetic drugs, the latter of which tends to be systemically administered. On a whole-individual scale, epigenetic regulatory drugs target various cell types, acting on immune cells in addition to tumor cells, as well as normal tissue cells. This non-specificity can lead to off-target effects and other side effects(Issa et al., 2004). In the context of the TME, epigenetic regulation has two aspects, meaning that epigenetic drugs may have both tumor-suppressive and tumor-promoting effects. Treatment with decitabine in a pancreatic ductal adenocarcinoma model led to a 5-fold escalation in the intratumoral frequency of CD8+ T lymphocytes with potential anti-tumor activity, alongside a roughly 17-fold augmentation in the infiltration of M2 macrophages, which are often associated with tumor-promoting phenotypes(Gonda et al., 2020). In individual cells, epigenetic regulators function in complex ways and may be required to maintain the expression of a number of key target genes. If these regulators are universally targeted, this balance may be disrupted enough to lead to a cellular catastrophe. Inhibition of H3K27ac by BRD4 inhibitor increases the sensitivity of tumor cells to EZH2 inhibitors, but blocking H3K27ac simultaneously activates the MAPK signaling pathway, which is closely linked to cancer formation, through the downregulation of the transcriptional feedback of ERK1 (Huang et al., 2018).

To sum up, although the overall effect observed in the aforementioned studies with the use of epigenetic drugs is the suppression of tumor growth, the changes in epigenetic modifications induced by these treatments also exhibit a complex dual role and carry the potential risk of promoting tumorigenesis. These studies underscore the importance of carefully considering the dosage, administration regimen, and combination strategies in the clinical application of epigenetic drugs, in order to maximize therapeutic efficacy and minimize potential side effects. More studies are needed to elucidate the characteristics of TME remodeling induced by various epigenetic modifications to develop highly selective epigenetic inhibitors with improved targeting efficiency. In addition, the recent emergence of CRISPR/dCas9 and dCas13 techniques is expected to address this challenge. These techniques enable site-specific epigenetic editing of DNA and RNA and substantially reduce the proportion of incorrectly edited cells, while guaranteeing editing yields for precision therapy(Wang et al., 2022a; Wilson et al., 2020).

5.2. Clonal expansion of CAR T cells caused by epigenetic gene editing

Owing to the high targetability of epigenetic modifications, several studies have been conducted to achieve epigenetic regulation by editing a single gene. As mentioned above, TET2 sequentially oxidizes 5-methylcytosine in DNA, mediates DNA demethylation, and activates gene expression(Fraietta et al., 2018). Epigenetic programming of TET2 in CD19 CAR T cells can enhance the killing of leukemia cells(Fraietta et al., 2018). However, *TET2*-deficient CD19 CAR T cells establish an epigenetic state that upregulates *BATF3* and *MYC* expression, leading to T cell antigen-independent overexpansion, which increases the risk of accumulating secondary mutations(Jain et al., 2023a). Moreover, in older adults who are more likely to have *DNMT3A* mutations, *DNMT3A* mutations can synergistically act with *TET2* deletions to contribute to T cell carcinogenesis(Jain et al., 2023a).

Although studies on editing of other epigenetic regulatory genes have not found anything similar for the time being, this potential risk cannot be ignored. A new system of bidirectional epigenetic editing called CRISPRai can help to investigate the complex genetic interactions of genes and non-coding elements, and may assist in identifying targets to solve this problem(Pacalin et al., 2024). Screening for pre-existing mutations that predispose individuals to hyperproliferation or carcinogenesis would help mitigate this hazard. Lastly, transient or partial inhibition of TET2 during CAR T cell production could avoid this risk. Further confirmation of this possible problem is needed before the corresponding research results can be used in clinical trials.

5.3. Disappointing anti-tumor efficacy in most solid tumors

CAR T cell therapy and epigenetic drugs were first used primarily for the treatment of hematologic tumors(Dai et al., 2021; Maude et al., 2018). To date, the combination of epigenetic modulation with CAR T cell therapy has achieved excellent efficacy in preclinical studies, with clinical trials now underway (Table 2). However, the success achieved in hematological tumors is difficult to replicate in solid tumors, and the efficacy of both CAR T cell therapy and epigenetic drugs in solid tumors is limited and often short-term(Morel et al., 2017; Morel et al., 2020) (Table 3). Researchers have carried out many studies to find out what limits the effect of CAR T cell therapy in solid tumors. These limitations have been attributed to several mechanisms of CAR T cell resistance, including limited transport of CAR T cells to solid tumors, poor *in vivo* persistence, antigen escape, and an immunosuppressive TME. The reason why epigenetic drugs do not work well in solid tumors is still not fully understood. One possible explanation is that solid tumors tend to be derived from more differentiated or even terminally differentiated cells, which have low epigenetic reprogramming capacity. In addition, unlike blood cancers, solid tumors usually have a more complex TME, which contains many different types of cells and extracellular matrix. Heterogeneity in the TME leads to a marked difference in cell proliferation rates, which in turn show different sensitivities to drugs(Trédan et al., 2007). In addition, the TME is characterized by hypoxia, which not only changes the epigenetic state of tumor cells, but also correlates with increased drug resistance(Hammond et al., 2014; Ramachandran et al., 2015). Another important reason for drug resistance is poor drug penetration into the tumor, which is similar to what happens in CAR T cell therapy. For a drug to reach all viable cells in a tumor, it must be efficiently transported through the tumor vasculature, across the vessel wall, and through the extracellular matrix, which prevents the drug from reaching all tumor cells at potentially lethal concentrations. Finally, the non-specificity of epigenetic drugs may further contribute to the formation of a pro-tumor TME, all of which are possible mechanisms leading to drug resistance in solid tumors.

This issue remains a great challenge, and for a specific class of solid tumors, new biomarkers may need to be explored to identify the subpopulations that could benefit from treatment. A recent study found that the transcription factor odd-skipped related 2 (Osr2) induced biomechanical stress in CD19 CAR T cells in colon cancer tissues, a process in which Osr2 remodeled the epigenetic program by recruiting HDAC3, thereby leading to CAR T cell exhaustion(Zhang et al., 2024). This finding indicates that Osr2 may serve as an important target. In addition, epigenetics act through complex mechanisms that contribute to the dynamic plasticity of tumor cells. There are compensatory networks between epigenetic regulators, and the crosstalk between epigenetic and other factors, such as epigenetics and metabolism, also creates a compensatory regulation. Therefore, combination therapy remains a promising solution. Localized administration, which can bypass the physical barriers of solid tumors, is another option. In addition, targeting VEGF and its receptors can induce normalization of tumor vasculature and improve hypoxia in the TME(Tong et al., 2004), which is expected to increase CAR T cells and drug delivery to tumor tissue. Other means of targeting hypoxia, such as the use of nanomaterials and biocarriers, or increasing the TME oxygen concentration by employing targeted hypoxia-inducing factors and downstream targets, can also improve the sensitivity of solid tumors to treatment(Chen et al., 2023).

5.4. Instability of epigenetic biomarkers for predicting prognosis

Despite the great potential of epigenetic status as a biomarker for predicting the prognosis of CAR T cell therapy, some limitations need to be emphasized. The previously mentioned studies utilizing epigenetic biomarkers to predict patient prognosis lacked information regarding long-term follow-ups. Consequently, they were unable to assess the effectiveness of these epigenetic characteristics in predicting long-term

Table 2
Completed or ongoing clinical trials of epigenetic drugs combined with CAR T cell therapy in tumors.

ID	Cancer types and conditions	Trial Phase	Therapy	Patients enrolled*	Results/Status	Reference
NCT03614858	Relapsed/refractory B cell acute lymphoblastic leukemia	I/II	Decitabine; Tandem CD19/CD22 CAR T cells (third-generation, CD28 and OX40)	26	Three-year OS was 92.3 %, and 3-year LFS was 92.9 %.	(Ma et al., 2023)
NCT03196830	Relapsed/refractory DLBCL	II	Decitabine; Tandem CD19/CD22 CAR T cells (second-generation, 4-1BB)	33	OR and CR rates were 90.9 % and 63.6 %, respectively. The median PFS was 10.2 months, and OS was undefined. The 2-year OS and PFS rates were 54.3 % and 47.2 %, respectively.	(Qu et al., 2022b)
NCT04697940	Relapsed/refractory B cell NHL	I/II	Decitabine; Tandem CD19/CD22 CAR T cells (N.A.)	33	Recruiting	ClinicalTrials.gov
NCT04850560	Relapsed/refractory B cell lymphoma	I	Decitabine; CD19 PD-1/CD28 CAR T cells (N.A.)	30	Recruiting	ClinicalTrials.gov
NCT04553393	Refractory/relapsed B cell NHL with huge tumor burden	I/II	Decitabine; Chidamide; Tandem CD19/CD22 CAR T cells (N.A.)	80	Recruiting	ClinicalTrials.gov
NCT05370547	Relapsed/refractory B cell NHL	I/II	Chidamide; CD19 CAR T cells (N.A.)	120	Recruiting	ClinicalTrials.gov
ChiCTR-OPN-16008526 ChiCTR-OPN-16009847	Refractory/relapsed aggressive large B cell lymphomas	0	Chidamide; Sequentially infused CD19/CD22 CAR T cells (N.A.)	104	Neither PFS nor OS was reached. The expected 2-year OS and PFS rates were 89 % and 77 %, respectively.	(Wang et al., 2024)
NCT05934838	Previously treated DLBCL, follicular lymphoma, and mantle cell lymphoma	I	Tazemetostat; CAR T cells (N.A.)	15	Recruiting	ClinicalTrials.gov
NCT06242834	Aggressive B cell NHL	II	Tazemetostat; CAR T cells (N.A)	32	Not yet recruiting	ClinicalTrials.gov
NCT06078306	High-risk and Ph-negative (pH-) B cell acute lymphoblastic leukemia	II	Azacitidine; CD19/CD22 CAR T cells (N.A.)	20	Recruiting	ClinicalTrials.gov
NCT05797948	Relapsed/refractory B cell NHL	N/A	Azacitidine; Tandem CD19/CD22 CAR T cells (N.A.)	20	Enrolling by invitation	ClinicalTrials.gov
NCT05823701	Relapsed/refractory DLBCL	II	Chidamide; Azacitidine; CD19/CD22 CAR T cells (N.A.)	23	Recruiting	ClinicalTrials.gov

Abbreviations: LFS, leukemia-free survival; OR, overall response; CR, complete remission; PFS, progression-free survival; OS, overall survival; NHL, non-Hodgkin's lymphoma; DLBCL, diffuse large B-cell lymphoma; CAR, chimeric antigen receptor.
The generation and the costimulatory domains of CAR T cells are labeled in parentheses sequentially after the CAR T cells. The "N.A.". stands for the information about generation and costimulatory domains was not provided.
* Note: For completed clinical trials, the number refers to the actual patients enrolled; for ongoing clinical trials, the number refers to the estimated patient enrollment.

outcomes. It is worth noting that although epigenetic features associated with treatment response have been identified, how these features specifically impact the mechanism of CAR T cell therapy is still unclear. Treatment resistance may involve multiple factors, including tumor heterogeneity, TME, immune escape mechanisms, and the persistence of CAR T cells(Dagogo-Jack and Shaw, 2018; Westcott et al., 2023). Epigenetic characteristics may be just one of the many factors affecting treatment response. Furthermore, the treatment itself leads to changes in the epigenetic landscape, and because CAR T cell therapy is often not used as a first-line treatment, the stability and accuracy of epigenetic biomarker testing for prognostic prediction require further validation. Finally, the costly and cumbersome steps of epigenetic testing make it difficult to use in routine clinical diagnostic processes, and the testing procedures need to be simplified to reduce testing costs.

In summary, although these studies suggest that epigenetic features have potential in predicting CAR T cell therapy responsiveness, further exploration is needed to address the aforementioned issues and translate these findings into practical applications in clinical practice.

6. Conclusions and perspectives

The entry of CAR T cell therapy into the clinic marks a new milestone in immunotherapy, but numerous safety and efficacy concerns remain. With extensive progress in understanding epigenetics over the past decades, an increasing number of studies have attempted to investigate T cells from an epigenetic perspective and have found that the epigenetic modification landscapes of different T cell differentiation subpopulations are substantially different. Thus, sensitizing CAR T cell therapy through epigenetic reprogramming has promising applications. Epigenetic gene editing and the combined application of epigenetic drugs can considerably improve the persistence and effector function of CAR T cells, increase the residence of T cells in the TME, improve the immunosuppressive TME, and inhibit immune escape. The epigenetic state can help to predict the prognosis of patients who undergo CAR T cell therapy.

Despite the major breakthroughs, several issues deserve further exploration. The non-specificity of epigenetic drugs may result in off-target effects, which may have a counterproductive effect on the TME or even cause catastrophe in normal cells. In addition, epigenetic gene editing may cause the uncontrolled proliferation of T cells, raising concerns regarding where epigenetic gene editing is going. Furthermore, the complexity of tumors and their microenvironments makes it ineffective to treat solid tumors from a single perspective, and the question of the poor efficacy of epigenetic strategies and CAR T cell therapy in

Table 3
Completed clinical trials of CAR T cell therapy in solid tumors.

ID	Cancer types and conditions	Trial Phase	Therapy	Patients enrolled	Anti-tumor efficacy	Reference
NCT03874897	Previously treated, CLDN18.2-positive advanced gastrointestinal cancers	I	CLDN18.2-targeted CAR T cells (second-generation, CD28)	98	The ORR and DCR in all 98 patients were 38.8 % and 91.8 %, respectively. The median PFS and OS were 4.4 and 8.8months, respectively.	(Qi et al., 2024)
NCT05660369	Recurrent glioblastoma, EGFRvIII(+)	I	CARv3-TEAM-E T cells (second-generation, 4-1BB)	3	After a single infusion of CARv3-TEAM-E T cells, the tumors all rapidly regressed, with 2 cases having a short-lived efficacy, and 1 case having a sustained efficacy as of 150 days post-infusion	(Choi et al., 2024)
NCT03089203	Metastatic castration-resistant prostate cancer	I	PSMA CAR T cells armored with a dominant-negative TGFβ receptor (second-generation, 4-1BB)	13	Five patients (38.5 %) maintained SD at the 3-month imaging assessment. Tumor regression was observed in 1 patient (7.7 %). The median OS was 477 days (15.9 months), and PFS was 132 days (4.4 months).	(Narayan et al., 2022)
NCT02416466	Measurable unresectable CEA-expressing LM	Ib	Anti-CEA CAR T cells (second-generation, CD28)	8	Median survival time was 8 months (mean 11, range 4–31)	(Katz et al., 2020)
NCT03198546	Patients with advanced HCC/PC/OC with glypican-3 or mesothelin expression	I	Anti-GPC3/anti-MSLN CAR T cells secreting human IL-7 and CCL19 (third-generation, CD28 and TLR2)	6	One patient with PC (1/6, 16.7 %) achieved CR; one patient with HCC (1/6, 16.7 %) achieved PR; and 2 patients (2/6, 33.3 %) achieved steady disease (SD).	(Pang et al., 2021)
NCT03545815	Adult patients with measurable MLSN (+) (≥10 % of tumor cells expressing MLSN) locally advanced or metastatic solid tumors	I	MSLN-directed CAR T cells with PD-1 and TCR disruption (second-generation, CD28)	15	Seven of the 15 patients achieved SD 3–4 weeks after infusion, but the response was maintained only in 2 out of 7 patients at a follow-up duration of 8–12 weeks. The median PFS of those 7 patients with SD was 7.1 weeks (range, 2.9–20.1)	(Wang et al., 2021b)
NCT02159716	Chemotherapy-refractory malignant pleural mesothelioma, OC, and pancreatic ductal adenocarcinoma	I	Anti-MLSN CAR T cells (second-generation, 4-1BB)	15	SD observed in 11 of 15 patients at 28 days and in 3 of 8 patients on follow-up at 2–3 months. Median PFS was 2.1 months	(Haas et al., 2019)
ACTRN12613000198729	Metastatic melanoma, colorectal cancer fibromyxoid sarcoma	I	Anti-GD2 CAR T cells (third-generation, CD28 and OX40)	14	Of patients receiving anti-GD2 CAR T cells alone (7 of 12 patients), 2 had transient stabilization of their previously progressing disease, and 5 had progressive disease. Five of 12 patients received BRAF and MEK inhibition concurrent with anti-GD2 CAR T infusion and achieved a PR.	(Gargett et al., 2024)
NCT03220347	Advanced or unresectable solid tumors and relapsed/refractory NHL	I	BET inhibitor CC-90010	69	One patient with grade 2 astrocytoma achieved a CR, one patient with endometrial carcinoma had a PR, and six patients had prolonged SD ≥11 months.	(Moreno et al., 2020)
NCT01987362	Advanced solid malignancy, testis carcinoma or advanced aggressive DLBCL with abnormal MYC expression	I	BET inhibitor RO6870810	74	ORR were 25 % (2/8), 2 % (1/47), and 11 % (2/19) for patients with testis carcinoma, other solid tumors and DLBCL, respectively.	(Shapiro et al., 2021)
NCT03035591	High-grade serous OC, HER2 negative breast cancer or any other solid tumor predicted to have a substantially higher likelihood of response to ODM-207 (such as MYC amplified tumors)	I	BET inhibitor ODM-207	35	No PR or CR were observed. Six patients had SD. The median (range) duration of SD was 16.3 (8.0–23.0) weeks.	(Ameratunga et al., 2020)
NCT02875223	Advanced solid tumors and relapsed/refractory lymphoma	I	LSD1 inhibitor CC-90011	69	One patient achieved a durable CR. One patient achieved a PR. Thirty-two patients had SD.	(Hollebecque et al., 2022)
NCT01537744	Metastatic or unresectable solid tumors	I	DNMT inhibitor CC-486 with HDAC inhibitor romidepsin	14	Five patients had SD, 2 of which were > 4 cycles; no responses were observed.	(Gaillard et al., 2019)
NCT01422499	children and adolescents with relapsed/refractory solid tumor, lymphoma, or leukemia	I/II	HDAC inhibitor vorinostat	50	Five patients achieved prolonged disease control (>12 months). Best overall response (combining 4 PR and 2 SD, no CR observed) rate was 6/27 (22 %) with a median PFS and OS of 5.3 and 22.4 months, respectively.	(van Tilburg et al., 2019)
NCT02269943	Previously treated patients with locally advanced or metastatic nasopharyngeal carcinoma	II	DNMT inhibitor CC-486 (azacitidine)	36	ORR was 12 %. The median PFS and OS were 4.7 and 18.0 months, respectively.	(Mesia et al., 2019)

Abbreviations: ORR, overall response rate; DCR, disease control rate; OS, overall survival; PFS, progression-free survival; CR, complete response; PR, partial response; SD, stable disease; CI, confidence interval; HCC, hepatocellular carcinoma; PC, pancreatic carcinoma; OC, ovarian carcinoma; TCR, T cell receptor; BET, bromodomain and extra-terminal; NHL, non-Hodgkin's lymphoma; DLBCL, diffuse large B-cell lymphoma; LSD1, lysine-specific demethylase 1; DNMT, DNA methyltransferases; HDAC, histone deacetylases; CAR, chimeric antigen receptor; MLSN, mesothelin.
The generation and the costimulatory domains of CAR T cells are labeled in parentheses sequentially after the CAR T cells. The "N.A." stands for the information about generation and costimulatory domains was not provided.

solid tumors remains open. The instability of epigenetic biomarkers to predict prognosis poses a challenge for the use of epigenetic biomarkers in the clinic.

Recently, genome-wide epigenomic profiling techniques have become the best method for studying epigenetically regulated landscapes(Yang et al., 2023). With advancements in epigenetic analytical techniques, multi-omics and multi-dimensional epigenetic analyses have become possible. Spatialomics, such as spatial-CUT&Tag, spatial ATAC, can help in the spatially resolved genome-wide profiling of epigenetic modifications(Deng et al., 2022). Single-cell epigenomic profiling enables assessment of epigenetic regulatory landscapes within individual CAR T cells for spatiotemporal trajectory analysis(Yang et al., 2023). These developments will help overcome technical barriers and greatly facilitate the exploration of epigenetic reprogramming in CAR T cell therapy. In terms of clinical translation, advances in high-throughput compound screening platforms will accelerate the development of new drugs and pave the way for clinical translation of epigenetic research.

In the future, many directions are worth exploring for in-depth research on epigenetics and CAR T cell therapy. Previous studies on epigenetics have focused on "writers", "erasers", and "readers", with less research on "remodelers" involved in chromatin remodeling, which could provide more targets for future research. It is worth mentioning that CAR T cell therapy is personalized, and the epigenetic modifications of different tumors and individuals also differ. The exploration of utilizing epigenetic information to tailor personalized CAR T cell therapies represents a promising avenue of research. This approach has the potential to enable the development of precision medicine strategies, thereby enhancing the therapeutic options and clinical outcomes for patients with various malignancies. Furthermore, epigenetic biomarkers for predicting prognosis should be further evaluated for their predictive effects on the long-term immune status and therapeutic efficacy in patients treated with CAR T cell therapy. In addition, CAR T cell therapy may be accompanied by severe side effects, such as CRS and neurotoxicity. Further studies are necessary to evaluate whether epigenetic signatures can predict the occurrence of these side effects. In-depth and extensive studies are required before the implementation and popularization of epigenetic strategies combined with CAR T cell therapy.

Authors' contribution

A.Z., Y.W., and L.Y. provided conception and design of the study. Y. Z. and Q.Z. collected and organized the literature and prepared the initial draft. X.L. and T.G. assisted in table preparation. A.Z. and L.Y. revised the manuscript. A.Z., R.J., and S.W. supervised the study and provided guidance throughout the preparation of this manuscript. All the authors conducted a final review and approved the manuscript before submission.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Not applicable.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Not applicable.

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