Multidimensional Engineering of CAR-based Cellular Immunotherapies for Ovarian Cancer

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***Abstract*—Chimeric Antigen Receptor (CAR)-based cellular immunotherapy has demonstrated remarkable efficacy in hematological malignancies; however, its clinical translation to solid tumors, particularly ovarian cancer, remains limited by multiple factors. Using ovarian cancer as a representative model, this review focuses on the key challenges related to the limited efficacy and suboptimal safety profiles of CAR therapy in solid tumors. This research systematically synthesizes recent multidimensional engineering strategies designed to optimize CAR-based interventions. Drawing upon principles of tumor immunology, cellular engineering, gene editing, and epigenetic regulation, we critically evaluate targeted optimization approaches, including dual-antigen targeting, chemokine and metabolite receptor modification, gene editing, epigenetic reprogramming, armored cytokine secretion, universal modular CAR designs, and allogeneic "off-the-shelf" platforms. Current evidence indicates that these multimodal engineering strategies can synergistically enhance tumor homing, intratumoral infiltration, metabolic fitness, and persistence of CAR-engineered cells, while effectively mitigating antigen escape and T-cell exhaustion to improve safety profiles. Furthermore, adjunctive strategies such as intraperitoneal delivery and the identification of predictive biomarkers are discussed for their potential to augment therapeutic efficacy and facilitate clinical translation. In conclusion, this review underscores that a multidimensional engineering framework provides a safe, scalable, and standardized paradigm to overcome treatment resistance and immune evasion, thereby laying a solid theoretical and practical foundation for the development of potentially curative CAR immunotherapies for ovarian cancer and other solid tumors.**

***Keywords*—Chimeric Antigen Receptor (CAR)-T cell therapy, ovarian cancer, multidimensional engineering, tumor microenvironment, solid tumor immunotherapy**

I. Introduction

Chimeric Antigen Receptor (CAR) T-cell therapy has demonstrated unprecedented efficacy in hematologic malignancies, establishing the redirection of engineered immune cells as a transformative paradigm in oncology. Nevertheless, its therapeutic impact in solid tumors remains substantively constrained, particularly in ovarian cancer—the most lethal gynecologic malignancy characterized by pervasive peritoneal dissemination and acquired platinum resistance. Principal impediments encompass inadequate tumor trafficking and infiltration, an immunosuppressive Tumor Microenvironment (TME) that induces metabolic dysfunction and T-cell exhaustion, significant antigenic heterogeneity facilitating immune escape, and safety concerns including Cytokine Release Syndrome (CRS), Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS), and Graft-versus-Host Disease (GvHD) in allogeneic contexts, alongside manufacturing scalability limitations inherent to autologous platforms. To surmount these barriers, next-generation CAR-based immunotherapies have evolved through multidimensional engineering strategies. These include dual-antigen targeting to mitigate immune escape, chemokine and metabolite receptor modification to enhance tumor trafficking, CRISPR-mediated gene editing, epigenetic and metabolic reprogramming, armored cytokine delivery, universal modular CAR architectures, and allogeneic "off-the-shelf" platforms. Furthermore, the integration of non-viral transposon systems and locoregional intraperitoneal delivery offers promising avenues to improve safety profiles, T-cell persistence, and local antitumor potency. Although individual modalities have demonstrated preclinical potential, a comprehensive synthesis of integrated multimodal engineering approaches remains lacking. This review centers on ovarian cancer to elucidate state-of-the-art CAR T-cell engineering strategies, delineate their synergistic mechanisms for enhancing tumor infiltration, cytotoxicity, and persistence, and critically evaluate emerging clinical evidence, including trials involving T Cell Receptor-mimetic (TCR-mimetic) CAR-T cells. Ultimately, this work aims to establish a rational framework for the development of safe, scalable, and potent CAR immunotherapies for ovarian cancer and other refractory solid tumors.

II. Critical Barriers Impeding the Clinical Efficacy of CAR T-Cell Immunotherapy in Ovarian Carcinoma

A. Impaired Tumor Homing and Infiltrative Capacity

Impaired trafficking and limited infiltration of CAR-T cells into peritoneal metastases constitute a primary obstacle to realizing their clinical efficacy in ovarian cancer [1, 2]. This challenge is exacerbated by deficient tumor-specific chemokine gradients, dense extracellular

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matrix deposition, fibrotic stromal barriers, and a profoundly immunosuppressive peritoneal microenvironment. These factors impede CAR-T cell extravasation, chemotactic migration, and deep intratumoral penetration, ultimately compromising antitumor activity.

B. Metabolic Dysregulation and Functional Exhaustion of Immune Cells within the Tumor Microenvironment

Characterized by profound nutrient deprivation, hypoxia, and extracellular acidosis, the TME imposes severe metabolic constraints on CAR-engineered immune cells, inducing mitochondrial dysfunction, apoptosis, and progressive functional exhaustion [3, 4]. Consequently, the in vivo persistence and tumor-specific cytotoxicity of adoptively transferred effector cells are markedly diminished within this immunosuppressive milieu, ultimately undermining the therapeutic efficacy of CAR-based therapies against solid tumors.

C. Tumor Antigen Heterogeneity and Mechanisms of Immune Evasion

Ovarian carcinoma is characterized by profound intratumoral heterogeneity in the expression profiles of Tumor-Associated Antigens (TAAs), with Mesothelin (MSLN), Mucin 16 (MUC16), and Tumor-Associated Glycoprotein 72 (TAG-72) identified as the most predominantly aberrantly expressed targets. Consequently, monospecific CAR-T cell therapies frequently fail to achieve sustained eradication of the heterogeneous tumor cell population. This incomplete clearance exerts potent selective pressure, driving the clonal expansion of antigen-negative variants and facilitating antigen escape. Ultimately, this evolutionary process culminates in disease progression, acquired therapy resistance, and clinical relapse [5, 6].

D. Safety Profiles and Considerations for off-the-Shelf Therapeutic Development

Clinically approved autologous CAR-T cell therapies are frequently complicated by severe, potentially life-threatening toxicities, notably Cytokine Release Syndrome (CRS) and Immune effector Cell-Associated Neurotoxicity Syndrome (ICANS). Furthermore, the logistically complex, cost-prohibitive, and patient-specific manufacturing processes inherent to autologous products significantly constrain scalability, standardization, and timely clinical access. These limitations underscore a critical unmet clinical need for off-the-shelf cellular immunotherapies characterized by optimized safety profiles, enhanced scalability, and sustained therapeutic durability [7, 8].

III. Multidimensional Engineering Strategies for CAR-Engineered Immune Cells

A. Cell Trafficking Programs: Chemokine and Metabolite Receptor-Mediated Guidance

The synergistic coordination between chemokine receptors and metabolite-sensing G-Protein-Coupled Receptors (GPCRs) is pivotal for orchestrating directional immune cell trafficking and enabling robust infiltration into the solid Tumor Microenvironment (TME). Specifically, GPR183 functions as a high-affinity receptor for 7α, 25-dihydroxycholesterol (7α, 25-OHC), a bioactive oxysterol abundantly secreted by ovarian carcinoma cells. This ligand-receptor interaction confers distinct tumor tropism and promotes potent intratumoral infiltration of engineered CAR-T and CAR-NK cells [1]. In parallel, ectopic overexpression of CCR2 renders CAR-T cells responsive to tumor-derived CCL2 chemokine gradients, significantly augmenting chemotactic migration and selective accumulation within peritoneal metastatic lesions [2]. Moreover, intraperitoneal (i.p.) administration enhances local retention and tumor-specific enrichment of these engineered CAR immune cells, thereby optimizing therapeutic bioavailability within the peritoneal tumor niche.

B. Metabolic and Epigenetic Programming: Mitigating Exhaustion and Sustaining T Cell Persistence

The dynamic interplay between metabolic reprogramming and epigenetic remodeling orchestrates cellular homeostasis, mitochondrial fitness, and functional persistence. Significantly, accumulating evidence substantiates that Neo-2/15-armored CAR-NK cells potently activate the c-Myc-NRF1 transcriptional axis, thereby preserving mitochondrial integrity, maintaining bioenergetic homeostasis, and conferring resilience against TME-mediated functional exhaustion [3]. Membrane-bound interleukin-15 (mbIL-15) confers robust long-term persistence and cytokine-independent survival to CAR-NK cells, eliminating the need for exogenous cytokine supplementation [4]. Consequently, this strategy mitigates the rapid attrition and functional exhaustion that typically constrain the durability of conventional CAR-NK cells in both in vitro and in vivo contexts. Valproic Acid (VPA) functions as a multifaceted epigenetic and metabolic modulator that orchestrates comprehensive transcriptional and post-translational reprogramming in CAR-T cells. Mechanistically, VPA promotes the accumulation of histone propionylation marks, specifically H3K56pr, and induces the expression of pivotal effector genes such as LOX and GUCY1B3. These alterations significantly augment CAR-T cell migratory capacity, Extracellular Matrix (ECM) invasion, and tumor infiltration, while concurrently enhancing mitochondrial bioenergetics, glycolytic flux, and metabolic plasticity [9]. This coordinated epigenetic-metabolic engineering strategy synergistically enhances CAR-NK cell fitness, mitigates TME-induced functional exhaustion, and circumvents antigen escape, thereby establishing a robust framework to overcome the inherent limitations of conventional CAR-based immunotherapies in solid tumors.

C. Genetic and Signal Transduction Modulation: Potentiation of Cytolytic Activity

Targeted genomic engineering represents a robust strategy to augment intracellular signal transduction and enhance the cytolytic potency of CAR-T cells. Specifically, CRISPR-Cas9-mediated ablation of diacylglycerol kinase α and ζ (DGKα/ζ) abrogates a

pivotal intrinsic negative regulatory checkpoint within the T cell signaling cascade. By precluding the enzymatic conversion of Diacylglycerol (DAG) to Phosphatidic Acid (PA), DGKα/ζ ablation sustains DAG-mediated downstream signaling, thereby potentiating T cell activation, proliferation, and effector functions. This reprogramming of intracellular signaling architecture enables CAR-T cells to circumvent exhaustion, maintain long-term persistence, and elicit durable, potent antitumor immunity even within the immunosuppressive tumor microenvironment [10]. JNK knockdown selectively enhances NFATc1-dependent transcriptional activity in CAR-T cells, thereby significantly upregulating Granzyme B (GZMB) expression and enhancing cytotoxic degranulation. Consequently, this strategy selectively amplifies antitumor efficacy while preserving intrinsic cellular fitness [11]. CRISPR-Cas9-mediated site-specific integration of therapeutic transgenes into genomic safe harbor loci, such as the T-cell Receptor Alpha Constant (TRAC) locus, substantially circumvents the risks of insertional mutagenesis and genotoxicity inherent to stochastic integration mediated by conventional viral vectors. Furthermore, this precision genome engineering strategy facilitates the stable and coordinated expression of optical and Positron Emission Tomography (PET) imaging reporter's concomitant with CAR constructs. Consequently, this approach enables real-time, longitudinal, and noninvasive in vivo monitoring of CAR-T cell trafficking, tumor infiltration, persistence, and biodistribution [12].

D. Next-Generation CAR Architectures: Mitigating Intratumoral Antigen Heterogeneity

Next-generation CAR architectures are rationally engineered to overcome therapeutic resistance caused by tumor antigen heterogeneity and immune evasion. Specifically, tandem CAR-T cells co-targeting MSLN and MUC16 utilize an optimized spatial arrangement of single-chain variable Fragments (scFvs) and refined linker spacing to mitigate steric constraints between antigen-binding domains. By concurrently addressing antigen-loss variants and mixed antigen expression profiles, tandem dual-targeting CAR-T cells elicit durable and potent cytolytic activity against ovarian and pancreatic carcinomas, effectively counteracting antigen escape phenomena and diminishing the likelihood of disease relapse [5]. TCR-mimetic CAR-T cells integrate the high specificity of antibody-based antigen recognition with the physiological signaling cascade of native T cell receptors, thereby conferring enhanced functional stability, reduced tonic signaling, and prolonged in vivo persistence. Compared with conventional CAR-T constructs, this innovative architecture also drives superior tumor-directed migration, deep intratumoral infiltration, and enhanced resilience within the hostile solid tumor microenvironment [13]. Universal modular CAR platforms—exemplified by the GA1CAR-Fab system and Chimeric Fcγ Receptor T (CFRT) cells—enable dynamic, on-demand antigen retargeting without the need for repeated genetic engineering of host T cells. By decoupling antigen specificity from the constitutive CAR construct, these versatile systems enable rapid transitions between tumor-specific targets. Consequently, this strategy prevents tumor antigen escape, optimizes manufacturing protocols, and improves safety profiles through tunable effector functions [6, 14].

E. Engineering Armored CAR-T Cells for Tumor Microenvironment-Specific Immune Potentiation

Armored CAR-T cells constitute a next-generation therapeutic paradigm designed to remodel the immunosuppressive TME while mitigating the risk of systemic toxicities. CAR-T cells engineered to secrete anti-PD-L1-IL-12 bifunctional fusion proteins facilitate tumor-localized, PD-L1-targeted cytokine retention. This strategy substantially attenuates systemic cytokine spillage and associated inflammatory adverse events while concentrating immunostimulatory signals within the tumor niche [15]. By concurrently blocking PD-L1-mediated immune checkpoint suppression and delivering IL-12-driven pro-inflammatory remodeling, this armored architecture reverses the immunologically "cold" phenotype characteristic of solid tumors-enhancing CAR-T cell infiltration, augmenting intratumoral IFN-γ production, promoting dendritic cell maturation, and reducing the abundance of immunosuppressive myeloid populations. Collectively, these mechanistic actions sustain potent, durable antitumor immunity and significantly extend survival in preclinical models of ovarian carcinoma and other solid malignancies.

F. Emerging Cell Therapeutic Modalities: Allogeneic "Off-the-Shelf" Platforms with Enhanced Safety Profiles

Alternative cellular platforms represent promising off-the-shelf therapeutic modalities characterized by enhanced safety profiles. Allogeneic CAR-NKT cells mediate dual cytotoxic mechanisms via CAR-directed tumor targeting and invariant TCR-dependent ablation of immunosuppressive tumor-associated macrophages (TAMs) and Myeloid-Derived Suppressor Cells (MDSCs). This approach is associated with a negligible risk of Graft-versus-Host Disease (GvHD) and reduced Cytokine Release Syndrome (CRS) toxicity [7]. CAR-modified Natural Killer (NK) cells, $\gamma\delta$ T cells, and macrophages demonstrate superior infiltration into solid tumors and enhanced metabolic fitness within the immunosuppressive TME. Moreover, these innate immune effector cells exhibit an improved safety profile, characterized by a reduced incidence of CRS, ICANS, and GvHD. These platforms enable streamlined allogeneic "off-the-shelf" manufacturing, thereby significantly expediting the clinical translation of solid tumor immunotherapy [8, 16].

IV. Advancements in Clinical Translation and Pivotal Evidence

A. Clinical Efficacy, Optimization of Administration Protocols, and Synergistic Combination Therapies

First-in-human clinical evaluation of TCR-mimetic CAR-T cells in patients with platinum-resistant advanced

ovarian cancer has demonstrated promising preliminary antitumor efficacy, evidenced by a Disease Control Rate (DCR) of 80% [13]. Notably, this investigational therapy exhibited a favorable safety profile, with no incidence of grade ≥ 3 CRS or ICANS observed across all dose cohorts. Intraperitoneal (i.p.) administration has emerged as an advantageous delivery modality for CAR-engineered cells in ovarian cancer, a strategy validated by multiple preclinical models. This localized approach significantly enhances local biodistribution and cellular persistence in the peritoneal metastatic microenvironment while reducing systemic dissemination and off-target exposure, thereby maximizing the therapeutic index and antitumor efficacy of CAR-based cellular therapies [1, 2, 7]. Furthermore, synergistic combination regimens potentiate clinical outcomes in platinum-resistant ovarian cancer [13]. Specifically, the concomitant administration of carboplatin and nanoparticle albumin-bound paclitaxel, coupled with lymphodepleting conditioning, markedly promotes the expansion, persistence, and tumor infiltration of TCR-mimetic CAR-T cells, resulting in substantial improvements in therapeutic response. Additionally, circulating biomarkers-including KLK13, KLK14, and CXCL17-exhibit robust predictive value for treatment response, duration of disease control, and clinical benefit, thereby enabling the prospective stratification of patient subgroups most likely to benefit from TCR-mimetic CAR-T therapy.

B. Manufacturing Innovations and Clinical Development of Armored CAR Cell Platforms

Membrane-bound interleukin-15 (mbIL-15)-armored CAR-NK cell therapy constitutes a promising next-generation modality that significantly enhances in vivo persistence, proliferative capacity, and antitumor efficacy. Nevertheless, rigorous safety profiling remains paramount. Potential adverse events encompass localized inflammatory responses, transient hematologic abnormalities, on-target/off-tumor toxicity mediated by the recognition of constitutively expressed NKG2D ligands on healthy tissues, as well as theoretical risks associated with prolonged immune cell persistence or systemic immune hyperactivation. Non-viral gene delivery systems, exemplified by the piggyBac transposon platform, offer distinct advantages for clinical manufacturing. These vectors enable high-efficiency, stable transgene integration while reducing the risk of viral insertional mutagenesis. Furthermore, they support scalable, Good Manufacturing Practice (GMP)-compliant ex vivo production and enable robust expansion of engineered NK and T cells [4]. To date, numerous investigational CAR-NK and armored CAR-T constructs have advanced to clinical trials targeting both hematologic malignancies and solid tumors, underscoring the accelerated translational trajectory and broad therapeutic potential of next-generation CAR-based cellular immunotherapies.

V. Conclusion

Ovarian cancer, particularly in the context of platinum resistance and peritoneal dissemination, remains a formidable clinical challenge, compounded by a limited therapeutic arsenal. While CAR T-cell immunotherapy holds transformative potential, its clinical utility is significantly hindered by distinct biological and technical barriers. These include suboptimal leukocyte trafficking and infiltration into the immunosuppressive TME, accelerated T-cell exhaustion driven by metabolic constraints and inhibitory signaling, extensive tumor antigen heterogeneity facilitating immune escape, and safety concerns regarding systemic toxicity and off-target effects. Multidimensional engineering strategies have exhibited remarkable synergistic efficacy in overcoming these longstanding therapeutic barriers. Key innovations encompass GPR183- and CCR2-mediated chemokine receptor engineering to boost tumor-directed migration [1, 2]; metabolic fitness augmentation via Neo-2/15 and membrane-bound IL-15 to sustain persistence and effector function [3, 4]; targeted disruption of DGKα/ζ and JNK inhibitory pathways to amplify cytotoxicity [10, 11]; dual-target tandem CARs and universal modular architectures to mitigate antigen escape [5, 6]; and off-the-shelf allogeneic platforms utilizing CAR-NKT, CAR-NK, $\gamma\delta$ T, and CAR-macrophage cells to minimize GvHD and improve safety [7, 8, 16].

Early-phase clinical studies, particularly those utilizing TCR-mimetic CAR-T therapies, have achieved encouraging disease control in heavily pre-treated, platinum-resistant ovarian cancer patients [13], further confirming the translational feasibility of multidimensional cellular programming. Nevertheless, significant hurdles persist, including the rigorous assessment of long-term response durability, standardized GMP-compliant manufacturing, personalized engineering protocols to address inter- and intra-tumor heterogeneity, and the rational design of combinatorial regimens. Future research priorities should focus on non-invasive in vivo tracking, adaptive safety regulatory circuits, scalable allogeneic production, and clinically actionable combination strategies. Ultimately, multifunctionally engineered CAR-based immunotherapies represent a promising paradigm in next-generation oncology, offering renewed hope and transformative therapeutic potential for patients with advanced, recurrent, and refractory ovarian carcinoma.

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Y. M. Kim, M. K. Tsai, C. Sun *et al*., "Engineering NK and T cells with metabolite-sensing receptors to target solid tumors," *Nature Immunology*, vol. 27, no. 5, pp. 1039–1052, 2026.

[2] R. Kumar, I. E. Veillard, M. Xu, L. Al-Alem, B. R. Rueda, and O. O. Yeku, "CCR2 improves tumor directed CAR-T cell trafficking in ovarian cancer," *Frontiers in Pharmacology*, vol. 16, 1651526, 2025.

[3] J. Luo, M. Guo, M. Huang *et al*., "Neoleukin-2/15-armored CAR-NK cells sustain superior therapeutic efficacy in solid tumors via c-Myc/NRF1 activation," *Signal Transduction and Targeted Therapy*, vol. 10, no. 1, 78, 2025.

[4] D. Feng, L. Sun, D. Hu *et al*., "Expression of membrane-bound Interleukin-15 sustains the growth and survival of CAR-NK cells," *International Immunopharmacology*, vol. 166, 115577, 2025.
[5] D. Salas-Benito, F. Birocchi, S. Park *et al*., "Tandem CAR-T cells targeting mesothelin and MUC16 overcome tumor heterogeneity by targeting one antigen at a time," *Journal for ImmunoTherapy of Cancer*, vol. 13, no. 9, e012822, 2025.
[6] A. Arina, E. Arauz, E. Masoumi *et al*., "A universal Chimeric Antigen Receptor (CAR)-Fragment Antibody Binder (FAB) split system for cancer immunotherapy," *Science Advances*, vol. 11, no. 27, eadv4937, 2025.
[7] Y. R. Li, Z. Li, Y. Zhu *et al*., "Overcoming ovarian cancer resistance and evasion to CAR-T cell therapy by harnessing allogeneic CAR-NKT cells," *Med*, vol. 6, no. 10, 100804, 2025.
[8] A. L. Portillo, M. Mehboob, G. Snyder *et al*., "IL-21-reprogrammed Vδ1 T cells exert killing against solid tumors which is enhanced by CAR arming for off-the-shelf immunotherapy," *Oncoimmunology*, vol. 14, no. 1, 2562210, 2025.
[9] J. Chen, W. Li, S. Wang *et al*., "Sodium valproate drives propionylation-mediated epigenetic reprogramming to enhance mesothelin CAR-T cell therapy in solid tumors," *Molecular Therapy*, vol. 34, no. 3, pp. 1448–1464, 2026.
[10] V. J. Evtimov, N. N. Nguyen, M. V. Hammett *et al*., "CRISPR-Cas9 knockout of DGKα/ζ improves the anti-tumor activities of TAG-72 CAR-T cells in ovarian cancer," *Molecular Therapy Oncology*, vol. 33, no. 2, 200962, 2025.
[11] C. J. Kuhlmann, C. E. Jepson, M. T. Blucas *et al*., "JNK knockdown enhances CAR-T cell cytotoxicity through elevated NFATc1-dependent transcription in preclinical ovarian cancer models," *Journal for ImmunoTherapy of Cancer*, vol. 13, no. 11, e012968, 2025.
[12] R. E. Sanchez-Pupo, J. J. Kelly, N. Shalaby *et al.*, "Imaging CRISPR-edited CAR-T cell therapies with optical and positron emission tomography reporters," *Theranostics*, vol. 16, no. 7, pp. 3227–3245, 2026.
[13] Y. Shan, B. Ding, F. Ji *et al*., "T-cell receptor-like chimeric antigen receptor T cells targeting mesothelin: A first-in-human dose-escalation trial for platinum-resistant advanced ovarian cancer," *Cancer*, vol. 132, no. 3, e70279, 2026.
[14] J. Zhao, M. Chen, X. Li *et al*., "Construction and characterization of chimeric FcγR T cells for universal T cell therapy," *Experimental Hematology & Oncology*, vol. 14, no. 1, 6, 2025.
[15] J. P. Murad, L. Christian, R. Rosa *et al*., "Solid tumour CAR-T cells engineered with fusion proteins targeting PD-L1 for localized IL-12 delivery," *Nature Biomedical Engineering*, vol. 10, no. 4, pp. 613–629, 2025.
[16] Y. Chen, X. Zhu, Y. Chen *et al*., "IL-21 promotes the anti-tumor effect of anti-CD47 chimeric antigen receptor macrophages in ovarian cancer," *Communications Biology*, vol. 9, no. 1, 63, 2025.