Overcoming Resistance to CD19 CAR-T Cell Therapy in B-cell Malignancies: Mechanisms and Emerging Strategies

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Abstract. Chimeric antigen receptor T-cell (CAR-T) therapy, especially CD19-targeted therapy, has revolutionized B-cell malignancy treatment, achieving unprecedented remission in relapsed/refractory patients—many of whom previously had limited therapeutic options. However, disease recurrence and drug resistance remain major challenges, necessitating deeper understanding of resistance mechanisms and effective solutions. This paper outlines key CD19 CAR-T resistance mechanisms: CD19 loss via genetic (e.g., deletions) or epigenetic (e.g., promoter methylation) changes, CAR-T cell exhaustion from sustained stimulation (marked by reduced cytokine secretion), immunosuppressive tumor microenvironments (with Tregs/MDSCs and TGF-β/IL-10), and abnormal activation of JAK/STAT3 or PI3K/AKT/mTOR pathways (enhancing tumor survival/immune escape). Proposed strategies to overcome resistance include dual/multi-target CARs, combining immune checkpoint inhibitors (e.g., anti-PD-1), CRISPR-engineered "armored" CAR-T cells, optimizing T-cell metabolism/memory, and regulating the tumor microenvironment. Future research should address long-term toxicity (e.g., neurotoxicity), develop predictive biomarkers, and advance allogeneic universal CAR-T platforms, with solid tumor CAR-T experience providing references. These findings lay a foundation for next-generation cellular immunotherapy.

Keywords: Chimeric antigen receptor T cell, B-cell malignancies, resistance mechanisms, tumor microenvironment.

1. Introduction

Chimeric antigen receptor (CAR) is a synthetic receptor that has been artificially modified to specifically guide T cells to recognize tumor associated antigens and activate their targeted killing effect on tumor cells. Its typical structure consists of three parts: an antigen binding region located outside the cell (usually an antibody unit called a single chain variable fragment scFv), a transmembrane region, and one or more intracellular signaling modules, which work together to effectively activate T cells [1]. It is worth noting that CAR-T cells do not rely on HLA compatibility when recognizing targets, thus having broader applicability potential. At present, the US Food and Drug Administration (FDA) has approved six CAR-T cell therapies, most of which target CD19 and are mainly used to treat B-cell malignancies. The approved CD19 targeted CAR-T products include axicabtagene ciloleucel (trade name Yescarta), tisagenlecleucel (trade name Kymriah), lisocabtagene maraleuucel (trade name Breyanzi) and brexucabtagene autoleucel (trade name Tecatus) [2].

CD19 has become an ideal target in CAR-T therapy due to its stable expression and widespread distribution on the surface of B cells. This characteristic enables CAR-T cells targeting CD19 to demonstrate significant clinical efficacy in the treatment of B-cell derived malignancies, such as B-cell acute lymphoblastic leukemia (B-ALL) and diffuse large B-cell lymphoma (DLBCL). In addition, CD19 expression covers all developmental stages from progenitor B cells to mature B cells, while it is not expressed in other important normal tissues, thereby minimizing the risk of off target toxicity and improving the safety of treatment. Furthermore, CD19 expression remains relatively stable, reducing the risk of antigen loss during therapy. Crucially, the risk of immune escape is minimal because CD19 is less prone to downregulation than other antigens, such as CD20. This stability makes it a more reliable target for immunotherapies [3].

CAR-T therapy targeting CD19 has shown excellent efficacy in treating refractory or relapsed B-cell lymphomas such as follicular lymphoma (FL), mantle cell lymphoma (MCL), and large B-cell lymphoma (LBCL), with some patients even achieving long-term remission and survival. Based on

these encouraging results, CAR-T cell therapy has gradually been promoted from first-line treatment to more advanced treatment options, becoming an important milestone in the domain of precision immunotherapy for hematological malignancies. Nevertheless, disease relapse and resistance remain significant barriers, as the majority of patients eventually experience progression despite sustained CAR-T persistence in vivo. This observation suggests that mechanisms beyond antigen loss contribute to therapeutic failure [4, 5].

2. Mechanisms of Resistance

2.1. Antigen Escape

Loss or structural alteration of CD19 is the most frequent and well-documented resistance mechanism, as it directly prevents CAR-T recognition and killing of tumor cells. Pioneering studies in relapsed B-ALL demonstrated that resistant clones emerge via alternative splicing of CD19 exon 2 or intron retention, producing isoforms that escape recognition by FMC63-derived scFv, or sequester CD19 intracellularly instead of presenting it on the cell surface. In addition, somatic mutations and immune selection pressure further consolidate this phenotype (including exon deletions, missense mutations, and epigenetic downregulation). In certain cases, lineage switch (from B-cell to myeloid/early progenitor-like phenotype) results in functional loss of CD19 as well. Clinical cohorts show that a substantial proportion of relapses after CAR-T therapy are associated with CD19-negative/low relapse (reported in ~30–90% of cases, depending on study design, detection methods, and patient population) [6].

2.2. T Cell Exhaustion

Long term antigen stimulation and persistent inflammatory or inhibitory microenvironment signals can induce CAR-T cells to gradually exhibit a depletion phenotype, characterized by decreased cytokine secretion ability, weakened proliferation potential, and impaired cytotoxic function. This functional impairment state is accompanied by the upregulation of multiple inhibitory receptors (such as PD-1, LAG-3, TIM-3, and CTLA-4) and is closely related to the activation of exhaustion related transcriptional regulatory pathways (such as TOX and NR4A families) and metabolic reprogramming. In clinical practice, T cell depletion is significantly associated with poor persistence of CAR-T cells in the body and increased risk of early recurrence in patients.

The current strategies for this mechanism mainly include PD-1/PD-L1 checkpoint blockade, co stimulatory domain optimization, and improving in vitro manufacturing processes to reduce excessive stimulation during amplification. It is worth emphasizing that clinical efficacy is closely related to the good expansion and long-term persistence of CAR-T cells in vivo [5,7]. In patients with no response or early recurrence, low peak amplification levels of CAR-T cells, rapid clearance, or inability to achieve long-term stable implantation can usually be observed. These clinical manifestations are influenced by multiple factors, where 4-1BB costimulation typically supports superior persistence compared with CD28, which drives stronger immediate cytotoxicity; the intrinsic quality of the infused T cells, with enrichment of stem or central memory-like subsets predicting more durable efficacy; and host-related influences such as tumor burden, cytokine milieu, and the broader tissue microenvironment. Consistent findings across clinical cohorts highlight that CAR-T products enriched with memory-like transcriptional and metabolic features are linked to sustained B-cell aplasia and long-term remission, whereas exhausted or apoptosis-prone profiles predict suboptimal therapeutic outcomes.

2.3. Tumor Immunosuppressive Microenvironment (TME)

The B-cell lymphoma microenvironment exerts profound inhibitory pressure on CAR-T efficacy. It is enriched with myeloid-derived suppressor cells (MDSCs), regulatory T cells (Tregs), and immunosuppressive cytokines such as IL-10 and TGF-β. Additionally, upregulation of PD-L1, metabolic competition (lactate, adenosine, tryptophan metabolites), and stromal barriers collectively

impair CAR-T infiltration, proliferation, and function. MDSCs directly suppress effector T cells through production of ARG1, ROS, IL-10 and TGF-β, while IL-10/TGF-β signaling broadly weakens T-cell activity. Preclinical data suggest that targeting these axes—through MDSC depletion/reprogramming, TGF-β blockade, metabolic reconditioning, or modifying CAR-T cells to either secrete specific cytokines or express chemokine receptors—can restore CAR-T potency [7].

2.4. Activation of Resistant Signaling Pathways

The JAK/STAT3 pathway is constitutively activated in several B-cell malignancies (DLBCL, CLL, MCL), driving expression of IL-10, PD-L1, and other suppressive mediators while promoting tumor cell survival. Hyperactivation of STAT3, therefore, reduces CAR-T cytotoxicity and promotes relapses. Targeting STAT3 or its downstream effectors has shown preclinical promise in sensitizing tumors to CAR-T therapy [8]. The PI3K/AKT/mTOR pathway enhances tumor cell survival and proliferation, inhibits mitochondrial apoptosis, and rewires metabolism to withstand immune attack. It also shapes the TME by fostering immunosuppressive myeloid cells. Selective inhibition of PI3K-δ/γ not only blocks BCR signaling but also remodels T cell differentiation and the immunosuppressive niche, supporting combination strategies.

3. Strategies to Overcome Resistance

3.1. Dual- or Multi-Target CAR Designs

One of the most promising strategies to address the common resistance mechanism in CAR-T therapy, antigen escape, is to develop dual or multi-target CAR structures. This type of design enhances the targeting and clearance ability of immune escaping tumor cells by simultaneously recognizing two or more tumor associated antigens (such as CD19/CD22 or CD19/CD20), thereby reducing the risk of recurrence due to loss or downregulation of a single antigen. Clinical studies have confirmed that CD22 targeted or CD19/CD22 bispecific CAR-T therapy can still induce good therapeutic responses in patients who experience relapses after CD19 targeted CAR-T therapy due to CD19 antigen deficiency, highlighting its important translational medical value. Moreover, the development of multispecific CARs offers a strategy to address the heterogeneity of antigen expression within malignant clonal populations. By broadening the spectrum of tumor cell recognition, these constructs enhance overall tumor coverage and substantially lower the likelihood of immune escape [7, 9].

3.2. Combination with Immune Checkpoint Blockade

Another highly promising treatment strategy is to combine CAR-T cell therapy with immune checkpoint blockers to reverse T cell depletion, which is one of the key mechanisms leading to poor response to CAR-T therapy in patients with recurrent or refractory B-cell malignancies. Both preclinical studies and early clinical trial data indicate that activation of the PD-1/PD-L1 signaling pathway in the tumor microenvironment significantly inhibits the proliferation and cytotoxicity of CAR-T cells, thereby weakening their anti-tumor efficacy. By using PD-1 or PD-L1 blocking antibodies in combination with CAR-T cell infusion, T cell function can be effectively restored, cytokine secretion can be enhanced, and the duration of anti-tumor response is expected to be prolonged. While initial clinical trials indicate improved outcomes with this combinatorial strategy, careful consideration of toxicity and cytokine release syndrome is essential, as the synergistic immune activation may exacerbate systemic inflammation [9].

3.3. CRISPR-Enhanced CAR-T and Other "Armored" Edits

Genome editing can intrinsically resist exhaustion or immunosuppression. First-in-human trials of a virus-free, site-specific CD19 CAR integrated at the PDCD1 (PD-1) locus (PD1-19BBζ) report feasibility with early signs of efficacy, aligning with preclinical data showing PD-1 disruption

improves CAR-T degranulation and clearance of PD-L1$^+$ targets. Multiplex editing (e.g., PD-1/TCR) is also clinically testable and may reduce tonic signaling, alloreactivity (for allo-CAR-T), and checkpoint sensitivity.

Beyond PD-1, "armoring" strategies—cytokine co-expression (IL-12, IL-15, IL-18), dominant-negative TGF-βR, or switch receptors—aim to enhance proliferation, persistence, and resistance to TME suppression. IL-15-armed CARs show improved expansion/persistence in early clinical experience and preclinical CD19 systems; membrane-bound IL-15/IL-15Rα designs may mitigate systemic toxicity. HDAC inhibitors and related epigenetic agents can increase tumor antigen presentation and sensitize malignant B cells to CAR-T killing in preclinical models and early reports, but dosing/schedule must avoid impairing T-cell fitness.

3.4. Enhancing T-Cell Metabolism/Activity and Memory Phenotype

Engineering CAR-T cells to sustain a durable memory phenotype and robust metabolic fitness has emerged as a key strategy to overcome limited persistence and functional exhaustion. Comparative studies have demonstrated that the choice of costimulatory domain is central: 4-1BB–based CARs typically induce slower expansion but confer superior mitochondrial fitness, oxidative metabolism, and long-term persistence, whereas CD28–based CARs promote rapid cytotoxicity with shorter durability. These inherent differences reflect distinct transcriptional and epigenetic pathways that drive T cells toward either effector or memory states, emphasizing the importance of tailoring CAR design to the specific disease context. Beyond construct design, ex vivo manufacturing conditions strongly influence the resulting product. By incorporating IL-7 and IL-15 during cell culture, temporarily inhibiting PI3K signaling, and selectively enriching for less differentiated T cell subsets, such as T-SCM or T-CM, a memory-like program can be established. This process can promote CAR-T cells to exhibit stronger expansion ability and more persistent functional activity in vivo, thereby enhancing the persistence and stability of their anti-tumor effects. In addition, transgenic cytokine support, most notably IL-15 co-expression, has been shown to boost proliferation and persistence in both preclinical models and early clinical trials, although strategies that tightly regulate cytokine release are necessary to minimize systemic toxicity. Together, these approaches converge on the principle that maintaining a stem-like, metabolically adaptable state is essential for long-term disease control following CAR-T therapy [9].

3.5. Modulating the Tumor Microenvironment (TME)

Reversing the immunosuppressive properties of tumor microenvironment is another key strategy for enhancing the therapeutic effectiveness of CAR-T cell treatment in B-cell malignancies. The microenvironment in which lymphoma is located is typically rich in Tregs, MDSCs, and inhibitory cytokines, while also exhibiting characteristics such as metabolic competition, local hypoxia, and matrix barrier, which collectively hinder T cell infiltration and function.

Currently, various treatment strategies are being explored to overcome these microenvironmental limitations. For example, in chronic lymphocytic leukemia (CLL) and MCL, the Bruton tyrosine kinase (BTK) inhibitor Ibrutinib has been shown to improve T cell adaptation, reduce Treg activity, and promote in vivo expansion of CAR-T cells. Clinical studies have shown that simultaneous or sequential use of Ibrutinib during CAR-T infusion can significantly improve treatment response rates and demonstrate good synergistic effects. Targeting myeloid compartments is also gaining traction, with CSF-1R blockade capable of depleting or reprogramming immunosuppressive macrophages, thereby facilitating CAR-T infiltration and effector function. Furthermore, strategies directed against suppressive signaling and metabolic checkpoints—such as dominant-negative TGF-β receptors, adenosine A2A receptor antagonists, and IDO inhibitors—offer additional opportunities to dismantle the protective niche that tumors construct. While most of these interventions remain in preclinical or early clinical testing, the growing evidence supports the concept that effective CAR-T therapy requires not only intrinsic engineering of the cells but also concurrent remodeling of the tumor ecosystem to unlock their full therapeutic potential [10].

4. Challenges and Future Outlooks

A major clinical challenge moving forward is the issue of "resistance after resistance" and the long-term management of CAR-T-related toxicities. Many patients who initially respond well to CD19 CAR-T eventually relapse, and some will experience further relapses even after salvage treatments, such as repeat CAR-T, alternative antigen CAR-T, or checkpoint blockade. This creates a cycle where successive therapies select for increasingly resistant tumor clones and host conditions that impair the effectiveness of future cellular treatments.

At the same time, long-term toxicities—such as persistent B-cell aplasia leading to hypogammaglobulinemia, ongoing cytopenia, delayed or prolonged neurotoxicity (ICANS), and a heightened risk of infections—continue to present significant challenges for patient survivorship. These late effects require continuous monitoring, including regular assessments of immune reconstitution, infection surveillance, and appropriate timing for vaccinations and immunoglobulin replacement when necessary. Additionally, they pose difficult decisions when considering retreatment or more aggressive combination therapies that could heighten immune activation and toxicity. Therefore, the field needs to develop reliable biomarkers (e.g., minimal residual disease (MRD), immune phenotype of persisting CAR-T cells, host inflammatory markers) to guide decisions on whether to retreat patients or pursue alternative therapies. Standardized long-term follow-up protocols are also critical for assessing and managing late adverse events, ensuring that antitumor effectiveness is maintained while minimizing harm.

The shift from autologous to allogeneic "off-the-shelf" CAR-T products offers the potential to improve accessibility, reduce treatment delays, and provide more consistent cell quality at the start. However, it also introduces immunologic and practical challenges that still need to be addressed. Allogeneic CAR-T therapies face two primary immune hurdles: graft-versus-host disease (GVHD), where donor TCRs recognize host alloantigens, and host-mediated rejection of donor cells. To mitigate these risks, strategies are being explored, including precise genome editing to knock out the endogenous T cell receptor (TRAC knockout), disrupting HLA expression or introducing nonclassical HLA molecules to minimize allorecognition, and using transient or long-term lymphodepletion regimens to reduce host rejection. Gene editing can also be applied to modify molecules like CD52 or PD-1, which affect the persistence and toxicity of CAR-T cells. However, edited allogeneic CAR-T products often display shorter persistence than well-manufactured autologous products, and residual risks of GvHD, immune rejection, and unforeseen off-target editing effects require long-term safety data and careful regulatory oversight. Manufacturing scale, cost of editing, and the need for broad HLA coverage or universal immune-evasive modifications are additional translational bottlenecks; solving these will require coordinated progress in editing safety, conditioning strategies, and immune-evasive engineering.

Finally, drawing lessons from CAR-T work in solid tumors offers both opportunities and caveats for B-cell malignancies. Many innovations developed to address the hostile solid-tumor microenvironment—armored CARs that secrete cytokines or express dominant-negative receptors, chemokine receptor engineering to improve trafficking, local or regional delivery, metabolically tuned T cells, and combination therapies targeting stromal or myeloid suppressors—are conceptually attractive for lymphomas with complex microenvironments (for example, nodal or extranodal disease with a dense suppressive niche). Indeed, some TME-modulating strategies (e.g., targeting MDSCs or TGF-β resistance, or combining with small-molecule agents that normalize T-cell function) have already shown promise in B-cell contexts. Yet direct translation is not guaranteed: hematologic malignancies differ from solid tumors in antigen accessibility, spatial constraints, tumor burden kinetics, and patterns of immune infiltration, and interventions that improved trafficking or overcame physical barriers in solid tumors may be less relevant in circulation-rich diseases.

In addition, strategies aimed at enhancing the intrinsic efficacy of CAR-T cells, such as mandatory co expression of cytokines or knockout of immune checkpoint molecules, have raised new safety concerns in the therapeutic management of hematologic malignancies. Due to the existing clinical risks of systemic cytokine release and severe B cell regeneration disorders, such

reinforcement strategies may further exacerbate treatment-related toxicity. Looking ahead to the future, this field should focus on promoting the rational translation and disease-specific adaptation of innovative strategies developed for solid tumors in lymphoma treatment. This requires the use of mechanism specific preclinical studies (such as validation in representative lymphoma models) and rigorously designed translational clinical trials to systematically assess the therapeutic effectiveness and durability of immune-related toxicities associated with these strategies. Through interdisciplinary collaboration - integrating solid tumor immunology, gene editing safety technology, and clinical expertise in hematological malignancies - we can accelerate treatment progress while effectively controlling potential risks and promoting the safe and efficient development of the forthcoming generation of CAR-T cell therapies.

5. Conclusion

CAR-T cell therapy directed against CD19 has fundamentally reshaped the therapeutic landscape for B-cell malignancies., bringing new hope and clinical benefits to patients with limited treatment options in the past. However, the issues of recurrence and drug resistance, including mechanisms such as antigen escape, CAR-T cell depletion, and inhibitory tumor microenvironment, still persist, and a comprehensive strategy is urgently needed to achieve lasting therapeutic effects. In recent years, innovative approaches such as dual/multi-target antigen recognition, immune checkpoint blockade, armored CAR design, T cell programming, and tumor microenvironment regulation have shown broad prospects in preclinical and early clinical research. However, these strategies still need to be carefully balanced between their immune activation intensity and potential toxicity risks to ensure the safety and effectiveness of treatment. Future challenges include addressing "resistance after resistance," ensuring long-term safety with monitoring/biomarkers, refining allogeneic CAR-Ts for accessibility, and adapting solid tumor CAR-T lessons to hematologic malignancies. Ultimately, integrating mechanistic insights, engineering, and precision strategies will advance CD19 CAR-Ts as standard care and shape cellular immunotherapy.

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