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ABSTRACT

Adoptive cell therapy (ACT) is one of the most transformative advances in modern oncology, using genetically engineered T lymphocytes to achieve targeted and long-lasting tumor elimination. Chimeric antigen receptor (CAR) and T-cell receptor (TCR) therapies have shown remarkable success in treating blood cancers, but their application to solid tumors is still limited by multiple resistance factors, including antigen diversity, immunosuppressive microenvironments, and limited T-cell persistence.

This review summarizes the mechanistic barriers and engineering innovations shaping the next generation of adoptive T-cell therapies. We compare structural and functional differences between CAR- and TCR-based systems, explore major resistance mechanisms such as antigen escape, metabolic restrictions, and T-cell exhaustion, and discuss emerging strategies like dual and logic-gated CARs, armored constructs, and TCR mimic designs. We highlight how systems biology, artificial intelligence, and advanced modeling tools are transforming receptor optimization, preclinical testing, and manufacturing scalability.

Although progress is rapid, key mechanistic questions remain about dynamic antigen evolution, cytokine control over space and time, and the long-term safety of multi-circuit constructs. Future advances will require integrating computational feedback, adaptive signaling, and modular receptor designs to create precise, self-optimizing T-cell therapies. These developments collectively mark a shift from static receptor engineering to intelligent, adaptive immune treatments capable of sustained control across diverse and resistant cancer environments.

INTRODUCTION

Adoptive cell therapy (ACT) has become a groundbreaking method in cancer immunotherapy, using ex vivo expanded and genetically modified T lymphocytes to specifically seek out and destroy cancer cells[1]. Unlike vaccine-based or checkpoint-targeted immunotherapies, which rely on activating existing immune responses, ACT supplies patients with pre-prepared effector T cells capable of attacking tumors even in severely immunosuppressed environments[1].

This approach bypasses many limitations of natural antitumor immunity, providing a direct and programmable immune intervention. Although promising, ACT still faces significant biological and translational hurdles.

A major obstacle is immune tolerance to self-derived tumor-associated antigens (TAAs), which are common targets in both solid and blood cancers. Since these TAAs are often unmutated and resemble normal tissue antigens, naturally occurring tumor-reactive T cells usually have low-affinity T cell receptors (TCRs), resulting in weak activation, limited proliferation, and low cytotoxicity[1]. To overcome this, synthetic receptor engineering was developed to bypass natural tolerance mechanisms and improve tumor recognition while balancing efficacy and safety. This led to the development of chimeric antigen receptors (CARs), synthetic molecules that reprogram T-cell specificity toward tumor surface antigens independently of MHC.

CARs combine the extracellular antigen-binding domain of monoclonal antibodies, usually a single-chain variable fragment (scFv), with intracellular T-cell signaling domains from CD3ζ and costimulatory molecules like CD28, 4-1BB, or OX40[2]. This design allows T cells to recognize surface antigens directly, without needing MHC presentation, thus avoiding one of the main tumor immune evasion tactics, loss or downregulation of MHC class I molecules. Clinically, CAR-T cell therapy has transformed treatment for blood cancers. Tisagenlecleucel, the first FDA-approved CD19 CAR-T therapy, demonstrated complete remission rates of 81–90% in pediatric and young adult patients with relapsed/refractory B-cell acute lymphoblastic leukemia (ALL)[3]. Notable cases, such as the sustained remission of pediatric patient Emily Whitehead[4], highlight the curative potential of this approach. However, these advances have also revealed the risks, including severe toxicities like cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS), which show how potent immune activation can cause systemic harm[4].

The success in hematologic cancers has led to exploring TCR-engineered T cells (TCR-T) as a complementary method that can target intracellular antigens presented on MHC molecules. Unlike CAR-Ts, which target surface epitopes, TCR-T therapies can access a wider range of tumor-related peptides or neoantigens[5]. Nevertheless both techniques face shared issues, including antigen heterogeneity, immune escape, and an immunosuppressive tumor microenvironment (TME)[2]. These challenges have so far limited their effectiveness against solid tumors. Overall, these differences highlight the need for integrated receptor engineering approaches that optimize activation, specificity, and safety.

Understanding the comparative advantages and constraints of CAR-T and TCR-T systems (Table 1), alongside innovations in circuit design, metabolic programming, and safety modulation, will be essential for extending the benefits of ACT beyond hematologic cancers and into the far more complex landscape of solid tumors.

TCR and CAR T-Cells in Adoptive Cell Therapy: Structure, Function, and Mechanisms

ACT encompasses several immune-engineering modalities, among which T-cell receptor (TCR)-engineered T cells and chimeric antigen receptor (CAR)-T cells represent the most advanced and clinically validated platforms[1]. While both aim to redirect T cells toward tumor antigens, they differ fundamentally in antigen recognition, signaling architecture, and translational potential. Understanding these structural and mechanistic distinctions is essential to explain their respective successes in hematologic malignancies and the persistent barriers faced in solid tumors.

TCR-Engineered T Cells: Structure and Mechanism

T-cell receptors (TCRs) are naturally occurring αβ heterodimeric proteins composed of variable (V) and constant (C) domains. Antigen recognition occurs through the complementarity-determining regions (CDRs) within the V domains, particularly CDR3, which engages peptide fragments presented by major histocompatibility complex (MHC) molecules on target cells. This interaction is central to adaptive immunity, allowing discrimination between self and non-self antigens[5]. Importantly, the TCR itself lacks intrinsic signaling capacity; instead, activation depends on its association with the CD3 complex, which contains ten immunoreceptor tyrosine-based activation motifs (ITAMs) responsible for downstream signaling[5].
Upon binding a peptide-MHC (pepMHC) complex, the

Feature	CAR T-Cells	TCR T-Cells	Citations
Antigen recognition	Extracellular surface antigens via scFv; MHC-independent	Intracellular peptides via TCR–MHC; MHC-dependent	6
Antigen repertoire	Limited to surface proteins (e.g., CD19, HER2)	Broad; includes intracellular and viral antigens (e.g., NY-ESO-1, KRAS)	7
MHC requirement	None	Requires a patient HLA match	8
Engineering complexity	Synthetic receptor insertion	αβ TCR modification; mispairing prevention	9
Tumor coverage	Hematologic cancers; limited solid-tumor efficacy	Potential for solid tumors; limited by antigen presentation	10
Toxicities	CRS, ICANS, off-tumor effects	CRS, ICANS, off-tumor effects	11
Manufacturing	Autologous standard; allogeneic emerging	Autologous; limited allogeneic feasibility	12
Key advantages	Potent, MHC-independent, proven efficacy	Access to intracellular targets, physiologic signaling	13
Key limitations	Surface-only targeting, cytokine toxicity	MHC-restriction, complex engineering	14

***Table 1**: Structural, functional, and translational features of CAR-T and TCR-T cell therapies*

TCR initiates a multi-step activation cascade involving phosphorylation of CD3 ITAMs by Lck, recruitment of ZAP70, and subsequent activation of adapter proteins such as LAT. This culminates in transcriptional programs that drive cytokine secretion, proliferation, and cytotoxicity[15]. The strength of the TCR-pepMHC interaction is relatively low (Kd ~1–100 μM), yet through serial triggering and co-receptor amplification (CD4/CD8) TCRs exhibit remarkable sensitivity, capable of responding to only a few antigenic complexes per cell[16]. From a therapeutic perspective, TCR engineering enables recognition of intracellular tumor antigens, including mutated neoantigens and cancer-testis antigens, which are inaccessible to antibody-based receptors. This

expands the range of targetable malignancies, particularly for tumors lacking unique surface markers.

However, MHC restrictions remain: while it allows exquisite specificity, it also limits the applicability of a given TCR to patients with compatible HLA alleles and renders tumors vulnerable to immune escape via MHC downregulation. Clinically, TCR-T cells have achieved encouraging responses in tumors such as synovial sarcoma and melanoma, yet their translation is constrained by on-target/off-tumor toxicities and HLA-dependent variability, underscoring the need for improved antigen selection and affinity tuning.

CAR-T Cells: Modular Design and Function

Chimeric antigen receptors (CARs) are synthetic, modular constructs that endow T cells with the ability to recognize target antigens independently of MHC presentation. Structurally, a CAR integrates several functional domains: an extracellular single-chain variable fragment (scFv) derived from an antibody, a hinge or spacer providing flexibility, a transmembrane domain ensuring receptor stability, and one or more intracellular signaling modules responsible for activation[15].

The evolution of CAR design has dramatically influenced therapeutic outcomes. First-generation CARs, containing only the CD3ζ signaling domain, induced cytotoxicity but lacked robust cytokine secretion and persistence; second-generation CARs, incorporating costimulatory motifs such as CD28 or 4-1BB, achieved enhanced proliferation and survival, marking the foundation for today's FDA-approved CAR therapies; and third-generation CARs, which combine multiple costimulatory domains (e.g., CD28 and 4-1BB), generate stronger signaling but have shown mixed clinical advantages, suggesting that excessive stimulation can predispose to exhaustion and toxicity rather than improved efficacy[17].

Functionally, CAR-T cells operate as "living drugs." Upon antigen engagement, they activate, proliferate, release proinflammatory cytokines, and exert cytolytic activity through perforin and granzyme release. Some persist as memory-like populations, contributing to long-term immune surveillance[2]. However, antigen density, tumor microenvironmental factors, and receptor design critically modulate this activity. High antigen expression favors potent killing but also increases the risk of CRS and ICANS, highlighting the delicate balance between potency and safety.

Mechanisms of Resistance to CAR and TCR Therapies

Engineered T-cell therapies have demonstrated remarkable efficacy in hematologic malignancies, yet they face substantial and multifactorial resistance barriers in solid and relapsed cancers[18]. Resistance arises through a convergence of biological processes that erode cytotoxic function, persistence, and antigen recognition fidelity, ultimately compromising therapeutic durability. Understanding these mechanisms is central to the rational design of next-generation immune engineering strategies capable of overcoming tumor evolution and immune evasion.

Up to 60% of relapses are characterized by CD19 antigen loss after CAR-T therapy[19]. A major axis of failure lies in antigen-related resistance, which remains one of the most fundamental obstacles to sustained clinical response[20]. Tumor cells can evade immune recognition through antigen loss, mutation, or transcriptional downregulation of target epitopes[21]. In CAR-T therapies, epitope masking and alternative splicing of canonical targets such as CD19 have been shown to drive relapse following initially successful treatment[22]. Similarly, in TCR-T therapies, tumor cells often downregulate MHC class I molecules or disrupt antigen processing and presentation pathways, rendering themselves invisible to TCR-mediated recognition[23].

Beyond complete antigen loss, intra- and intertumoral heterogeneity allows for the coexistence of antigen-negative or low-density subclones[24]. These resistant subpopulations survive immune pressure and later expand, forming a reservoir for tumor relapse. Such antigenic plasticity underscores the need for multi-antigen targeting and adaptive recognition systems rather than reliance on single epitope specificity[24]. Resistance is further compounded by the immunosuppressive TME, which represents one of the most formidable barriers to effective ACT. Structural and metabolic constraints within solid tumors, including dense extracellular matrix, aberrant vasculature, and hypoxic gradients, physically restrict T-cell infiltration[25]. At the same time, soluble factors such as TGF-β, IL-10, and VEGF, alongside regulatory immune cells including Tregs, myeloid-derived suppressor cells, and

tumor-associated macrophages, collectively reprogram infiltrating T cells toward an exhausted or anergic phenotype. These suppressive interactions diminish cytokine secretion, proliferation, and cytolytic function, creating an immunological "trap" that blunts effector activity even when tumor cells are recognized.

A related but distinct mechanism of dysfunction involves cell-intrinsic exhaustion, a state of progressive dysfunction induced by chronic antigen stimulation and sustained signaling through CAR or TCR constructs[26]. Prolonged activation triggers epigenetic and transcriptional reprogramming, locking T cells into a terminally exhausted state characterized by high expression of inhibitory receptors such as PD-1, TIM-3, and LAG-3, diminished effector cytokine production, and poor recall responses upon re-encountering tumor antigen. The TME further amplifies this exhaustion through metabolic competition for glucose and amino acids, oxidative stress, and exposure to immunoregulatory metabolites like adenosine and kynurenine. Together, these pressures create a self-reinforcing feedback loop that erodes T-cell persistence and long-term functionality. In addition to immune evasion and exhaustion, deficient trafficking and limited persistence remain key practical barriers to therapeutic success[27]. Engineered T cells frequently fail to home efficiently to tumor sites, particularly in solid tumors that lack the chemokine gradients required for guided migration. Even when T cells reach the tumor, nutrient deprivation, hypoxia, and oxidative stress compromise their survival, while macrophage-mediated clearance and host immune rejection further limit persistence[25].

These findings highlight the importance of engineering strategies that enhance chemokine receptor compatibility, metabolic fitness, and stress resistance to sustain antitumor function in the hostile tumor niche. Finally, manufacturing and scalability limitations introduce a less visible but clinically significant layer of heterogeneity. Autologous CAR- and TCR-T cell products vary widely in transduction efficiency, differentiation state, and metabolic profile, leading to inconsistent expansion and potency across patients[28]. Such manufacturing variability complicates both clinical outcomes and mechanistic interpretation, as differences in cell composition or exhaustion state can obscure true therapeutic performance.

Current engineering solutions tend to focus on isolated resistance mechanisms, for instance, developing dual-antigen CARs to counter immune escape or armored CARs to resist TGF-β suppression, without fully addressing the dynamic and interdependent feedback loops that tumors exploit to evade immune pressure. The next frontier in CAR and TCR engineering should therefore embrace systems-level, adaptive designs capable of sensing and responding to evolving tumor signals. Integrative strategies combining antigen multiplexing, metabolic resilience, and programmable regulatory circuits could enable engineered cells to dynamically adjust their behavior in hostile environments. A shift toward such holistic, adaptive frameworks, rather than single-variable modifications, will be essential to achieve durable remissions across genetically heterogeneous and evolutionarily adaptable cancer types.

Engineering Strategies to Overcome Antigen Escape

Antigen escape remains one of the most frequent and devastating mechanisms of relapse following CAR or TCR T-cell therapy[29]. Quantitatively, CD19 antigen loss accounts for 40–60% of post-CAR-T relapses, underscoring its clinical relevance as a dominant resistance mechanism[30]. Loss or mutation of the target epitope, antigen downregulation, and heterogeneous expression within the tumor mass all conspire to reduce recognition and killing efficacy[22,29]. Modern synthetic biology approaches are now enabling the design of multifunctional, logic-gated, and adaptive immune receptors that respond to this complexity with enhanced precision and resilience. These strategies (Table 2) aim to extend the durability and flexibility of engineered T cells beyond the static, single-target paradigm of early CAR constructs.

Multi-targeting and Logic-Gated CARs

Conventional CAR-T cells are limited by their reliance on a single antigen target, making them vulnerable to clonal escape when that antigen is lost or mutated[6]. To counter this, multi-targeting and logic-gated CAR architectures such as tandem CARs (TanCARs), DualCARs, and SynNotch systems have been developed to enable combinatorial antigen recognition[31,32,33]. TanCARs link two single-chain variable fragments (scFvs) within one receptor, allowing simultaneous binding to two antigens, which enhances recognition breadth and reduces relapse probability[33].

CAR Type	Core Engineering Principle	Biological Goal / Targeted Limitation	Key Drawbacks or Risks	Citations
Tandem (bi-scFv) CARs	Two scFvs in a single receptor chain	Simultaneous engagement of two antigens with one receptor	Steric hindrance, variable binding affinity	31
SynNotch CARs	Synthetic Notch receptor triggers the expression of a secondary CAR after detecting Antigen A	Boolean "A AND B" logic to enhance tumor selectivity	Requires dual-antigen co-expression; complex circuit tuning	32
Dual CARs	Two separate CAR constructs expressed in the same T cell	Expands recognition spectrum; mitigates antigen escape	Signal imbalance; greater metabolic stress	33
Armored CARs	Incorporate genes for cytokines (e.g., IL-12, IL-15, IL-18) or costimulatory ligands	Reinforce T-cell activation and remodel the immunosuppressive TME	IL-12 gives strong cytotoxicity but systemic toxicity; IL-18 is safer but weaker	8, 34
iCasp9 / safety-switch CARs	Inducible suicide system or reversible small-molecule control (e.g., dasatinib)	Rapid termination of severe CRS/ ICANS or uncontrolled activation	Adds complexity; cannot prevent early cytokine spike	35
iCARs (inhibitory CARs)	Contain inhibitory domains (PD-1, CTLA-4) triggered by off-target antigen	"A NOT B" logic to prevent normal-tissue attack	Precise inhibitory thresholds are hard to calibrate	36

***Table 2**: Functional CAR Architectures and Engineering Strategies for Overcoming Tumor Resistance and Enhancing Safety*

DualCAR designs employ separate CARs expressed within the same T cell, each targeting distinct antigens to create redundant activation pathways[33]. Antigen heterogeneity is the principal cause of relapse in solid and hematologic malignancies. Dual and TanCAR designs, which encode two or more scFv binders, reduce immune escape but introduce new structural and signaling complexities. Dual-CAR systems enhance the breadth of recognition yet risk tonic signaling and exhaustion when both receptors are co-expressed at high density[33]. In contrast, TanCARs, which integrate two scFvs within one receptor chain, improve immune synapse stability when both targets are co-expressed but lose potency if one antigen is absent. Recent trispecific and "CAR pool" approaches further extend valency, but their translation is impeded by increased vector size, steric interference, and compounding on-target/off-tumor risks. Universal or switchable CARs, using antibody–CAR bridges (e.g., CD16-based, SpyTag-SpyCatcher, or small-molecule adaptors), offer a modular solution, one cell product for multiple diseases, but hinge on the pharmacologic behavior and immunogenicity of the adaptor molecule. Critically, universal CARs may offer dose-dependent tunability, yet long-term persistence without ligand engagement remains unpredictable, and manufacturing complexity remains a barrier to scalability[37].

SynNotch systems, by contrast, introduce Boolean logic into immune signaling: the recognition of one antigen through a synthetic Notch receptor induces the expression of a second CAR targeting another antigen[32,38]. Traditional CARs operate on a binary "on–off" paradigm, where antigen engagement directly triggers activation. However, this model lacks contextual sensitivity and often results in off-tumor toxicity. Logic-gated CARs, including split-signal AND, NOT, and synNotch IF/THEN systems, represent the next conceptual leap. Split-signal AND-CARs distribute CD3ζ and co-stimulatory domains across separate receptors, requiring two antigens for full activation, minimizing false-positive engagement.

Yet, their stringency may compromise efficacy in tumors with heterogeneous antigen expression, increasing the risk of escape. The synNotch architecture adds conditional control: recognition of one antigen activates a transcriptional program that induces a second CAR specific for another antigen, functioning as an "IF/THEN" circuit. This approach localizes cytotoxicity to antigen-rich regions, mitigating systemic toxicity. Nevertheless, preclinical data reveal leakage of transcriptional activity and incomplete silencing in healthy tissues, suggesting the need for improved temporal precision and humanized synNotch scaffolds to prevent immunogenicity. The co-LOCKR system further extends this concept by combining AND/OR/NOT logic within modular protein switches, though translational feasibility remains limited by the non-human protein components and complex pharmacokinetics[32,38]. This logic-gated approach offers conditional activation that minimizes off-tumor toxicity and enhances tumor specificity. However, despite these advances, most existing platforms remain static in their antigen recognition capacity. Tumor antigen profiles evolve dynamically under immune pressure, yet current CAR configurations cannot adapt to those shifts. This gap underscores the need for adaptive receptor systems capable of reprogramming in situ without full re-engineering or reinfusion.

Future directions include developing modular CAR libraries that allow real-time exchange or tuning of scFv domains through switchable docking modules or universal adaptor scaffolds. Adjusting scFv affinity can selectively spare normal tissues expressing low antigen levels, while extended hinge domains and modified CD3ε motifs dampen cytokine release without compromising cytotoxicity. Such "mechanical tuning" of CAR conformation represents an underappreciated but powerful layer of control, one that, when integrated with transcriptional and pharmacologic systems, may achieve multi-tier safety without sacrificing potency. These could enable clinicians to "retarget" existing CAR-T populations as new resistance patterns emerge. Integration of AI-guided epitope prediction could further refine antigen selection by identifying evolutionarily stable, lineage-restricted neoantigens less prone to immune escape. Moreover, CRISPR-based screening platforms can be employed to perform high-throughput testing of antigen-pair combinations to determine optimal co-targeting strategies for individual tumor types[39]. Collectively, these approaches aim to transform CAR-T therapy from a static therapeutic into a dynamic, evolving immunologic system capable of maintaining surveillance against tumor plasticity.

TCR Engineering to Broaden Recognition

While CAR-T cells are confined to surface-expressed antigens, TCR - engineered T cells extend immune

recognition into the intracellular proteome by detecting peptide fragments presented via MHC molecules[7]. This property allows access to previously "undruggable" intracellular oncoproteins, thereby broadening the therapeutic landscape well beyond surface antigen availability. However, despite this conceptual advantage, TCR-based therapies continue to face significant resistance barriers, including MHC downregulation, defective antigen processing, and cross-reactive alloreactivity, all of which can reduce specificity or lead to severe off-target toxicity[40]. To address these limitations, several engineering innovations have been explored. Affinity-tuned TCRs aim to strengthen recognition of tumor-specific peptides while preserving tolerance to self-antigens, minimizing autoimmune risk[41]. Meanwhile, TCR-mimic CARs (TCRm-CARs) have emerged as hybrid constructs that merge the MHC-dependent specificity of TCRs with the robust signaling of CARs, allowing antibody-derived scFv domains to recognize peptide–MHC complexes directly[42]. These hybrids may represent a practical bridge between traditional TCR and CAR modalities, potentially achieving intracellular antigen targeting with CAR-like kinetics and amplification. In parallel, data-driven and AI-assisted receptor discovery pipelines are rapidly advancing. Deep learning–trained TCR libraries can predict antigen–HLA binding affinities and potential cross-reactivity profiles, accelerating the discovery of safe, high-avidity receptors capable of maintaining recognition across mutating tumor variants.

Yet, despite this progress, key translational challenges persist. Tumor-induced MHC downregulation and disruptions in antigen processing continue to limit the presentation of intracellular peptides, while the high diversity of HLA alleles among human populations hinders universal application[43]. Therefore, the future of TCR engineering may depend on integrating synthetic biology principles to reprogram signaling modules for partial or complete MHC independence. This shift would blur the functional boundary between CAR and TCR systems, giving rise to hybrid or context-dependent receptors capable of sustaining recognition even in antigenically unstable tumors. Ultimately, the integration of patient-specific immunopeptidome profiling with AI-driven antigen selection may lead to personalized, broad-spectrum T cells that retain adaptability and precision in real time. Together, these emerging strategies signal a paradigm transition, from rigid, single-target constructs to adaptive, evolution-aware immune platforms capable of co-evolving with the tumor ecosystem.

Engineering for Tumor Microenvironment (TME) Resistance

TME forms a major axis of resistance against adoptive T-cell therapies, imposing physical, biochemical, and metabolic constraints that blunt immune activation[44]. Within this hostile ecosystem, suppressive cytokines, nutrient deprivation, hypoxia, and inhibitory checkpoints converge to induce T-cell dysfunction and exhaustion[26]. Overcoming these layered inhibitory signals requires not only persistence against suppression but also the capacity to actively remodel and reprogram the TME. Recent bioengineering advances have led to the development of T cells that secrete immunostimulatory factors, rewire their metabolic machinery, or operate through synthetic circuits capable of sensing and adapting to local environmental cues.

Armored CAR/TCR Cells

CAR-T failure in solid tumors is largely due to metabolic, immunosuppressive, and physical barriers within the TME[45]. Armored CARs, engineered to secrete cytokines or express resistance modules, represent one of the most intensively studied yet clinically underperforming solutions. Constructs secreting IL-12 or IL-18 enhance macrophage activation and antigen spreading but frequently trigger systemic inflammation, reflecting the narrow therapeutic window of constitutive cytokine expression. Conditional systems, such as NFAT-inducible cytokine expression, reduce baseline toxicity but remain susceptible to "leakiness" and unpredictable kinetics.

Metabolic reprogramming through overexpression of arginine-synthetic enzymes (ASS1, OTC) or resistance to inhibitory cytokines (dominant-negative TGFβRII, A2A receptor knockdown) has shown enhanced persistence in murine models, but human data remain sparse, and the pleiotropic effects of TGFβ or adenosine signaling complicate their safety assessment[45]. Overall, while armored CARs demonstrate mechanistic rationale, their clinical translation demands more precise spatial and temporal regulation, a role potentially filled by logic-gated or sensor-integrated constructs.

One of the most direct approaches to counteract the immunosuppressive milieu involves engineering "armored"

CAR and TCR T cells capable of secreting cytokines or checkpoint-blocking agents directly within the tumor. Cells engineered to release IL-12, IL-15, IL-18, or PD-L1-blocking single-chain antibodies (scFvs) locally can enhance cytotoxicity, recruit endogenous immune effectors, and reshape the TME toward a proinflammatory phenotype[34]. IL-12–secreting CAR-T cells, for instance, have demonstrated enhanced persistence and resistance to Tregs, while IL-15 promotes memory-like phenotypes and sustains metabolic fitness. Local secretion of checkpoint inhibitors also allows autonomous blockade of PD-1/PD-L1 signaling, avoiding the need for systemic antibody administration[34].

However, a persistent gap remains in the spatiotemporal regulation of these immune mediators. Constitutive cytokine expression can trigger systemic inflammation and off-target toxicity, limiting translational feasibility[8]. Future engineering directions therefore emphasize inducible cytokine expression systems that respond to drug cues or microenvironmental conditions such as hypoxia or high PD-L1 expression. For example, drug-responsive promoters can allow transient activation under physician control, while synthetic gene circuits incorporating AND/NOT logic gates could restrict IL-12 or IL-18 release exclusively to regions exhibiting both immunosuppressive and hypoxic signals. These programmable systems aim to provide localized immunostimulation with minimal systemic burden, transforming T cells into precision-controlled immunologic actuators.

Metabolic and Epigenetic Reprogramming

T cells entering solid tumors encounter severe metabolic stress characterized by glucose and amino acid depletion, high lactate levels, and oxygen scarcity, all of which impair effector function and survival[9]. Engineering metabolically resilient T cells has thus become a critical focus in overcoming TME-induced dysfunction. Overexpression of peroxisome proliferator–activated receptor gamma coactivator 1-alpha (PGC1α) enhances mitochondrial biogenesis and oxidative phosphorylation, improving persistence under nutrient limitation[10]. Similarly, HIF-2α overexpression promotes adaptation to hypoxia and sustains cytotoxic activity in oxygen-deprived niches[11]. Beyond metabolic rewiring, epigenetic engineering offers a means to prevent terminal exhaustion by resetting dysfunctional chromatin landscapes. Targeted editing using dCas9-fused epigenetic modifiers, such as dCas9-TET1 (for demethylation) or dCas9-KRAB (for gene repression), can modulate exhaustion-associated loci and restore transcriptional flexibility.

Yet despite these promising directions, a major knowledge gap persists: there are no robust predictive models linking metabolic or epigenetic modifications to long-term antitumor outcomes. The complexity of metabolic, epigenetic crosstalk and interpatient variability complicate rational design. Moving forward, integration of computational metabolic modeling and multi-omics longitudinal profiling is essential to forecast how engineered pathways influence persistence, proliferation, and exhaustion trajectories in vivo. Such insights could guide the development of rationally tuned metabolic circuits that adapt dynamically to TME stressors while maintaining controlled activation states.

TME-Responsive Sensors

An emerging frontier in T-cell engineering involves embedding synthetic biosensors capable of detecting and responding to tumor-specific molecular cues, thereby allowing conditional activation or adaptive behavior[12,13]. These biosensors can monitor environmental signals such as reactive oxygen species (ROS), lactate, hypoxia, or immunosuppressive cytokines like TGF-β, and trigger predefined transcriptional responses such as cytokine release, costimulatory activation, or self-regulation[13]. For example, hypoxia-sensitive CARs activate signaling only in low-oxygen environments, minimizing off-tumor cytotoxicity in healthy tissues.

Real-time sensing CAR-T platforms integrated with microelectronic feedback systems or reporter circuits are needed to enable continuous monitoring and adaptive dosing. Such feedback-controlled designs could modulate therapeutic intensity in response to changing TME conditions, functioning as closed-loop immunotherapy systems. Furthermore, microfluidic tumor-on-a-chip technologies can be employed as preclinical testing platforms to simulate tumor architecture, gradient dynamics, and cellular interactions, allowing the refinement of conditional circuits before advancing to animal or human studies[46]. These technologies represent a critical translational bridge between synthetic immunology and clinical oncology, enabling safe, programmable, and context-aware immune therapeutics.

Overcoming Trafficking and Persistence Barriers

Effective tumor eradication requires engineered T cells not only to recognize and attack malignant cells but also to efficiently traffic to, infiltrate, and persist within the tumor microenvironment. Many adoptive cell therapies fail due to insufficient homing, limited tissue penetration, or premature exhaustion following infiltration[27]. Solid tumors often exclude immune cells through disorganized vasculature, dense extracellular matrices, and mismatched chemokine signaling[20,21,26]. In addition, chronic antigen exposure, metabolic deprivation, and inhibitory checkpoint signaling compromise the longevity of infused cells. Therefore, recent engineering strategies have focused on optimizing both spatial navigation and long-term persistence within the hostile TME.

Chemokine receptor engineering represents one of the most direct approaches to improve tumor-directed migration. Many solid tumors secrete chemokines such as CXCL1, CCL5, and CX3CL1, which are not efficiently recognized by unmodified T-cells[14]. To exploit these chemotactic gradients, CAR and TCR T cells can be engineered to express corresponding receptors such as CXCR2, CCR5, or CX3CR1, aligning their migratory profiles with the tumor's chemokine landscape[14,47]. For example, CXCR2 expression enhances trafficking toward melanoma and ovarian carcinoma, while CCR5 facilitates migration to CCL5-rich environments characteristic of certain breast and pancreatic cancers[48,49]. This receptor reprogramming allows effector cells to localize more effectively within tumor cores where immunosuppression is most profound.

Once T cells arrive at the tumor site, the extracellular matrix (ECM) imposes a major physical barrier to effective infiltration. The ECM, enriched in collagen, proteoglycans, and fibronectin, restricts T-cell motility and limits cytotoxic interactions with tumor cells[50]. To overcome this obstacle, engineered lymphocytes have been equipped with degradative enzymes such as heparanase or matrix metalloproteinases (MMPs) to facilitate ECM remodeling and deeper penetration. For instance, heparanase-expressing CAR T-cells demonstrate improved tumor infiltration and clearance in preclinical models[50]. However, maintaining a delicate balance between matrix degradation and stromal integrity remains crucial, as excessive proteolysis could damage healthy tissues or promote metastasis.

Beyond physical infiltration, sustained T-cell persistence is essential for durable tumor control. Genetic modulation of exhaustion pathways and metabolic reprogramming can reinforce cell survival and effector function. Incorporation of cytokine support systems, such as IL-15 or IL-7 transgenes, enhances memory differentiation and long-term activity, while silencing inhibitory receptors like PD-1 or transcription factors such as TOX mitigates exhaustion. Additionally, metabolic enhancement strategies, such as overexpressing PGC1α to boost mitochondrial biogenesis, improve energy utilization and resilience within nutrient-depleted tumor niches. Together, these approaches promote functional persistence and prevent premature T-cell attrition in the TME.

Despite these promising advances, a key limitation persists: existing preclinical models often fail to replicate the spatial and molecular heterogeneity of human tumors. Conventional two-dimensional cultures oversimplify stromal architecture and do not capture the dynamic gradients that govern chemokine signaling and matrix density[51]. Moreover, murine models differ from human tumors in vascular organization, stromal stiffness, and chemokine repertoire, limiting translational predictability. Future directions point toward integrating three-dimensional bioprinted tumor constructs that incorporate stromal, vascular, and immune compartments, allowing for high-fidelity evaluation of T-cell infiltration and retention under physiologically relevant conditions. Complementarily, computational agent-based and multiscale models can simulate CAR/TCR cell migration, antigen encounters, and cytotoxic dynamics within patient-specific tumor geometries. These digital reconstructions, or "immune digital twins", could guide personalized receptor designs and dosing regimens that maximize infiltration while minimizing off-target effects[52]. By integrating experimental and computational modeling, next-generation adoptive T-cell therapies can be rationally engineered not only for antigen recognition and signal potency but also for efficient spatial navigation and durable persistence within the complex architecture of solid tumors.

Engineering for Controlled Activation and Safety

Achieving a precise balance between potent antitumor

efficacy and controlled immune activation remains one of the most formidable challenges in adoptive T-cell therapy. Conventional CAR and TCR architectures are largely governed by binary activation logic; once antigen recognition occurs, the signaling cascade proceeds irreversibly[35]. While this design has driven remarkable success in hematologic malignancies, it presents considerable safety risks in solid tumors, where heterogeneous antigen expression increases the likelihood of off-tumor reactivity and CRS. The clinical burden of immune-related adverse events, particularly CRS and ICANS, underscores the urgent need for tunable and reversible activation mechanisms[41].

To address these limitations, several molecular safety mechanisms have been developed to allow precise pharmacological control of T-cell activity. The inducible caspase-9 (iCasp9) suicide switch remains the most clinically validated, providing a rapid, small-molecule-triggered apoptotic shutdown of infused cells during severe toxicity[53]. Other strategies, including Tet-inducible CAR systems and protease-cleavable constructs, enable temporal modulation or selective deactivation of CAR function. However, the persistence of resistant subpopulations, variability in transgene expression, and increased vector complexity highlight the need for simplified yet robust safety architectures[53].

Despite these innovations, preclinical safety evaluation remains constrained by the poor predictive power of murine and xenograft models, which fail to capture the complexity of human immune and vascular responses. Consequently, cytokine storms and neurotoxic events often emerge only during clinical trials, after preclinical safety benchmarks were met. Future progress depends on humanized preclinical platforms capable of more accurately reflecting systemic immune dynamics. Immune–organ-on-chip systems, integrating human endothelial, neural, and immune cell components, offer a promising solution by reproducing organ-specific phenomena such as blood–brain barrier permeability and pulmonary inflammation[46]. These models could substantially improve prediction and mitigation of ICANS and CRS before clinical application. At the molecular design level, logic-gated inhibitory CARs (iCARs) represent a paradigm shift toward conditional activation. By applying Boolean logic principles, such as “AND,” “OR,” and “NOT” gates, engineered receptors can selectively activate or suppress cytotoxicity depending on multi-antigen recognition patterns[36]. For instance, a CAR may trigger full activation only in the presence of a tumor-specific antigen while being inhibited by the recognition of a self-antigen, creating a “therapeutic safe zone” that prevents off-target damage[36].

Beyond biochemical circuits, optogenetic CAR platforms enable precise spatiotemporal control of immune activation using light-sensitive signaling domains[54,55]. These systems can reversibly switch CAR activity on or off through external light stimuli, providing clinicians with the capacity to fine-tune immune engagement within defined anatomical regions. Although currently preclinical, optogenetic control holds the potential to inaugurate a new era of on-demand immunotherapy, allowing real-time modulation of immune responses within delicate or inaccessible tissues.

In summary, next-generation CAR and TCR engineering must evolve from static to adaptive and predictive frameworks that integrate systems biology, bioengineering, and computational modeling. Combining mechanistic modeling, organ-on-chip validation, and dynamic control circuitry will enable not only safer but also smarter immunotherapies, capable of responding autonomously to tumor microenvironmental cues and patient-specific variables.

Modeling, Manufacturing, and AI Integration

As CAR and TCR T-cell therapies advance from experimental success to clinical reality, the next transformative leap will arise from the convergence of experimental modeling, computational prediction, and scalable manufacturing. This integration marks the beginning of a new engineering paradigm in cellular immunotherapy, one that relies not only on molecular innovation but also on data-driven design, automation, and precision modeling of human immune dynamics.

While substantial progress has been achieved in receptor optimization, safety control, and enhancement of functional persistence, these advances remain constrained by a fragmented research ecosystem. Preclinical models, computational frameworks, and manufacturing processes continue to evolve independently, limiting reproducibility and translational predictability[17]. The next frontier demands the unification

of these once-isolated domains into a cohesive, closed-loop system that enables predictive, reproducible, and patient-specific cellular therapy design.

Advanced Experimental Models

Traditional murine and xenograft systems, though indispensable for initial validation, inadequately replicate the complexity of the human tumor–immune interface[56,57]. Recent innovations seek to overcome these limitations through models that more faithfully emulate human immunobiology. Humanized mouse platforms reconstituted with complete hematopoietic and myeloid lineages now enable more accurate evaluation of T-cell persistence, exhaustion trajectories, and toxicity under a human-like immune context. Complementary to these in vivo systems, perfused microfluidic tumor-on-chip technologies provide dynamic representations of tumor physiology, incorporating vascular flow, oxygen and nutrient gradients, stromal barriers, and cytokine-driven immunosuppression[46]. These microphysiological systems allow real-time imaging of T-cell infiltration, killing efficiency, and immune evasion within controlled, physiologically relevant environments.

In parallel, long-term co-culture systems combining engineered T cells with tumor organoids and stromal components are emerging as valuable tools for investigating chronic activation, exhaustion kinetics, and the formation of durable memory subsets[58]. Nevertheless, no existing platform effectively captures the temporal coevolution between tumors and immune effectors. Processes such as tumor immunoediting, antigen escape, and metabolic adaptation remain poorly represented in static or short-term models[59]. Future efforts should therefore aim to develop integrative, longitudinal frameworks that combine humanized in vivo systems with in vitro microfluidic co-cultures. In effect, these models would evolve from validation tools into predictive simulators of immune adaptation, allowing experimental data to guide next-generation receptor designs with improved translational reliability.

Computational and Systems-Biology Modeling

The synthesis of computational modeling with experimental biology is redefining the conceptual foundation of adoptive cell therapy. Multi-scale systems models now link receptor-level signaling events to cellular decision-making and, ultimately, to tumor-ecosystem behavior, offering a quantitative bridge between molecular design and clinical outcome[60]. These mechanistic frameworks allow researchers to simulate how variations in co-stimulatory domains, ligand affinity, or signaling thresholds shape cytotoxicity, persistence, and exhaustion across heterogeneous tumor architectures.

Artificial intelligence (AI) adds a powerful predictive dimension by enabling rapid in-silico design of receptor structures using large sequence–function datasets[61]. Deep learning models trained on experimental libraries can forecast CAR/TCR configurations that optimize affinity, specificity, and safety, compressing years of experimental iteration into computational hours. Parallel developments in digital twins, which are virtual patient avatars constructed from integrated clinical, genomic, and immunologic data, provide a means to simulate personalized therapy outcomes[62]. By modeling how an individual's tumor microenvironment and immune repertoire interact with a proposed CAR or TCR design, digital twins could predict both efficacy and toxicity before clinical infusion.

Yet, a gap is the lack of standardized, large-scale datasets that correlate in vitro and in silico predictions with in vivo outcomes. Furthermore, most modeling efforts exist in isolation, either mechanistic or AI-based, without feedback integration. The next stage requires hybrid modeling frameworks that unite mechanistic pathway simulations with machine-learning prediction engines, allowing adaptive refinement as new experimental data emerge. Standardized ontologies, interoperable databases, and cross-platform data-sharing agreements will be essential to close the current divide between computational predictions and biological validation. Ultimately, this fusion will enable a self-improving system in which experimental feedback continuously enhances model accuracy, advancing predictive immunotherapy design from theoretical potential to clinical reliability.

Manufacturing and Scalability

Even the most advanced receptor designs depend on efficient, reliable, and economically viable manufacturing systems to achieve clinical translation. The vein-to-vein manufacturing time for autologous CAR-T products can extend up to 30 days, often exceeding the clinical stability window for patients with rapidly advancing disease[63].

Conventional production pipelines, dominated by viral transduction, manual handling, and batch variability, remain significant bottlenecks. To overcome these limitations, automated closed-loop biomanufacturing platforms are being developed that integrate real-time monitoring of cell viability, phenotype, and metabolic state under good manufacturing practice (GMP) conditions[64]. Such automation reduces human error, enhances reproducibility, and ensures consistency across production batches[64].

Simultaneously, non-viral gene-delivery technologies, including mRNA electroporation, Sleeping Beauty, and PiggyBac transposon systems, are transforming genetic engineering by minimizing insertional mutagenesis risk, shortening production time, and reducing cost[65,66]. These technologies allow both transient and stable transgene expression without the biosafety constraints of viral vectors. In addition, CRISPR-mediated editing of MHC molecules is enabling the creation of universal "off-the-shelf" CAR and TCR platforms, bypassing donor-specific limitations and supporting scalable allogeneic therapy production[67].

Despite these promising advances, manufacturing pipelines still lack predictive tools capable of assessing product potency, persistence, and exhaustion risk before infusion. Current quality-control assays rely on static surface or cytokine markers that often fail to forecast in vivo performance. Future development should focus on integrating multi-omics analytics and machine-learning algorithms into real-time process monitoring. By correlating metabolic flux, transcriptomic signatures, and bioreactor parameters, AI-driven manufacturing frameworks could autonomously adjust culture conditions to sustain optimal T-cell functionality. Such adaptive manufacturing would not only standardize product potency but also enable sustainable, cost-efficient production across global therapeutic networks.

To synthesize these complex developments, Table 3 summarizes major domains of ongoing innovation in CAR and TCR engineering, highlighting their current limitations and outlining future directions that could enhance translational efficacy.

Domain	Innovation	Key Gap	Future Direction
Experimental Models	Humanized mice, microfluidic tumor chips, organoid co-cultures	Lack of dynamic tumor–immune coevolution	Integrate longitudinal multi-omics and metabolic profiling
Computational Modeling	Multi-scale and AI-driven prediction systems	Weak correlation with in vivo outcomes; poor data standardization	Hybrid mechanistic + AI frameworks with interoperable datasets
Manufacturing	Automated closed-loop platforms, non-viral systems, universal chassis	No predictive potency/ exhaustion analytics	AI-guided bioprocess monitoring and adaptive production control

***Table 3**: Summary of innovation domains, key gaps, and future directions in CAR and TCR cell engineering*

Future Perspectives

Despite the remarkable advances achieved in receptor design, signaling optimization, and manufacturing, current engineering strategies in adoptive cell therapy remain largely static and compartmentalized, addressing isolated challenges such as antigen escape or cytokine regulation without integrating their interdependent biological consequences[9,22]. Tumors, in contrast, are dynamic ecosystems that constantly evolve through antigenic drift, metabolic reprogramming, and immunosuppressive remodeling[68]. Bridging this asymmetry requires a paradigm shift: engineered T-cells transition from fixed constructs into adaptive, self-optimizing systems capable of sensing, interpreting, and responding to tumor evolution in real time.

A closed-loop engineering framework embodies this next stage. In such a system, high-dimensional clinical, genomic, and immunologic data continuously inform computational models that predict optimal receptor configurations and activation circuits. These designs are iteratively tested in advanced preclinical models, humanized mice, tumor-on-chip systems, and organoid co-cultures, and the resulting biological feedback refines future receptor generations. Over successive design cycles, this feedback loop could produce T cells with increasing specificity, persistence, metabolic resilience, and safety, ultimately achieving the conceptual goal of a self-learning cellular therapeutic. Technological convergence will be the key enabler of this transformation. Synthetic biology contributes modular logic circuits and inducible effector programs, multi-omics profiling reveals tumor vulnerabilities and immune adaptation pathways, and artificial intelligence accelerates receptor optimization and predictive modeling of therapy outcomes[61,69]. Together, these domains can turn T cells into context-aware biological devices, capable of conditional activation, controlled cytokine release, and autonomous modulation of their own exhaustion or metabolic states.

At the same time, next-generation receptor engineering must expand beyond single-target approaches. Platforms incorporating dual or tandem CARs, logic-gated synNotch systems, and mechanically tuned hinge domains should enable discrimination between malignant and healthy tissues while mitigating off-tumor toxicity. Integration of metabolic reprogramming modules, such as enhanced mitochondrial fitness or resistance to adenosine and TGF-β signaling, could further support T-cell survival within hostile tumor microenvironments[31,32,33,70]. These multilayered strategies represent a shift from designing "stronger" CARs toward constructing intelligent, adaptable immune agents that adjust activation thresholds according to antigen density, microenvironmental cues, and immune feedback. Yet, the path to translation remains constrained by scalability, regulatory complexity, and unpredictable in vivo behavior of multi-circuit systems. To ensure reproducibility and patient accessibility, manufacturing must evolve in parallel. Automated, closed-loop bioreactors, non-viral gene-delivery platforms, and CRISPR-based universal chassis can collectively shorten production time, reduce cost, and standardize product potency. Embedding real-time analytics, combining

Dimension	Short-Term Outlook	Long-Term Outlook
Experimental Models	Expansion of humanized mice, tumor-on-chip, and organoid co-cultures to validate in-silico predictions	Integration of longitudinal, multi-omics, and metabolic profiling to capture tumor–immune coevolution
Computational and AI Modeling	Early use of AI for receptor optimization; limited by small, isolated datasets	Hybrid mechanistic + AI frameworks with feedback loops and digital twin simulations enabling patient-specific therapy design
Manufacturing and Scalability	Adoption of automated closed-loop bioreactors, non-viral gene-transfer, and early real-time QC tools	Fully autonomous, AI-driven manufacturing with predictive potency and exhaustion analytics, enabling global scalability
Translational Integration	Improved reproducibility and standardized GMP pipelines.	Self-learning, adaptive systems combining computational modeling, manufacturing, and clinical feedback.

***Table 4**: A summary of the short- and long-term outlook for CAR/TCR T-cell engineering and translation*

transcriptomic, metabolic, and phenotypic data, will allow AI-guided quality control capable of predicting persistence and exhaustion risk before infusion. Ultimately, the field is moving from unidimensional receptor enhancement toward multi-layered, programmable immunotherapy. Overcoming resistance will not depend solely on inventing additional receptor formats but on rationally integrating existing modules, pairing antigen multiplexing with metabolic resistance, safety switches, and controlled apoptosis, to achieve reproducible, safe, and durable responses within the heterogeneous landscape of solid tumors.

Table 4 summarizes the short- and long-term outlook for CAR and TCR T-cell engineering, highlighting the evolving priorities across experimental modeling, computational design, manufacturing, and translational integration.

CONCLUSION

In conclusion, the future of CAR- and TCR-based therapies lies in intelligence, adaptability, and integration. By embracing closed-loop, data-driven engineering, adoptive cell therapy can progress from static molecular design to living, responsive, and self-optimizing immunologic systems, capable of anticipating tumor evolution, sustaining durable remission, and redefining precision oncology.

Ethical Considerations

This manuscript is a literature-based review and does not involve any new research with human participants or animals.

Conflict of Interest

The authors declare that there are no conflicts of interest relevant to the content of this manuscript.

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