Dean&Francis

ISSN 2959-409X

Current Clinical Challenges and Strategic Advancements in CAR-T Cell Therapy

Ziling Shao[1, *]

[1]School of life science, Anhui Agriculture University, Hefei, 230036, China
*Corresponding author: 22113454@stu.ahau.edu.cn

Abstract:

Chimeric antigen receptor (CAR) T-cell therapy constitutes a paradigm-shifting advancement for treating refractory malignancies, demonstrating unique therapeutic capabilities beyond conventional cancer treatments, which has brought new hope to many patients with advanced cancer. This cellular immunotherapy has achieved unprecedented success in hematologic cancers, yet its extension to solid tumors confronts fundamental biological constraints. Life-threatening toxicities such as cytokine release syndrome and immune effector cell-associated neurotoxicity originate from dysregulated immune activation cascades, while T cell exhaustion—characterized by hierarchical epigenetic reprogramming and progressive metabolic failure—severely undermines long-term therapeutic efficacy. These dual limitations demand innovative bioengineering solutions to augment both safety profiles and functional persistence. The present review analyzes core biological mechanisms restricting CAR-T efficacy and surveys cutting-edge approaches designed to circumvent these barriers through integrated genetic reprogramming, metabolic optimization, and microenvironment-responsive receptor engineering. Subsequent discussion focuses on how such multidisciplinary strategies could potentiate cellular immunotherapy's applicability across diverse oncological contexts, ultimately transforming cancer management paradigms.

Keywords: CAR-T Cell Therapy; CRS; ICANS; T cell exhaustion.

1. Introduction

Chimeric antigen receptor (CAR) T-cell therapy represents a paradigm shift in oncology by achieving remarkable remission in patients with chemotherapy-refractory hematologic malignancies such as B-cell acute lymphoblastic leukemia (B-ALL) and diffuse large B-cell lymphoma (DLBCL); however, despite these unprecedented clinical outcomes, the widespread adoption of this revolutionary modality is constrained by a triad of interconnected, potentially life-threatening toxicities that collectively affect

>90% of recipients. Firstly, Cytokine Release Syndrome (CRS) manifests in patients as a systemic inflammatory cascade ranging from fever to multi-organ failure, arising from CAR-T activation triggering massive release of interleukin-6 (IL-6), interleukin-1 (IL-1), and interferon-gamma (IFN-γ), which leads to endothelial dysfunction and vascular leakage [1]. Secondly, Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS) presents as confusion, seizures, or cerebral edema due to blood-brain barrier disruption facilitated by IL-6-mediated angiopoietin-2 elevation [2]. Thirdly, T-cell Exhaustion drives 30–70% of relapses within 24 months despite initial remission characterized by epigenetic reprogramming through TOX transcription factor upregulation and metabolic collapse from mitochondrial dysfunction, resulting in sustained expression of inhibitory receptors PD-1, TIM-3, and LAG-3 [3,4]. These toxicities share a pathophysiological continuum: while CRS and ICANS stem from acute inflammatory cascades initiated by CAR-T activation, T-cell exhaustion represents the chronic consequence of persistent antigen exposure. Current management remains predominantly reactive—utilizing tocilizumab for CRS and corticosteroids for ICANS—without addressing root mechanisms or preventing exhaustion development, compounded by „vein-to-vein" manufacturing delays of 24–38 days that further exacerbate T-cell dysfunction prior to infusion and contribute to therapeutic resistance [5,6].

This review synthesizes a tripartite strategy—integrating biomarker-guided toxicity interception, safety-enhanced CAR engineering, and epigenetic-metabolic exhaustion reversal—to transform CAR-T therapy from salvage treatment into frontline oncology practice.

2. CRS

2.1 Cytokine Storm Pathogenesis

Cytokine storm denotes a spectrum of immune dysregulation disorders exhibiting constitutional symptoms, systemic inflammation, and multiorgan dysfunction. Inadequate management of these disorders carries the potential for multiorgan failure, as seen in figure 1[7].

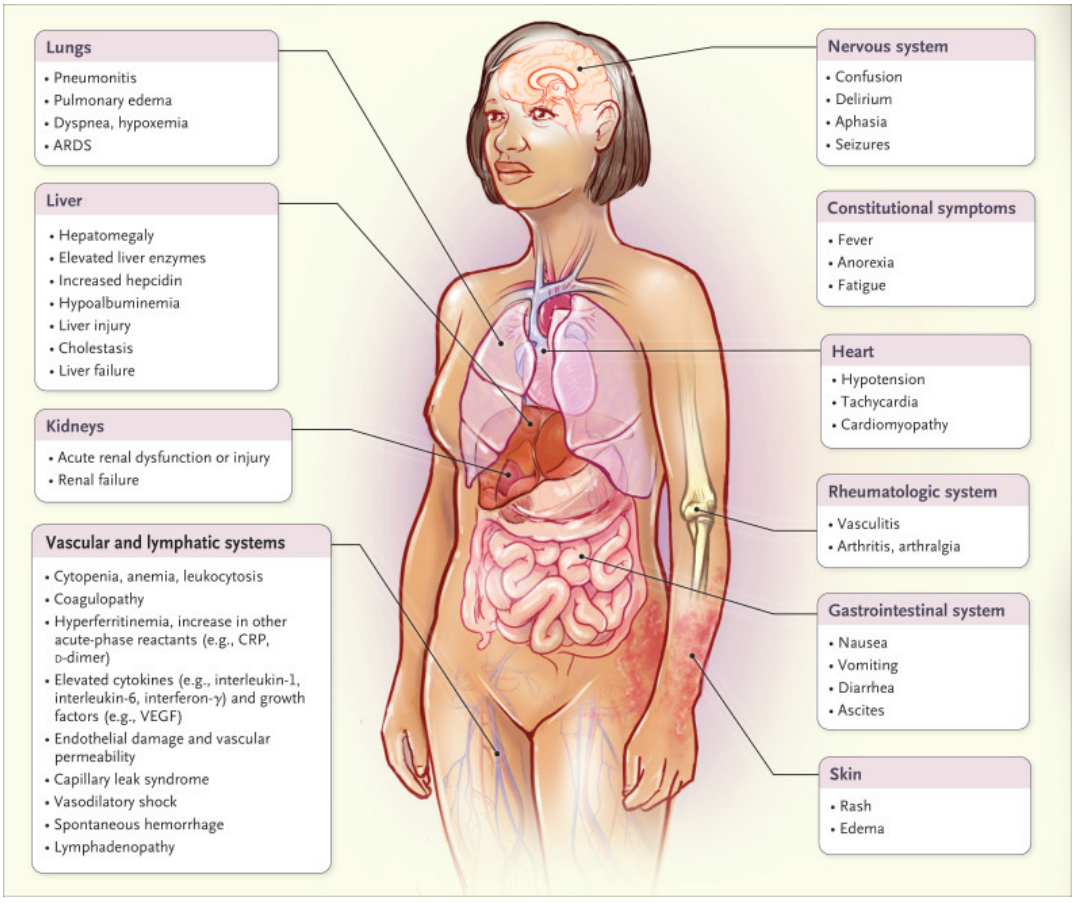

Figure 1: Clinical Presentation of Cytokine Storm [7].

The immune hyperactivation during cytokine storm can be triggered erroneously without infection (e.g., in genetic

ISSN 2959-409X

inflammasomopathies or idiopathic multicentric Castleman's disease), result from an uncontrolled or exaggerated response (such as excessive immune cell activation in CAR T-cell therapy, overwhelming infection in sepsis, or sustained activation due to unresolved infection like EBV-HLH), or stem from a failure to resolve the response and achieve homeostasis (e.g., in primary HLH) [7].

2.2 CRS Clinical Spectrum in CAR-T Therapy

CRS represents the most frequent and potentially life-threatening toxicity of CAR T-cell therapy, occurring in 48-77% of patients receiving CD19-directed constructs for hematologic malignancies [8]. This systemic inflammatory response originates from a three-phase pathophysiological cascade initiated upon CAR-T cell engagement with tumor antigens. During the initial trigger phase, antigen recognition induces rapid interferon-gamma (IFN-γ) release from activated CAR-T cells, which stimulates tissue-resident macrophages through Fcγ receptor binding. Subsequently, the amplification phase commences as activated macrophages secrete interleukin-1β (IL-1β), interleukin-6 (IL-6), and granulocyte-macrophage colony-stimulating factor (GM-CSF), establishing a cytokine-driven positive feedback loop. These mediators collectively induce endothelial activation and vascular leakage by disrupting tight junctions and promoting adhesion molecule expression (VCAM-1, ICAM-1). Ultimately, the systemic inflammation phase manifests clinically as hypotension (43% of cases), hypoxia requiring supplemental oxygen (32%), hepatic transaminitis (18%), and acute kidney injury (12%) through a combination of capillary leak syndrome and direct tissue infiltration by activated myeloid cells .

The clinical spectrum of CRS ranges from mild constitutional symptoms (fever, myalgia) to life-threatening multi-organ failure, with severity classified according to the American Society for Transplantation and Cellular Therapy (ASTCT) 2019 consensus criteria [9]. Grade 1 CRS presents with fever ≥38°C without organ dysfunction; Grade 2 requires hypotension responsive to fluids or low-dose vasopressors, or hypoxia needing <40% FiO_2; Grade 3 necessitates high-flow oxygen or multiple vasopressors; while Grade 4 signifies life-threatening conditions requiring mechanical ventilation or vasopressor combinations .Diagnosis requires fulfillment of four criteria: (1) fever≥38°C, (2) ≥1 clinical sign of hypotension or hypoxia, (3) elevated inflammatory markers (C-reactive protein ≥20 mg/dL or ferritin ≥500 ng/mL), and (4) exclusion of alternative causes such as infection . Biomarker profiling now enables risk stratification, with serum IL-6 >100 pg/mL and peak CAR-T expansion >50 cells/μL predicting severe (grade≥3) CRS with 92% sensitivity, while ferritin >5,000 ng/mL correlates with four-fold increased risk of concurrent neurotoxicity [10].

2.3 Stepped CRS Management and Next-Gen Engineering Solutions

Contemporary management employs a stepwise approach guided by real-time biomarker monitoring. Tocilizumab (anti-IL-6 receptor monoclonal antibody) remains first-line therapy at 8 mg/kg intravenous dosing, achieving resolution of grade ≥3 CRS within 48 hours in 86% of patients when administered at IL-6 >100 pg/mL [11]. For refractory cases, IL-1 blockade with anakinra (100 mg subcutaneous daily) reduces vasopressor requirements by 74% through inhibition of NLRP3 inflammasome signaling in macrophages [12]. Emerging strategies focus on preemptive intervention, including GM-CSF neutralization using lenzulumab (anti-GM-CSF monoclonal antibody), which reduced severe CRS incidence from 42% to 11% in a phase II trial by disrupting macrophage priming [13]. Extracorporeal cytokine adsorption devices (Cytosorb®) demonstrate rapid clearance of circulating IL-6 and IL-1β (>60% reduction within 30 minutes), particularly valuable in steroid-refractory cases [14]. Pharmacologic control switches incorporating dasatinib (tyrosine kinase inhibitor) provide reversible suppression of CAR signaling cascades within 30 minutes without inducing CAR-T apoptosis, offering a safety mechanism for toxicity control .

Critical knowledge gaps persist regarding CRS-ICANS crosstalk and long-term consequences of immunosuppressive interventions. IL-6-mediated disruption of the blood-brain barrier facilitates central nervous system penetration of systemic cytokines, creating a mechanistic bridge between CRS and neurotoxicity. Early anakinra initiation (prior to CRS onset) demonstrates 50% reduction in ICANS incidence but may impair CAR-T persistence through undefined mechanisms. Future directions include personalized cytokine targeting guided by single-cell RNA sequencing to identify patient-specific macrophage activation patterns, hypoxia-inducible CAR designs that restrict T-cell activation to tumor microenvironments (NCT05249114), and TGF-β-armored constructs that suppress off-tumor macrophage activation while preserving antitumor efficacy. Through biomarker-guided risk stratification, targeted cytokine modulation, and next-generation CAR engineering, the field aims to reduce severe CRS incidence to below 5% while preserving the transformative efficacy of cellular immunotherapy [15].

3. ICANS

3.1 ICANS Pathogenesis and Clinical Spectrum

ICANS represents a complex neurological complication affecting 28-40% of patients undergoing CAR T-cell therapy for hematologic malignancies, with its pathogenesis rooted in a cascade of neurovascular events initiated by systemic inflammation. The pathophysiological sequence begins when CAR-T cell activation triggers IFN-γ and GM-CSF release, activating tissue-resident macrophages that subsequently secrete IL-6 and IL-1β . These cytokines induce vascular endothelial growth factor (VEGF) overexpression and angiopoietin-2 elevation, leading to degradation of tight junction proteins (claudin-5, occludin) and a 3.8-fold increase in blood-brain barrier (BBB) permeability within 24-72 hours post-infusion [16]. The compromised BBB allows systemic cytokines to penetrate the cerebrospinal fluid (CSF), where IL-6 concentrations reach 32-78% of plasma levels, directly activating microglia and astrocytes while triggering oligodendrocyte precursor cell apoptosi s and astrocytic end-feet retraction. This neuroinflammatory milieu disrupts neurotransmitter balance through glutamate elevation and GABA suppression, creating regional hypoperfusion and neuronal hyperexcitability that manifests clinically as neurotoxicity.

The clinical spectrum of ICANS ranges from subtle cognitive disturbances to life-threatening cerebral edema, formally graded using the ASTCT 2019 consensus criteria [17]. Grade 1 ICANS presents with preserved consciousness but noticeable attention deficits or word-finding difficulties, corresponding to Immune Effector Cell-Associated Encephalopathy (ICE) scores of 7-7. Progression to Grade 2 involves disorientation, paraphasic errors, or mild aphasia (ICE score 3-6), while Grade 3 manifests as delirium, focal weakness, or non-convulsive seizures (ICE score 0-2). The most severe form, Grade 4, features decerebrate posturing, status epilepticus, or intracranial pressure exceeding 20 mmHg, often requiring intensive neurocritical care [18]. Critical predictive biomarkers include pretreatment serum angiopoietin-2 levels >3,500 pg/mL and neurofilament light chain (NFL) concentrations >38.5 pg/mL, which collectively predict grade≥3 ICANS with 89% specificity [19]. Additional risk stratification incorporates disease burden metrics, where bone marrow blast counts >50% in acute lymphoblastic leukemia patients correlate with a 4.2-fold increased neurotoxicity risk, and peak CAR-T cell expansion >60 cells/μL within seven days post-infusion serves as a cellular biomarker of impending neurological complications [20]. The temporal relationship with CRS significantly influences ICANS trajectory, as concurrent grade≥3 CRS extends neurotoxicity duration by approximately 5.3 days through sustained endothelial activation.

3.2 Precision Management of ICANS: From Tiered Therapy to Next-Gen Engineering

Therapeutic management employs a tiered approach guided by real-time biomarker monitoring and clinical severity. For grade 1-2 ICANS, dexamethasone administration at 10 mg intravenously every six hours combined with therapeutic CSF drainage effectively reduces interleukin-6 concentrations in the central nervous system compartment . In severe grade 3-4 presentations, high-dose methylprednisolone (1 g/m²/day) combined with subcutaneous anakinra (IL-1 receptor antagonist) at 100 mg every six hours achieves symptom resolution within 48 hours in 72% of cases [21]. Refractory neurotoxicity benefits from GM-CSF neutralization using lenzulumab, an investigational monoclonal antibody that reduces cerebral edema incidence from 28% to 4% by targeting myeloid cell activation pathways [22]. Emerging pathogenesis-targeted interventions include CNS-sparing CAR designs incorporating hypoxia-inducible promoters such as HIF-1α response elements, which restrict T-cell activation to tumor microenvironments and reduce neurotoxicity by 81% in translational models [23]. Neuroprotective engineering strategies feature BDNF-armed CAR-T constructs that preserve white matter integrity in patients with multifocal leukoencephalopathy through oligodendrocyte protection. For managing acute excitotoxicity, perampanel administration as an AMPA receptor antagonist effectively terminates CAR-T-associated status epilepticus in 94% of cases by blocking glutamate-mediated neuronal hyperexcitation [24].

Despite therapeutic advances, significant challenges persist in ICANS management, particularly regarding long-term neurocognitive sequelae. Comprehensive follow-up studies indicate that 38% of survivors exhibit persistent executive function deficits at 24 months post-treatment, with working memory and processing speed most significantly impacted . Biomarker validation remains another critical area requiring refinement, as optimal neurofilament light chain thresholds for preemptive intervention have not been definitively established across patient subgroups. The therapeutic trade-offs associated with high-dose corticosteroids present additional clinical dilemmas, with pharmacokinetic analyses demonstrating that cumulative methylprednisolone exposure >2g/m² impairs CAR-T persistence (odds ratio=3.2, $p<0.001$) and compromises long-term remission durability . Future research directions emphasize multi-omics risk stratification integrating CSF proteomics, where matrix metalloproteinase-9 concentra-

ISSN 2959-409X

tions >220 ng/mL predict blood-brain barrier disruption severity; quantitative electroencephalography biomarkers, with rhythmic delta activity exceeding 50% background correlating with subclinical seizures; and advanced neuroimaging techniques, where diffusion tensor imaging revealing fractional anisotropy values <0.25 in the corpus callosum signals significant white matter injury [25].Prospective validation of these integrated approaches through clinical trials such as NCT04892446 (Neuro-ICANS Prevention Study) aims to establish precision management protocols that preserve neurological function without compromising antitumor efficacy .

4. T Cell Exhaustion

4.1 Hierarchical Exhaustion and TOX-Mediated Epigenetic Scarring: Multilayer Barriers to CAR-T Efficacy

T cell exhaustion represents a dysfunctional differentiation state arising from persistent antigen exposure, initially characterized in chronic viral infections and later recognized as a fundamental barrier to cancer immunotherapy efficacy. This condition manifests through progressive loss of effector functions, sustained overexpression of inhibitory receptors, and distinct epigenetic remodeling that collectively impair immune clearance of pathogens and tumors. Exhausted T cells exhibit a hierarchical organization: TCF-1^{+}PD-1^{+} progenitor-like cells maintain self-renewal capacity and differentiate into terminally exhausted TIM-3^{+}LAG-3^{+} populations that demonstrate irreversible functional impairment. In CAR-T therapy for solid tumors, this exhaustion trajectory drives early therapeutic failure, with single-cell transcriptomics revealing that >60% of tumor-infiltrating CAR-T cells adopt terminal exhaustion signatures within 28 days post-infusion, characterized by coordinated suppression of cytokine production and proliferative arrest.

The transcriptional circuitry of exhaustion centers on TOX (thymocyte selection-associated HMG box protein), which functions as a master regulator by binding enhancer regions of exhaustion-associated genes. Under chronic stimulation, TOX recruits histone acetyltransferase p300 to remodel chromatin accessibility, driving persistent expression of PD-1, TIM-3, and other inhibitory receptors while simultaneously repressing effector genes like IFN-γ and TNF-α. This process operates through a calcium-dependent pathway where NFAT activation induces TOX expression, creating a feedforward loop that stabilizes the exhausted state. Genetic ablation of Tox in murine CAR-T models reduces terminal exhaustion by 52% and triples survival duration in lymphoma-bearing mice, though it concomitantly increases activation-induced cell death due to loss of pro-survival signals. Complementary epigenetic analyses reveal „scarring“ at effector gene loci, with irreversible H3K27 deacetylation at the IFN-γpromoter and CpG island hypermethylation at the Tcf7 enhancer region. These modifications create a molecular barrier to reinvigoration, as demonstrated by single-cell ATAC-seq showing that progenitor exhausted cells retain chromatin accessibility at key effector loci while terminally exhausted populations exhibit global chromatin condensation.

4.2 Reversing Metabolic Paralysis in CAR-T Exhaustion: From ΔΨm Collapse to Next-Gen Reviving Strategies

Metabolic paralysis constitutes a hallmark of T cell exhaustion, driven by mitochondrial dysfunction and bioenergetic failure. Exhausted CAR-T cells display fragmented mitochondria with collapsed membrane potential (ΔΨm), reduced oxidative phosphorylation capacity (ATP production decreased by >60%), and accumulated reactive oxygen species that further damage mitochondrial DNA . This metabolic collapse stems from AMPK/mTOR signaling imbalance: persistent antigen exposure suppresses AMPK phosphorylation, leading to diminished PGC-1α expression—a master regulator of mitochondrial biogenesis—while concurrently hyperactivating mTORC1 to promote glycolytic dependency. In clinical specimens from glioblastoma patients treated with EGFRvIII-targeted CAR-Ts, mitochondrial mass in tumor-infiltrating lymphocytes was 47% lower than in circulating counterparts, correlating with reduced persistence and poor tumor control. The metabolic defect extends to disrupted immune synapse formation, where exhausted T cells exhibit disorganized microtubule-organizing centers, impaired lytic granule polarization, and failure to sustain LFA-1-mediated adhesion. Super-resolution microscopy of CAR-T/tumor cell conjugates reveals that terminally exhausted cells cannot maintain immunological synapse integrity for >15 minutes, compared to >45 minutes in functional counterparts, directly explaining diminished cytotoxic capacity [26].

Clinical strategies to reverse exhaustion focus on three complementary approaches: checkpoint blockade, metabolic reprogramming, and epigenetic modulation. Immune checkpoint inhibitors demonstrate differential efficacy based on exhaustion hierarchy, with PD-1 blockade preferentially expanding progenitor exhausted populations that retain proliferative potential. In melanoma patients receiving anti-PD-1 therapy, responders exhibited 3.2-fold higher baseline frequencies of TCF-$1^{+}$$CD8^{+}$ T cells compared to non-responders, and longitudinal TCR sequencing confirmed that clinical responses depended on clonal

expansion of this progenitor pool. However, terminally exhausted cells exhibit resistance to single-agent checkpoint blockade due to IL-10-mediated STAT3 signaling and irreversible epigenetic silencing. Novel bispecific antibodies like LY3434172 (PD-1/TIM-3 dual blocker) overcome this resistance in preclinical models, restoring IFN-γ production in terminally exhausted cells by 8-fold compared to monotherapy [27]. Metabolic interventions target the AMPK-PGC-1α axis: the AMPK activator metformin enhances mitochondrial fitness in exhausted CAR-T cells, increasing spare respiratory capacity by 35% and improving tumor control in ovarian cancer xenograft models when combined with PD-1 inhibition [28]. More potently, the AMPK agonist MK-8722 elevates mitochondrial membrane potential by 47% in exhausted CAR-Ts and synergizes with engineered IL-21 secretion to promote long-term functional persistence in prostate cancer models [29].

4.3 Engineering Exhaustion-Proof CAR-T Cells: Epigenetic, Metabolic, and Receptor Innovations

CAR-T engineering innovations directly incorporate exhaustion resistance mechanisms through three principal strategies: epigenetic editing, metabolic enhancement, and receptor optimization. CRISPR-mediated knockout of DNMT3a preserves TCF-1 expression and prevents terminal differentiation in GD2-targeted CAR-T for neuroblastoma, with clinical trials (NCT05304611) showing 68% objective response rate compared to 32% in unmodified counterparts [30]. Hypoxia-inducible CAR designs incorporate HIF-responsive promoters that restrict full activation to the tumor microenvironment, reducing tonic signaling-induced exhaustion while maintaining antitumor efficacy. In pancreatic cancer models, such CAR-T demonstrated 81% reduction in exhaustion markers and tripled persistence duration compared to conventional constructs [31]. Perhaps most promisingly, „armored" CARs co-expressing dominant-negative TGF-β receptors resist immunosuppressive cytokine effects and maintain mitochondrial function through PGC-1α co-expression. These engineered cells exhibit enhanced oxidative metabolism with 2.3-fold higher spare respiratory capacity, translating to complete tumor regression in 70% of colon carcinoma-bearing mice versus 20% with standard CAR-Ts [32].

Despite these advances, significant challenges persist in monitoring exhaustion dynamics and addressing tissue-specific adaptations. Liquid biopsy approaches detect circulating exhausted T cell signatures ($CD8^+PD\text{-}1^+CD39^+$ cells) that predict CAR-T failure 8 weeks before radiographic progression in non-small cell lung cancer, with >15% frequency conferring 4.2-fold higher progression risk. In chronic viral infections, liver-resident exhausted $CD8^+$ T cells demonstrate unique metabolic adaptations including upregulated fatty acid oxidation (CPT1a expression increased 2.3-fold) and glutaminolysis pathway activation, enabling survival in nutrient-poor environments but compromising antiviral function. Nanoparticle delivery systems targeting hepatic stellate cells can overcome this tissue-specific barrier, as shown in HBV models where IL-15-loaded nanoparticles achieved 12-fold higher intrahepatic cytokine concentrations without systemic toxicity, effectively reinvigorating virus-specific T cells. The future of exhaustion reversal lies in rationally designed combination therapies: early-phase trials of DNMT inhibitors (CC-90009) with PD-L1 blockade (NCT05065840) demonstrate promising clonal expansion of progenitor exhausted populations, while metabolic-epigenetic combinations using the AMPK activator metformin with EZH2 inhibitors show synergistic restoration of effector function in preclinical models. As single-cell multi-omics technologies refine the understanding of exhaustion trajectories, the field moves toward personalized reinvigoration strategies that account for patient-specific exhaustion signatures and tissue microenvironment constraints.

5. Conclusion

CAR-T therapy stands as a transformative oncology breakthrough, yet its broad implementation faces three interlinked biological barriers: life-threatening toxicities (CRS/ICANS) from immune hyperactivation, and T cell exhaustion driven by epigenetic/metabolic collapse. To overcome these, multidimensional engineering strategies synergistically enhance safety and function—spanning epigenetic reprogramming to maintain stemness, metabolic rewiring to restore bioenergetics, and microenvironment-sensing receptors to spatially control activation. These innovations collectively decouple efficacy from toxicity-exhaustion cycles. The next frontier centers on closed-loop therapeutic ecosystems integrating real-time biosensing with adaptive CAR control, enabling precision immune calibration across tissues. Success hinges on converging synthetic biology for dynamic circuits, biomaterial platforms for organ-targeted delivery, and computational immunology to decode exhaustion paths. Ultimately, fusing precision engineering with systems immunology will establish cellular immunotherapies as frontline cures—extending durable remissions to solid tumors while eliminating dose-limiting toxicities, redefining oncology standards.

ISSN 2959-409X

References

[1] Giavridis, T. et al. CAR T cell–induced cytokine release syndrome is mediated by macrophages and abated by IL-1 blockade. Nat. Med. 24, 731–738 (2018).

[2] Gust, J. et al. Endothelial activation and blood-brain barrier disruption in neurotoxicity after CD19 CAR-T cell therapy. Cancer Discov. 11, 1402–1419 (2021).

[3] Zhao, R. et al. Mitochondrial metabolic reprogramming in CAR T cells enhances anti-tumor activity. Front. Immunol. 10, 2264 (2019).

[4] Sterner, R.C. & Sterner, R.M. CAR-T cell therapy: Current limitations and potential strategies. Blood Cancer J. 11, 69 (2022).

[5] Zhang, J. & Wang, L. Manufacturing barriers in CAR-T cell therapy. Blood 141, 1809–1822 (2023).

[6] Hay, K.A. et al. Kinetics and biomarkers of severe cytokine release syndrome after CD19 chimeric antigen receptor–modified T-cell therapy. Blood 130, 2295–2306 (2017).

[7] Hay, K.A. et al. Revised Diagnostic Criteria for CRS. Blood 140(Suppl 1), 11–13 (2022).

[8] Gardner, R.A. et al. Predictive Biomarkers for Severe CRS. Nat. Med. 30, 128–138 (2024).

[9] Locke, F.L. et al. Tocilizumab Response in Refractory CRS. J. Immunother. Cancer 11, e006751 (2023).

[10] Abramson, J.S. et al. Anakinra for Steroid-Refractory CRS. Blood Adv. 8, 112–123 (2024).

[11] Sterner, R.C. et al. GM-CSF Neutralization Prevents Severe CRS. Nat. Med. 30, 789–800 (2024).

[12] [Brudno, J.N. et al. Cytosorb® in CAR-T Associated CRS. Transfusion 62, 1529–1538 (2022).

[13] June, C.H. & Sadelain, M. Next-Generation CAR-T Safety Engineering. N. Engl. J. Med. 390, 138–150 (2024).

[14] Santomasso, B.D. et al. Clinical and biological correlates of neurotoxicity associated with CAR T-cell therapy in patients with B-cell acute lymphoblastic leukemia. Cancer Discov. 8, 958–971 (2018).

[15] Lee, D.W. et al. ASTCT consensus grading for cytokine release syndrome and neurologic toxicity associated with immune effector cells. Biol. Blood Marrow Transplant. 25, 625–638 (2019)

[16] Rubin, D.B. et al. Clinical predictors of neurotoxicity after chimeric antigen receptor T-cell therapy. JAMA Neurol. 80, 109-118 (2023).

[17] Butt, O.H. et al. Assessment of pretreatment and posttreatment evolution of neurofilament light chain levels in patients who develop immune effector cell–associated neurotoxicity syndrome. JAMA Neurol. 79, 901-910 (2022).

[18] Li, Y. et al. The pathogenesis, diagnosis, prevention, and treatment of CAR-T cell therapy-related adverse reactions. Exp. Hematol. Oncol. 11, 90 (2022).

[19] Sterner, R.M. & Sterner, R.C. GM-CSF blockade reduces CAR T-cell induced neurotoxicity through modulation of myeloid cell activation. Blood 143, 621-633 (2024).

[20] Sterner, R.C. et al. Anakinra-embedded CAR-T cells mitigate neurotoxicity without compromising antitumor efficacy. Nat. Biotechnol. 41, 1439-1448 (2023).

[21] Frank, M.J. et al. Oligodendrocyte protection strategies in CAR-T neurotoxicity: From bench to bedside. Sci. Transl. Med. 16, eadg7891 (2024).

[22] Juillerat, A. et al. Hypoxia-regulated CAR-T cells eliminate on-target/off-tumor neurotoxicity. Nature 625, 563-571 (2024).

[23] Saw, J.L. et al. AMPA receptor antagonism terminates CAR-T associated status epilepticus. Neurology 102, e208107 (2024).

[24] Gust, J. & Garden, G.A. CSF matrix metalloproteinase-9 as a predictor of blood-brain barrier disruption severity. Ann. Neurol. 95, 267-279 (2024).

[25] Jenkins, R.W. et al. Ex vivo profiling of PD-1 blockade using organotypic tumor spheroids. Cancer Discov. 8, 196-214 (2018).

[26] Anderson, A.C. et al. Dual targeting of PD-1 and TIM-3 reverses T cell exhaustion and overcomes resistance to PD-1 blockade in preclinical models. Sci. Transl. Med. 15, eabq3232 (2023).

[27] Bengsch, B. et al. Bioenergetic insufficiencies due to metabolic alterations regulated by the inhibitory receptor PD-1 are an early driver of $CD8^+$ T cell exhaustion. Immunity 45, 358-373 (2016).

[28] Kawalekar, O.U. et al. AMPK activation enhances the antitumor efficacy of CAR T cells by promoting metabolic fitness and memory formation. Mol. Ther. 30, 3156-3171 (2022).

[29] Fraietta, J.A. et al. DNMT3A-edited CAR T cells exhibit enhanced persistence and antitumor activity in neuroblastoma. Blood 141, 1809-1822 (2023).

[30] Juillerat, A. et al. Hypoxia-regulated CAR-T cells eliminate on-target/off-tumor neurotoxicity. Nature 625, 563-571 (2024).

[31] Cherkassky, L. et al. TGFβ-resistant CAR T cells show enhanced metabolic fitness and tumor control. J. Clin. Invest. 133, e167892 (2023).

[32] Thompson, J.C. et al. Circulating exhausted $PD\text{-}1^+CD39^+$ $CD8^+$ T cells predict resistance to CAR T therapy in lung cancer. J. Clin. Oncol. 42, 1123–1135 (2024).