International Journal of Multidisciplinary Research in Biotechnology, Pharmacy, Dental and Medical Sciences (IJMRBPDMS)

Conquering the Solid Frontier: Next-Generation CAR-T Cell Immunotherapy Beyond Hematologic Malignancies - Transformative Strategies, Emerging Targets, and Future Paradigms

Mohammad Anees Rather[1], Tawqeer Shafi[1], Pallavi Chandel[1]*, Sumairah Qadir[1].

[1]School of Pharmacy, Deshbhagat University, off to NH-44, Amloh Road, Mandi Gobindgarh District Fatehgarh Sahib, Punjab- 14, Mandi Gobindgarh, Punjab 147301, India.

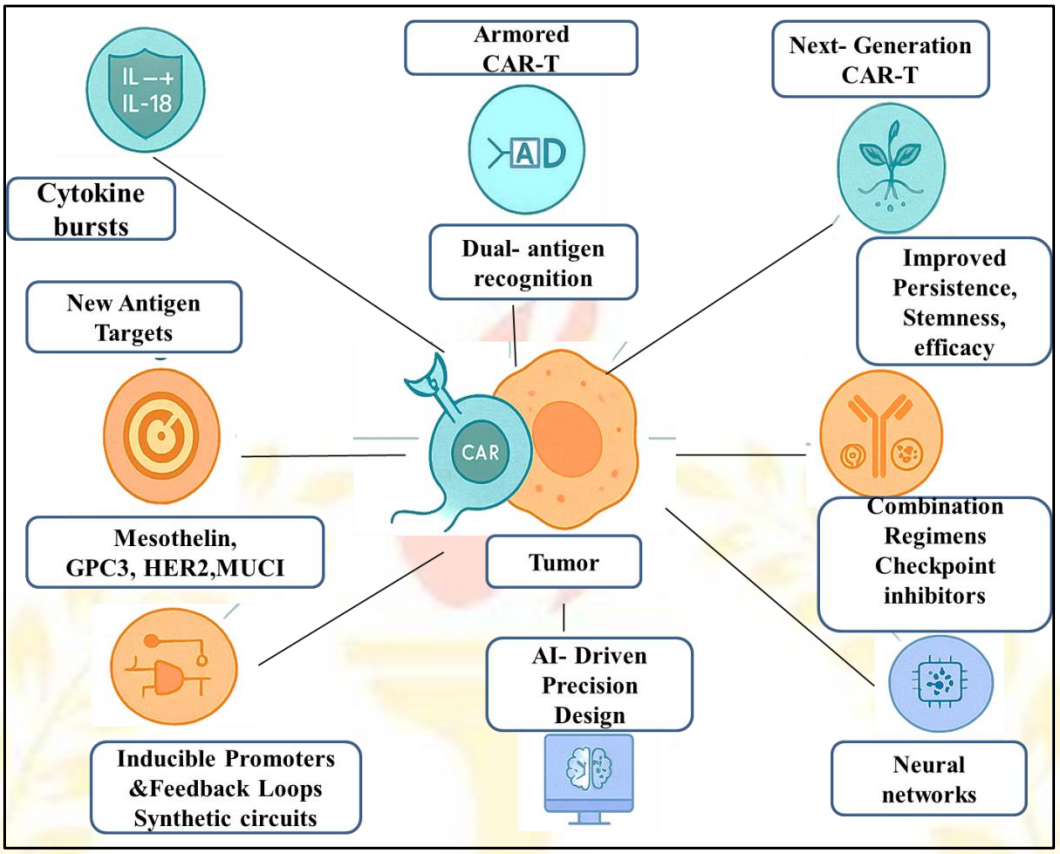
ABSTRACT

Chimeric antigen receptor T-cell therapy has revolutionized the management of blood cancers yet its application in solid tumors has been restricted because of the complicated tumor microenvironment, differences in tumor antigens and effective immune-evasion strategies. Recent developments are concerned with advanced engineering practices in order to overcome these problems. Cytokine releasing (interleukin-12 or interleukin-18) armored chimeric antigen receptor T cells activate immune responses in suppressive tumor microclimates. Designs of logic-gated chimeric antigen receptor which need two antigen recognition improve tumor specificity and minimize harm to normal tissues. Metabolic and epigenetic reprogramming strategies are being created to enhance the survival of chimeric antigen receptor T-cells, retain stem-like features, and be able to function in low-oxygen and nutrient-deprived environments. Mesothelin, glypican-3, human epidermal growth factor receptor-2, mucin-1, and B7-H3 are some of the new tumor-associated targets that are broadening therapeutic opportunities in various solid tumors. The tools of synthetic biology, such as inducible promoters and self-regulating feedback controls, can enable the accurate regulation of the activation of chimeric antigen receptor T- cells and enhance safety. Also, artificial intelligence, personalized antigen profiling, and optimization based on biomarkers are integrated to develop highly customized chimeric antigen receptor T-cell products. The combination of these innovations is a significant change to more potent, multi-purpose, and smartly designed T-cell therapies that can destroy the obstacles of solid tumor.

Keywords: ***Checkpoint inhibitors, Combination therapy, Biomarker development, Global healthcare impact, CAR-T cell therapy, Synthetic biology.***

***Corresponding Author:**
Miss. Pallavi Chandel
School of Pharmacy, Desh Bhagat University, off to NH-44, Amloh Road, Mandi Gobindgarh District Fatehgarh Sahib, Punjab- 14, Mandi Gobindgarh, Punjab 147301, India
Email; pallavichandel1921@gmail.com
ORCID ID: 0009-0000-5819-6016

Graphical Abstract: Advances in CAR-T engineering and combination strategies enhance tumor targeting and therapeutic efficacy



1. Introduction

Chimeric Antigen Receptor T -cell (CAR -T) therapy has swiftly become a ground-breaking offering in modern oncology, mainly because of its revolutionary achievement in the treatment of hematologic malignancies [1]. CARed-T therapy has demonstrated unprecedented remission rates in leukaemia's, lymphomas and multiple myeloma in patients who are exhausted with conventional treatment options, thus, defining a breakthrough of palliative to a potentially curative intervention [2]. This bench to bedside illustration of cellular immunotherapy is an extraordinary potential where T cells of patient origin are genetically engineered to target tumor antigens and trigger strong cytotoxic binomial [3]. The results obtained with hematologic cancers have made CAR-T therapy a clinical and scientific breakthrough, which confirms decades of studies on immune engineering and tumor biology [4]. The incredible progress of CAR-T therapy has not been replicated in the solid tumor setting, however, which represents the vast majority of cancer occurrence and death across the globe [5]. Clinical trials have never shown the limited efficacy in solid malignancies in spite of the encouraging preclinical studies. This impending promise points to one of the main gaps between scientific ambition and clinical reality that is marked by significant differences between biological and therapeutic aspects of hematologic and solid tumors [6]. Although hematologic malignancies may have access to easily available targets in permissive immune spaces, solid tumors are more complicated in nature. These are defined by the heterogeneity of the antigen expression, thick stromal barriers that block infiltration by immune cells, highly immunosuppressive microenvironment, and increased risks of on-target off-tumor toxicity because of overlaps in antigens with normal tissue [7]. These difficulties reveal the insufficiency of measures that worked in liquids and require a paradigm shift in therapeutic design, administration, and insertion in order to counteract the advantages of CAR -T to the solid tumor [8] .

This discussion will provide an in-depth analysis of the countless challenges that threaten the effectiveness of CAR -T cells in treating solid neoplasma, with the accompanying clarification of emerging opportunities and innovative approaches that promises a new direction in the field [9]. Recent studies are focused on strategies to overcome tumour resistance, like dual or multi-antigen targeting, and armour-type CARs with cytokine release to overcome immunosuppression, and new

delivery modalities, including regional infusion and implantable scaffold, which will improve tumour penetrance and survival [10]. Besides, combinations with checkpoint inhibitors, oncolytic viruses and traditional modalities (including chemotherapy and radiotherapy) are under investigation to synergize the overall antitumour effects [11]. The developments not only remodel the architecture of CAR-T therapeutics

but they also expand the conceptual basis of the application of immune-based interventions on complex carcinomas [12]. Clinically, effective translation of CAR-T therapy in solid tumours would be a paradigm shift, and this change would potentially mean life saving therapies to millions of patients who are now faced with a small number of available treatments and a poor prognosis [13]. The project is scientifically driven to advance the a fair impact[14] . Therefore, the area of work and the meaning is not limited to oncology but affects the health-care systems, technological advancement, and patient-care models worldwide. This analysis highlights the need to adopt a progressive paradigm, which is more amenable to innovation, integration, and adaptation, by voicing the situation of the challenges and prospects [15]. The future of CAR-T therapy in solid tumours is therefore based on its ability to become next-generation systems that overcome biological impediments, achieve lasting responses, and revolutionize the oncology clinical arena in the same way that it has achieved success in haematologic malignancies [10].

Figure 1 illustrates the contrasting outcomes of CAR-T therapy in hematologic cancers versus solid tumors. While blood cancers show high response rates due to accessible, well-defined targets, solid tumors present major obstacles including heterogeneous antigens, dense stroma, and an immunosuppressive microenvironment. The schematic highlights how these barriers limit CAR-T infiltration, persistence, and cytotoxicity within solid tumors. Overall, the figure underscores why translating CAR-T success from hematologic malignancies to solid tumors remains a significant challenge.

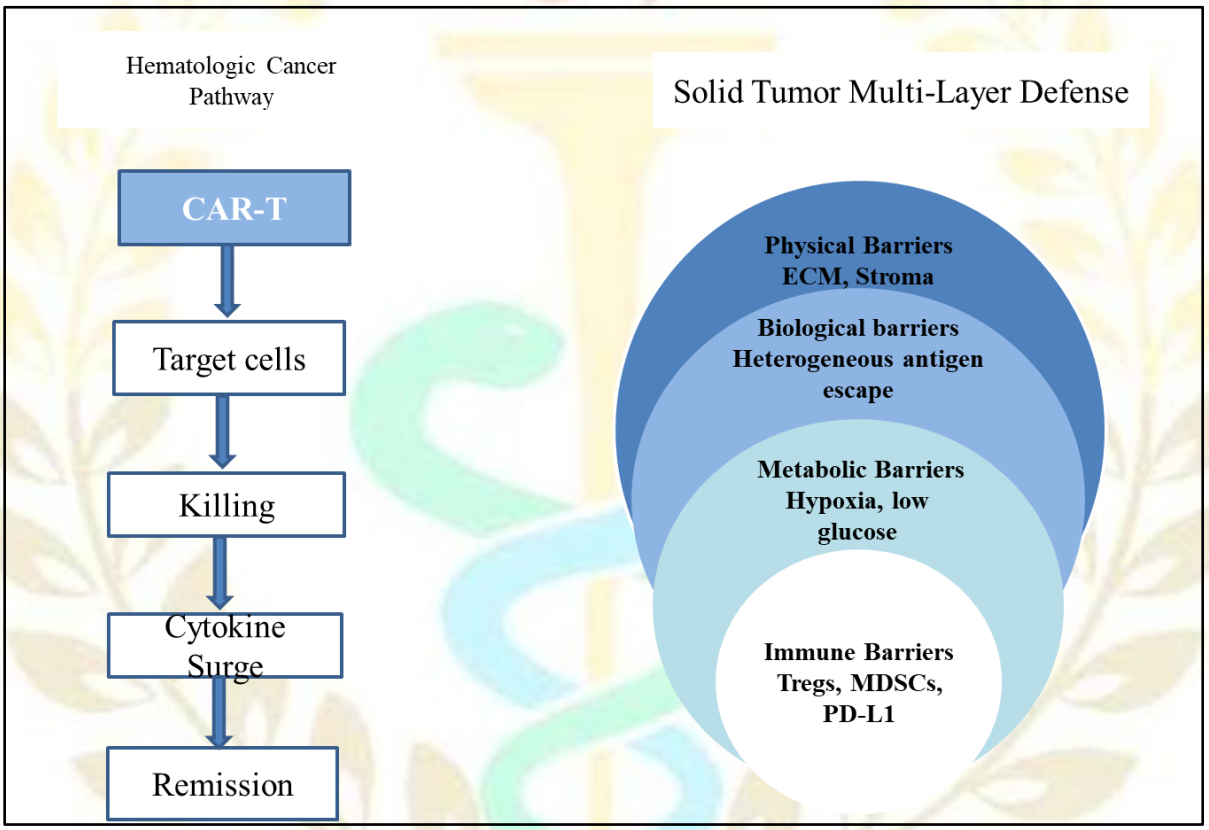


Figure 1: Figure 1: Schematic comparison showing high CAR-T success in hematologic cancers versus limited efficacy in solid tumors. *Comparison of CAR-T therapy outcomes in blood cancers and solid tumors. While CAR-T cells show strong clinical success in hematologic cancers, their efficacy in solid tumors is reduced due to tumor microenvironment challenges and antigen variability.*

2. The Current Landscape: CAR-T Cell Therapy Foundation

2.1 Mechanistic Overview

CAR-T cell therapy is based on the premeditated reprogramming of the innate immunity repertory of a patient with the aim of selective elimination of malignant cells through engineered receptors [16]. The key component of this paradigm is the chimeric antigen receptor (CAR), which is an artificial expression of an extracellular antigen-recognition domain (usually a single-chain variable fragment (scFv) of a monoclonal antibody) fused via a hinge and transmembrane anchor to intracellular signature of signalling modules [17]. The CD3zeta chain that primarily activates the cell is typically present in the intracellular components, and one or more co-stimulatory domains, including CD28 or 41BB, enhances the abilities to proliferate, persist, and become cytotoxic [18]. When expressed on T - cells, the CAR provides tumour-associated antigen

recognition independent of MHC, thus overcoming a major drawback of the traditional T -cell receptor. The interaction of antigen with the engineered cells leads to the proliferation and release of cytolytic effector molecules such as perforin and granzymes and pro-inflammatory cytokines which together are responsible in the targeted tumour lysis mechanism [19]. CAR -T therapy requires an advanced and highly regulated manufacturing process which itself requires leukapheresis to collect peripheral blood mononuclear cells of the patient [20]. The T-cells are then isolated and ex-vivo genetic manipulated the most common being through the use of viral vectors; lentiviruses or retroviruses and expanded under strictly controlled culture conditions to obtain clinically relevant cell numbers. Strict quality-control assays ensure the safety, potency, sterility and transduction efficiency before the reinfusion [21]. This careful production chain demonstrates the potential of the therapy as well as the logistic complexity of CAR -T therapy. The success of this depends on how perfectly the molecular engineering, immunobiology, and bio manufacturing skills are coordinated [22].

2.2 Hematologic Malignancy Success Story

CAR -T cell therapy has radically changed the way hematologic malignancies are treated, which is a watershed event in modern oncology. Its significant clinical response has led to the licensing of a number of FDA products, such as axicabtagene ciloleucel and lisocabtagene maraleucel to treat relapsed or refractory diffuse large B -cell lymphoma (DLBCL), brexucabtagene autoleucel to treat mantle cell lymphoma, and ciltacabtagene autoleucel to treat B -cell maturation antigen (BCMA) in multiple myeloma [23]. CAR-T treatments have patients with pediatric acute lymphoblastic leukemia (ALL) have continued to show superior response and remission rates over conventional chemotherapy or hematopoietic stem cell transplantation, even with patients who are heavily pretreated [24]. The long-term surveillance data have shown long-term remissions especially in ALL and DLBCL and this is a significant indicator of the potential transformation through therapeutic opportunities [25]. This is mostly due to the specific antigenic antigens of CD19, BCMA and CD22 which are highly expressed in malignant B cells and lowly expressed in normal tissues thus reducing off-tumor toxicity [26]. Clinical trials and real world evidence demonstrates both an extended lifespan and improved quality of life, and many patients achieved multi-year remission, instead of the repeated infusions, with the use of Myelosuppressive therapy [27]. In addition to quantifiable clinical metrics, the patient stories highlight the significant recovery and the re-establishment of normality, as an example of the life-changing effect of CAR-T therapy. Put together, these results solidify the CAR -T therapy as a novel standard of hematologic cancer care and an example of individualized cancer immunotherapy [27].

2.3 Generational Evolution

The development of CAR -T cell therapy has undergone a series of generation, each successive generation has been designed to overcome the defects of the previous one and to enhance the therapeutic effectiveness [28].The CARs of the first generation that only included the CD3zet signalling domain, supported antigen recognition but with limited persistence and with limited clinical efficacy. The next development of the second-generation CARs which incorporates the co-stimulatory domains of CD28 or 4-1BB significantly improved T-cell proliferation, survival and cytotoxicity, thus laying the basis of the modern FDA-approved therapies [29]. Third generation CARs expanded on this paradigm by incorporating two co-stimulatory domains; CD28 and 41BB therefore enhancing cytokine production and antitumor effect but their clinical efficacy is currently under study [30]. Fourth-generation CARs are also known as TRUCKs, supporting the release of cytokines (IL-12) that strengthened the operation in immunosuppressive tumour micro environments and enhanced the recruitment of innate immunity elements. Predicted closer in the future, the next-generation CAR-T platforms will use logic-gated circuits to target antigens more accurately, armour the constructs against inhibitory pathways, and use CRISPR-based genome engineering to enable safer and more sophisticated engineering [13]. Besides, the development of universal allogeneic off-the-shelf CAR-T cells, and advances in synthetic biology promises a significant step of scalability, affordability, and accessibility, thus becoming a critical advancement to personalized and widely accessible cancer immunotherapy [31].

Table 1 summarizes FDA-approved CAR-T therapies, outlining their targets, indications, and key clinical outcomes. It highlights consistently high response rates across hematologic malignancies, along with differences in durability, toxicity, and patient eligibility among products. Overall, the table captures the clinical impact and evolution of approved CAR-T treatments in blood cancers.

TABLE 1: FDA-approved CAR-T therapies and their clinical outcomes - recommended placement after Hematologic Malignancy Success Story

S. No.	Therapy (Trade Name)	Target	Approved Indication	Approval Year	ORR / CR (%)	Key Trial	References
1	Kymriah® (Tisagenlecleucel)	CD19	B-cell ALL, DLBCL	2017	83 / 63	ELIANA	[32]
2	Yescarta® (Axicabtagene ciloleucel)	CD19	Large B-cell lymphoma	2017	83 / 58	ZUMA-1	[33]
3	Tecartus® (Brexucabtagene autoleucel)	CD19	Mantle cell lymphoma, ALL	2020	93 / 67	ZUMA-2	[34]
4	Breyanzi® (Lisocabtagene maraleucel)	CD19	Large B-cell lymphoma	2021	73 / 54	TRANSCEND-NHL-001	[35]
5	Abecma® (Idecabtagene vicleucel)	BCMA	Multiple myeloma	2021	73 / 33	KarMMa	[36]
6	Carvykti® (Ciltacabtagene autoleucel)	BCMA	Multiple myeloma	2022	98 / 83	CARTITUDE-1	

3. The Solid Tumor Challenge: Biological and Technical Barriers

3.1 Tumor Heterogeneity and Antigen Landscape

The huge heterogeneity of tumor antigens presents a significant hindrance to the clinical efficacy of chimeric antigen receptor T-cell (CAR-T) therapy of solid tumours, making it difficult to target specifically and effectively to control the disease. Unlike hematological malignancies, which usually do express homogeneous lineage-specific antigens including CD19, solid tumours are characterized by high intra- and intertumoral heterogeneity, with specific tumour regions or individual patients of a given cancer subtype having distinct antigenic phenotypes. This heterogeneity allows subpopulations of malignant cells to escape single-antigen-targeted CAR -T cell leading to partial elimination and recurrence [32]. In addition, most solid tumours contain tumour associated antigens (TAAs) which are also present in normal tissues, which increase the likelihood of ontarget, offtumour toxicity; tumour-specific antigens (TSAs) are very rare. Tumour cells can also down-regulate or lose target antigens under immunologic pressure giving rise to resistant clones, as has been reported in glioblastoma, sarcoma and ovarian carcinoma [33]. The spatial heterogeneity, together with a dense stromal structure and disordered vasculature also contribute to the impairment of CAR-T cell trafficking and antigen access. This dynamic and complex antigenic environment combined restricts the performance of the clinical designs of CAR to date and highlights the need to develop improved designs containing dual or multi-antigen targets, switchable CAR designs, and versatile synthetic biology-based platforms to evade antigen variation and attain lasting therapeutic responses [34].

3.2 Immunosuppressive Tumor Microenvironment

One of the main challenges to the clinical success of CAR-T cell therapy in solid tumours is the highly immunosuppressive tumour microenvironment (TME) which actively suppresses T-cell functionality, growth, and survival [35]. A solid tumour attracts populations of immunosuppressive cells, including regulatory T-cells (Tregs) and myeloid-derived suppressor cells (MDSCs), that suppress the activity of cytotoxic T-cells by direct contact and by secreting inhibitory cytokines such transforming growth factor -b (TGF-b) and interleukin-10 (IL-10). This forms a cycle of immunosuppression that is self-perpetuating and that gradually restrains CAR-T cell growth and cytotoxicity [36]. In addition, the TME also causes metabolic stress secondary to low oxygen, glucose, and amino-acids and high levels of lactic acid produced

by tumour glycolysis leading to hypoxia-impaired T-cell metabolic and functional functions [37]. Abnormal vasculature, fibroblast network and dense stromal matrices are additional structural barriers hindering CAR-T infiltration and mobility, which, consequently, decreases their ability to interact and kill tumour cells. All of these biological, metabolic, and physical limitations lead to inefficient clinical outcomes [38]. To overcome these obstacles, new strategies are under development, such as TGF -b -resistant CAR -T cells, cytokine armoring, stromal targeting, and combination therapy with a checkpoint inhibitor, metabolic modulators, or oncolytic viruses with the objective of increasing infiltration, persistence, and overall antitumour effect [39].

3.3 Trafficking and Infiltration Obstacles

One of the most significant limitations to the therapeutic efficacy of CAR -T treatment in solid tumours is the poor delivery and penetration of engineered lymphocytes into the malignant microenvironment. Compromised, highly abnormal, and disorganised vasculature is a characteristic of solid neoplasms with consequent poor perfusion and hence restricted access of CAR-T cells to the entire tumour mass [40]. The migration of CAR -T cells occurs only if the tumour vasculature is infiltrated, and chemokine gradients and homing cues are misregulated in solid tumours. Numerous tumours do not have chemokines compatible with receptors found on CAR-T cells, or release inhibitory mediators that confuse the lymphocytes to the non-malignant areas [41]. On their arrival into the tumour, CAR-T cells are faced with a dense extra cellular matrix (ECM) consisting of collagen, fibronectin, and hyaluronan; the extra cellular matrix physically blocks cellular motility and limits the interaction with the malignant cells. The inflexibility of the ECM also excludes cytokines and growth factors, further postponing CAR-T activation and growth. In order to overcome these hindrances, new approaches are being explored [42]. These are regional (localised) CAR-T delivery, genetic engineering of cells to express ECM-degrading enzymes, chemical modification of chemokine receptors to promote tumour homing and combination with agents that normalize the vasculature or decrease stromal density [43]. All these strategies are designed to boost CAR -T infiltration, localisation, and cytotoxicity, which is a major issue that separates solid and haematologic malignancies (TABLE 2) [44].

TABLE 2: Comparison of tumor microenvironment characteristics between hematologic and solid malignancies- recommended placement after Immunosuppressive Tumor Microenvironment.

Feature	Hematologic Malignancies	Solid Tumors	Comparative Insight / Implication	References
Tumor Architecture	Dispersed, non-solid; malignant cells circulate in blood or bone marrow; lack of defined stromal framework	Dense, organized mass with distinct stromal and vascular compartments	Solid tumors exhibit structural barriers that limit immune infiltration and drug delivery, unlike hematologic malignancies.	[45]
Stromal Composition	Minimal stromal component; mainly hematopoietic and immune cell interactions	Rich stromal network of fibroblasts, endothelial cells, and extracellular matrix (ECM)	ECM and fibroblast density in solid tumors create physical and biochemical barriers to CAR-T cell activity.	[46]

Vascularization	High vascular access through bone marrow and peripheral circulation	Abnormal and heterogeneous vasculature; poor perfusion and hypoxia	Hypoxia in solid tumors contributes to metabolic stress and immunosuppression, limiting immune efficacy.	[47]
Immune Cell Infiltration	Naturally high due to immune-rich microenvironment of bone marrow and lymphoid tissues	Often low or spatially restricted due to exclusion by stromal and endothelial barriers	Immune exclusion is a major obstacle for adoptive cell therapies in solid tumors.	[48]
Antigen Accessibility	High surface antigen exposure in fluid compartments	Antigen expression is heterogeneous and sometimes intracellular	Limited accessibility and antigen heterogeneity reduce recognition and killing efficiency in solid tumors.	[49]
Cytokine and Chemokine Profile	Dominated by hematopoietic cytokines (e.g., IL-6, GM-CSF) that can promote CAR-T expansion	Enriched in immunosuppressive cytokines (e.g., TGF-β, IL-10, VEGF)	Solid tumors sustain an immunosuppressive milieu that inhibits cytotoxic cell activity.	[41]
Presence of Immunosuppressive Cells	Moderate levels of Tregs and MDSCs; often dynamically regulated by treatment	High infiltration of Tregs, MDSCs, and TAMs maintaining chronic suppression	Immunosuppressive cell density is significantly higher in solid tumor microenvironments.	[50]
Metabolic Landscape	Nutrient-rich, less hypoxic; supports immune effector functions	Hypoxic, acidic, glucose-depleted; favors tumor cell survival over immune activity	Metabolic competition in solid tumors reduces CAR-T persistence and function.	[51]
Immune Checkpoint Expression	PD-1/PD-L1 moderately expressed; variable across hematologic subtypes	High expression of PD-L1, CTLA-4, TIM-3, and LAG-3	Enhanced checkpoint activation in solid tumors contributes to T-cell exhaustion.	[52]
Therapeutic Implication	High efficacy of CAR-T and monoclonal antibody therapies	Limited success of CAR-T therapy; need for combination and engineering strategies	Future approaches focus on remodeling the solid TME to resemble the permissive hematologic niche.	[34]

4. Innovative Engineering Strategies: Overcoming the Barriers

4.1 Advanced CAR Design and Architecture

To address the complexity of solid tumours, new approaches to CAR -T engineering are being worked out to enhance specificity, efficacy, and safety. One of the major developments is in multispecific targeting, in which CAR constructs are designed to bind 2 or more tumour-associated antigens at a time [53]. Tandem CARs (TanCARs), Loop CARs, and others, allow the activation of T cells only when multiple antigens are presented, thus reducing tumour resistance due to antigen heterogeneity, and reducing off-tumour toxicity. These multispecific systems complement tumour selectivity and maintain cytotoxic attacks, particularly in neoplasms that have a heterogeneous expression of antigens [54]. At the same time, conditional activation modalities, including ON-switch CARs, are based on small-molecule or ligand-dependent triggers to activate CAR-T cells only in the presence of highly restricted conditions, which improves safety and cytokine-release and off-target effects [55]. Masked CARs regulated by proteases also enhance tumour specificity through the activation of proteins that are only activated when tumour-specific proteases or other micro environmental signals are present. These advanced architectures combine multispecific antigen recognition with conditional or protease based activation and thus overcome immune suppression, antigen

loss and toxicity [56]. These new generations of CAR designs represent a paradigm shift in adoptive cell therapy to reap the potential of synthetic biology and protein engineering to increase precision, controllability and durability - driving the remarkable success in haematological malignancies into effective cancer treatment [57].

4.2 Armoured CAR-T Cells and Cytokine Enhancement

Armoured chimeric antigen receptor T (CAR T) cells is an important developmental advancement in adoptive cell therapy, that has been engineered to enhance antitumor efficacy in solid malignancies by overcoming the tumour microenvironment (TME) immunosuppressive response and enhancing T -cell survival and performance [58]. This is an engineered cell type that is similar to the conventional CAR-T constructs except that it is endowed with the ability to release immuno stimulatory cytokines, such as interleukin-12 (IL-12), IL-15 and IL-18, directly into the tumour milieu. IL -12 overcomes the inhibitory effect of regulatory T cells and myeloid -derived suppressor cells, IL -15 promotes long-term persistence and memory development, and IL -18 enhances pro-inflammatory, tumour-icidal immune responses [59]. Armoured CAR-T designs are also capable of loading immune checkpoint inhibitors, including PD-1 or CTLA-4 blocks, locally into the tumour, increasing the activity of the effectors besides reducing overall toxicity in the system. Additional inventive therapeutics comprise activation of the STING pathway by co-expression of STING agonists, or innate immune sensors, which intensifies the demonstration of antigens, type I interferon generation, and CAR-T recruiting [60]. Moreover, cytokine -resistance pathways, such as disruption of TGF -receptor signalling, protect CAR -T cells against suppressive cytokines which are abundant in the TME, allowing them to maintain service in hostile environments. Preclinical models of glioblastoma, pancreatic, and ovary cancers show that armored CAR-T cells achieve a better tumour clearance, prolonged relapse and survival in comparison to conventional CAR-T therapies [13]. A combination of cytokine armoring, checkpoint modulation and resistance mechanisms can be viewed as the future of cellular immunotherapy as armored CAR-T cells can be used to overcome the inhibitory environment of solid tumours and to achieve durable and high-quality antitumour responses [61].

4.3 Metabolic and Epigenetic Reprogramming

Metabolic and epigenetic reprogramming of CAR-T cells is an important methodological development in an attempt to increase their persistence, functional activity and antitumour activity, particularly in the metabolically hostile microenvironment of the solid tumours [62]. Low oxygen conditions, lack of nutrients, and accumulation of inhibitory metabolites, especially lactate are seen in solid tumours and inhibit T-cell proliferation and cytokine release. In turn, researchers are optimizing CAR-T metabolic flux, namely, by increasing glycolysis, oxidative phosphorylation, and fatty acid oxidation, to maintain cellular energetics under nutrient limitation. CAR-T resilience and hypoxic tolerance is further strengthened by mitochondrial fortifications which includes increased biogenesis, high membrane potential, and enhanced efficiency of the electron transport chain [63]. Simultaneously, epigenetic reprogramming is the maintenance of CAR-T cells in a memory-like phenotype by alteration of histone changes and DNA methylation/acetylation, which prevent exhaustion, maintaining the effector activity. Self-renewal, multipotency and longevity of antitumor immune responses are increased with the induction of central or stem-cell-like memory phenotype [64]. These interventions have collectively demonstrated an enhanced CAR-T therapeutic efficacy in preclinical glioblastoma, pancreatic carcinoma and ovarian malignancy models [13]. Through incorporation of metabolic optimization, mitochondrial reinforcement, and continuous epigenetic regulation, next-generation CAR-T cells develop superior resilience and versatility, which continue to sustain cytotoxic activity and overcome antigenic heterogeneity and immune inhibition at the expense of which traditional immunotherapies diminish the effectiveness of CAR-T in solid malignancies [65].

Figure 2 illustrates the design of armored CAR-T cells, engineered to overcome tumor-induced immunosuppression. These cells incorporate cytokine-secreting modules (IL-12/15/18), enhanced co-stimulation domains, and checkpoint-resistant features such as PD-1 knockout or scFv. The schematic also highlights built-in safety switches (iCasp9) for controlled activation. Overall, armored CAR-T constructs are shown as multifunctional platforms optimized for improved antitumor potency and resilience in hostile tumor microenvironments.

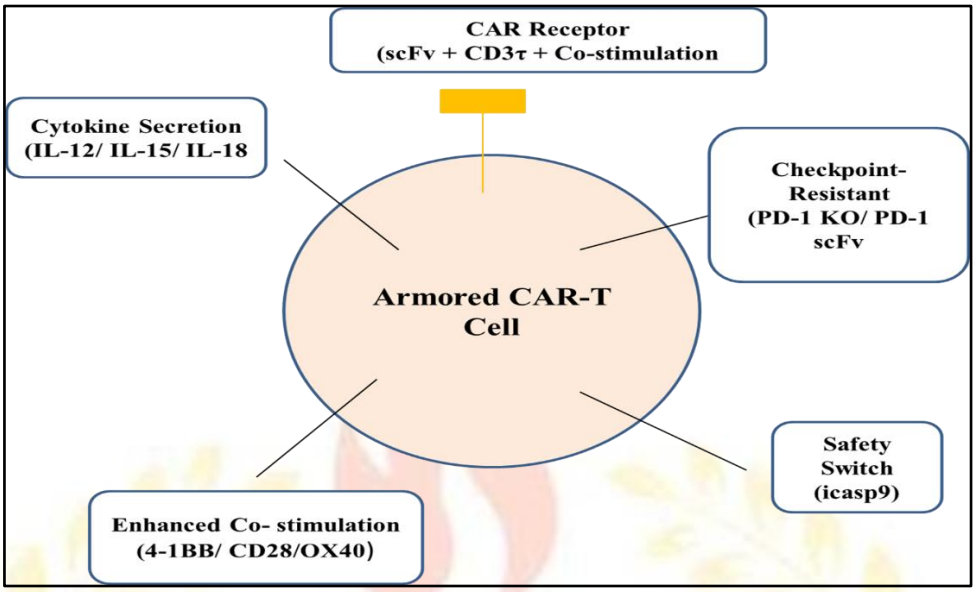


Figure 2: Schematic of next-generation armored CAR-T cell design - recommended placement after Armored CAR-T Cells section.

Schematic representation of next-generation armored CAR-T cells engineered to enhance antitumor potency through transgenic cytokine secretion, checkpoint inhibition, and resistance to immunosuppressive tumor cues. These advanced designs improve persistence, infiltration, and overall therapeutic durability in solid tumors.

5. CRISPR-Cas9 Revolution: Precision Engineering for Enhanced Function

5.1 Checkpoint Inhibitor Knockout Strategies

The use of CRISPR -Cas9 based knockout models has transformed the field of precision immunotherapy, as it has become possible to knock out the inhibitory receptors that tumours use to suppress T-cells, including PD-1, CTLA -4 and LAG-3. PDCD1 gene (PD-1) deletion enhances T-cell proliferation, cytokine release and cytotoxicity in the immunosuppressive tumour microenvironment, CTLA-4 deletion blocks inhibition of costimulatory signalling and LAG-3 deletion prevents early T-cell exhaustion [66]. However, tumours can counter this by using other inhibitory routes thus making individual knockouts inadequate. This has led to synergistic outcomes of multiplex CRISPR editing designs that target combinations of PD -1 / LAG -3 or PD -1 / CTLA -4, and increases T -cell persistence, suppressive resistance, and tumour clearance. Enhanced versions of Cas9 including high fidelity and optimised guide RNAs are being used to achieve better accuracy and reduce off-target effects, thus increasing the possibility of clinical use. However, issues of uncontrolled T-cell activation, which results in cytokine release syndrome or autoimmunity, are of primary importance [67]. The way through these risks that researchers are taking is by integrating inducible suicide genes, reversible editing systems and detailed preclinical safety modelling. To maintain the immune balance in the long term, continuous monitoring will also be inalienable [68] . In general, CRISPR-Cas9-mediated checkpoint gene editing is a ground breaking advancement on the next-generation T-cell therapies that can overcome tumour immune escape with greater specificity, longevity, and safety [69] .

5.2 Functional Enhancement Modifications

Functional improvement approaches based on CRISPR-Cas9 have completely revolutionized adoptive T-cell therapies by overcoming obstacles of the tumor microenvironment as well as enhancing the antitumor activity. These changes can be mainly classified into three major approaches: (i) knockout of the transforming growth factor- beta (TGF -b) receptor, (ii) increase in T -cell trafficking and (iii) improved immunosuppressive stimulus resistance [70]. TGFBR2 locus ablation via CRISPR breaks discontinuity of inhibitory TGF -B

signalling, maintaining T-cell proliferation, effector functions and metabolic stability in solid tumours. CRISPR is used to improve tumour infiltration by engineering homing to tumours by expressing chemokine receptors such as CXCR2, CCR2b, and CXCR5 to increase homing efficiency to tumours expressing cognate ligands. Further enhancement of the beneficial migratory effect is achieved by additional genetic modification to disrupt negative regulators of migration, or to insert adhesion molecules to enhance successful extravasation into tumour tissues [71]. Also, CRISPR -based disruption of inhibitory pathways, including adenosine signalling and indoleamine 2,3-dioxygenase (IDO) activity, enhances resistance of T-cells to immunosuppression; e.g. deletion of the adenosine 2A receptor (A2AR) eliminates adenosine-induced suppression, and regulation of glycolytic regulators maintains energy production in nutrient-deprived conditions [72]. Altogether, these changes allow T cells to stay antitumour active even in the state of physiological stress. Despite safety issues, such as off-target toxicity, being still present, the scalability and high accuracy of CRISPR -Cas9 makes it a highly potent platform in the creation of effective, long-lasting and tumour -targeting T-cell immunotherapies in the context of solid tumours [73].

5.3 High-Throughput CRISPR Screening

High-throughput CRISPR screening has become a ground-breaking technique in the field of cancer immunotherapy to enable the discovery of genes that affect immunocells and tumour resistance in an unbiased, genome-wide manner. Through this method, thousands of genes can be knocked-out or activated simultaneously by using pools of guide RNAs, which will reveal the molecular pathways that regulate T -cell proliferation, cytotoxicity, persistence, and immunosuppression resistance. The screens have revealed new regulators, metabolic checkpoints, transcription factors, and signalling molecules, which are essential in immune activity in the tumour microenvironment [74]. After discovery, CRISPR screening enhances target validation in primary T cells, patient-derived organoids and in-vivo cancer models, which guarantee the clinical relevance of the identified genes. Combination with single-cell RNA sequencing and epigenomic profiling can be used to trace the gene edits across transcriptional states and differentiation pathways and immune heterogeneity and tumour vulnerability can be studied with high-resolution detail. Notably, this technology has already led to the production of checkpoint-resistant CAR -T cells, small molecules that prevent tumour resistance, and programmable combination therapies [75]. As the CRISPR libraries and computational analytics are continuously improved, high-throughput CRISPR screening bridges discovery and clinical translation to offer a scalable, accurate, and data-driven platform to design the next-generation immunotherapy and clinical outcomes by enhancing patient outcomes in cancer treatment [76].

Table 3 summarizes key CRISPR-based genetic modifications used to enhance CAR-T cell performance. It highlights edits targeting inhibitory receptors, endogenous TCR components, and metabolic or persistence-related genes. Each modification is linked to functional outcomes such as improved cytotoxicity, resistance to exhaustion, enhanced proliferation, or reduced alloreactivity. Overall, the table underscores how CRISPR enables precise engineering of next-generation CAR-T cells with superior therapeutic potential.

TABLE 3: CRISPR modifications in CAR-T cells and their functional outcomes - recommended placement after Functional Enhancement Modifications.

Target Gene / Pathway	Type of CRISPR Modification	Rationale / Functional Goal	Observed Functional Outcome	Reference
PD-1 (Programmed Death-1)	Gene knockout (CRISPR/Cas9-mediated deletion)	Prevent T-cell exhaustion and enhance anti-tumor persistence	Increased cytokine secretion (IFN-γ, IL-2); improved tumor lysis and prolonged survival in xenograft models	[77]

CTLA-4 (Cytotoxic T-Lymphocyte Antigen-4)	Knockout	Block inhibitory checkpoint signaling to sustain CAR-T activation	Enhanced proliferation and cytotoxicity; reduced exhaustion phenotype	[78]
LAG-3 (Lymphocyte-Activation Gene 3)	Knockout	Reduce inhibitory receptor co-expression with PD-1	Improved effector function and reduced exhaustion in chronic antigen exposure models	[79]
TGFBR2 (Transforming Growth Factor-β Receptor II)	Knockout	Confer resistance to TGF-β–mediated immunosuppression in TME	Enhanced persistence and tumor regression in TGF-β–rich environments	[80]
FAS (CD95)	Knockout	Protect CAR-T cells from activation-induced cell death (AICD)	Increased survival and sustained cytotoxic function under repetitive stimulation	[81]
TRAC (T-Cell Receptor Alpha Constant Region)	Targeted integration of CAR into TRAC locus	Achieve uniform CAR expression; eliminate endogenous TCR to prevent GvHD	Reduced tonic signaling, enhanced potency, and minimized off-target reactivity	[82]
B2M (Beta-2 Microglobulin)	Knockout	Eliminate MHC-I to evade host immune rejection (for universal CAR-T)	Enabled generation of allogeneic, "off-the-shelf" CAR-T cells with reduced graft-versus-host risk	[83]
PDCD1 + LAG3 (Dual knockout)	Multiplex CRISPR editing	Synergistic checkpoint ablation for superior activation	Markedly increased cytokine output and anti-tumor activity compared to single knockouts	[84]
DNMT3A / TET2 (Epigenetic regulators)	Knockout or modulation	Promote stem-like, memory CAR-T phenotypes	Enhanced persistence, self-renewal, and anti-tumor efficacy	[85]
CXCR4 or CCR5 (Chemokine receptors)	Gene knock-in	Enhance tumor trafficking and infiltration into solid tumors	Improved homing to tumor sites and augmented local cytotoxicity	[86]

6. Emerging Targets and Novel Therapeutic Approaches

6.1 Next-Generation Target Antigens

Recent discoveries in next generation target antigen have redefined cancer immunotherapy to go beyond the traditional surface markers to more tumor specific and clinically versatile targets. Claudin18.2 has become one of the top candidates due to its limited expression in normal gastric mucosa, and high expression in gastric, pancreatic and esophageal malignancies and both CAR-T cell and monoclonal antibody therapies have shown strong efficacy and acceptable safety profile [87]. Equally, mesothelin, a protein that is up-regulated in mesothelioma, ovarian, pancreatic and lung cancers, is under investigation in CAR-T, antibody, and vaccine platforms because it has low levels in normal tissues. Another promising target is glypican-3 (GPC3) which is a proteoglycan that is over expressed in the hepatocellular carcinoma and tumours in children and is considered promising because of its low off-target toxicity. In addition to these tumor-associated antigens, precision oncology is advancing at a rapidly rising pace with neo-antigen-based approaches, which is enabled by next-generation sequencing and computational software to find patient-specific tumor mutations to design individual vaccines and engineered cell therapies [88]. Moreover, the Aberrant glycosylation or phosphorylation are post-translational modification (PTM)-derived epitopes which are specific to cancerous cells and which enhance the repertoire of antigens with exceptional specificity [89]. Together, Claudin 18.2, mesothelin, GPC3, neo -antigens and PTM -derived targets define a

new generation of immunotherapy antigens that have a greater tumour precision, less toxicity and the possibility of long-term clinical responses in a range of solid tumours [90].

6.2 Alternative Cell Platforms

The development of alternative cellular platforms other than conventional T cells is changing the world of immunotherapy, and it offers specific benefits in safety and scalability and therapeutic competence. CAR-based natural killer (NK) cells show a high antitumor potency and a significant reduction in the risks of cytokine release syndrome and graft-versus-host disease due to the intrinsic ability to perceive the malignant cells even in the case of antigen loss [91]. Similarly, CAR-modified macrophages (CAR-Ms) also take advantage of their innate tumor-infiltrating and phagocytic capabilities by eliminating neoplastic cells and also by generating an adaptive immunological response. Immune effector cells derived using induced pluripotent stem cell (iPSC) represent a renewable and standardized source of high-volume production of engineered cellular agents with superior persistence and resistance to exhaustion [92]. Allogeneic off-the-shelf products also increase the accessibility by facilitating the quick and cost-efficient implementation of the cell donor-derived or iPSC based. The insertion of genome-editing capabilities like CRISPR- Cas9 allow the rejection and optimisation of functional properties and consequently broaden the therapeutic spectrum of next-generation cellular modalities to include both hematologic malignancy and solid tumours [93].

6.3 Delivery and Administration Innovations

The new technologies in delivery and administration are transforming the clinical potential of engineered cell therapies by improving precision, safety, and efficacy using regional delivery, in vivo programming, nanotechnology and temporal control systems [94]. Locoregional or intratumoral infusions place therapeutic agents at the site of tumor and, thus, reduce systemic toxicity and enhance efficacy in solid tumors with minimal T - cell infiltration. Direct genetic modification of immune cells can be done in vivo by CAR programming using nanoparticles or viral vectors, and does not require the complexities of ex vivo manufacture [95]. Nanotechnology enhances the capacity of delivery through transfer of genes, regulation of immunomodulators discharge, and co-delivery of synergistic agents like CAR constructs and checkpoint inhibitors [96]. Inducible promoters, small-molecule switches, and optogenetic systems, which are examples of temporal control systems, allow the activation of cells to be controlled with high precision, avoiding excessive immune responses, such as cytokine release syndrome. The combination of these strategies will increase the scale, safety, and flexibility of the next-generation cellular immunotherapies by expanding the therapeutic window [97].

Figure 3 highlights emerging CAR-T targets across major solid tumor types, illustrating the expanding landscape of tumor-associated antigens under investigation. Different cancers—such as breast, ovarian, lung, prostate, pancreatic, and glioblastoma—are linked with promising targets including ROR1, mesothelin, PSMA, EGFRvIII, GD2, and MUC-family antigens. The schematic underscores the growing diversity of antigenic strategies aimed at overcoming heterogeneity and improving CAR-T specificity in solid tumors. Overall, the figure demonstrates how target discovery is driving next-generation CAR-T development for solid malignancies.

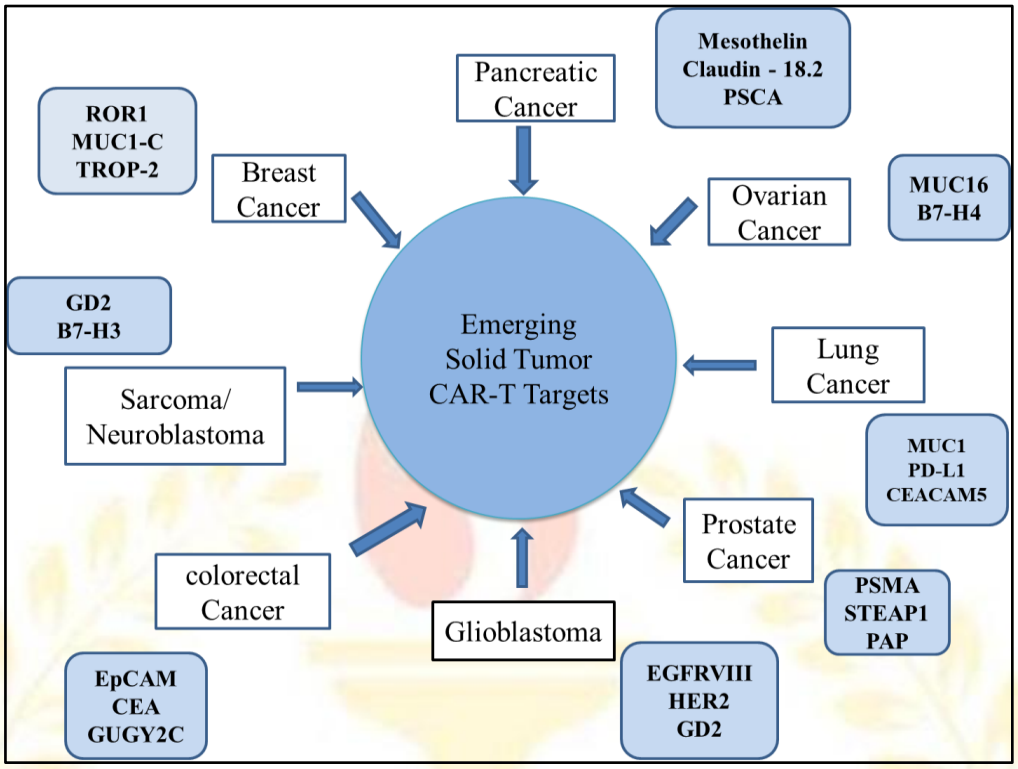


Figure 3: Emerging target antigens in various solid tumor types - recommended placement after Next-Generation Target Antigens.

Overview of emerging solid tumor–associated antigens under exploration for next-generation CAR-T therapies, including glycoproteins, cancer testis antigens, and tumor-specific neoantigens. These targets aim to improve specificity and reduce off-tumor toxicity in solid malignancies.

7. Combination Therapeutic Strategies: Synergistic Approaches

7.1 Checkpoint Inhibitor Combinations

A new system of combination checkpoint inhibitors has become a new paradigm in the modern immunotherapy of cancer, the goal of which is to increase antitumor effect and avoid immune resistance mechanisms through the use of a combination of checkpoint inhibitors and exploration of other checkpoint targets and timing/sequencing optimization of the therapeutic process [98]. Combination inhibition of PD -1 /PD -L1 and CTLA -4 has complementary effects, since PD -1/PD -L1 blockage reinvigorates exhausted effector T cells in the tumor microenvironment, whereas CTLA -4 blockage promotes prompt T -cell activation and clonal expansion in the lymphoid tissues. The complementary system has brought about long-term and high-quality clinical results especially in melanoma and other malignancies [99]. In addition to these axes, other novel checkpoints like LAG -3, TIM -3, TIGIT, and VISTA are under study to interfere with compensatory responses that tumors use after PD -1 or CTLA -4 blockade. This is also essential when it comes to the optimization of their sequencing in treatment, concomitant blockade may be more toxic, and sequential regimens, such as CTLA-4 blockage followed by PD-1 blockage, are better able to strike a balance between efficacy and safety. It is expected that ongoing research that combines biomarker profiling, real time immune measurement, and personalized treatment algorithm will further optimize these combination strategies to achieve maximum clinical effectiveness but with controllable adverse events associated with immunotherapies in the long-term [100].

7.2 Radiation and Chemotherapy Integration

Integration of radiotherapy with chemotherapy and immunotherapy has proved to be an effective approach to enhance antitumor immune response through the induction of immunogenic cell death (ICD), the tumor microenvironment remodeling and the release and presentation of antigens [101]. In addition to their direct cytotoxic activity, radiation, and some chemotherapeutic agents trigger ICD, the extracellular release of damage-associated molecular patterns (DAMPs) including calreticulin, high-molecular-weight-binding glycoprotein-1 (HMGB1), and ATP, stimulates dendritic cells and prime T cells. These mechanisms transform immunologically cold tumors to inflamed and hot microenvironments which promote immune infiltration and enhance the checkpoint blockade therapy. In addition to this, radiation and chemotherapy influence tumor microenvironment by reducing suppressive cellular components, such as regulatory T cells,

myeloid-derived suppressor cells, and suppressive cytokines, and promoting vascular permeability and chemokine gradients, which facilitates immune cell trafficking and immune cell persistence [102]. The tumor antigens discharged upon therapy widen the epitope repertoire, permitting the endogenous and engineered T cells to realize and exterminate the leftover tumor cells. A good balance between strong immune stimulation and immunosuppression is obtained by accurately timed and dosed forms of these modalities to produce the best synergy. Altogether, cytotoxic treatment combined with immunotherapy is a synergist and multifactorial method of remodeling the tumor milieu and stimulating long-term antitumor immunity [103].

7.3 Targeted Therapy Combinations

Integration of targeted therapy and immunotherapy is a new concept that increases antitumor effects and overcomes resistance using synergistic effects that include kinase inhibition, angiogenesis, epigenetics as well as oncolytic viral platforms [104]. Pathway blockers like MAPK, PI3K/AKT and JAK/STAT not only prevent malignant growth and survival, but also increase immune vulnerability by increasing antigen presentation, reducing immunosuppressive cytokines, and enhancing T -cell infiltration. Similarly, VEGF - targeted treatments normalize vasculature in tumors, reduce hypoxia, and encourage immune cell creep into tumor microenvironment and also, reduce the numbers of immunosuppressive cells, including regulatory T cells and myeloid-derived suppressive cells [105]. Epigenetic suppressors, such as DNA methyltransferase and histone deacetylase inhibitors, fail malignant and immune cells by restoring silenced tumor antigens, inducing the major histocompatibility complex expression, and promoting a pro-inflammatory environment that promotes immune activation [106]. Oncolytic viruses can also augment this synergy as they have a direct lysogenic effect on tumor cells, releasing tumor antigens, and activating innate immune responses and adaptive immune responses. Optimal sequencing, dose and combinatorial design still play a central role in the maximization of these synergistic effects and the reduction of toxicity. The combination of the above desensitized therapeutic interventions redefines the cancer microenvironment, increases immunogenicity and boosts the efficacy of immunotherapy in solid and hematologic malignancies [107].

This table 4 summarizes major CRISPR-based genetic modifications applied to CAR-T cells and their corresponding functional improvements. It highlights edits such as PD-1 knockout, TRAC insertion, TGF-βR or HLA deletions, and multiplexed gene editing. These modifications collectively enhance persistence, reduce exhaustion, improve antigen sensitivity, and mitigate alloreactivity. Overall, the table demonstrates how genome engineering is expanding CAR-T performance and safety for solid tumors.

TABLE 4: Ongoing clinical trials of combination CAR-T therapies for solid tumors - recommended placement after Checkpoint Inhibitor Combinations.

Combination Strategy	Target	Tumor Type(s)	Phase / Trial ID	Key Focus	Refrences
CAR-T + PD-1 Blockade	Mesothelin	Ovarian, Pancreatic, Mesothelioma	Ph I/II – NCT02414269	Overcome exhaustion, improve persistence	[108]
CAR-T + CTLA-4 Inhibitor	GD2	Neuroblastoma, Melanoma	Ph I – NCT03356782	Boost infiltration and T-cell activity	[109]
CAR-T + Oncolytic Virus	HER2	Glioblastoma, Breast	Ph I – NCT03740256	Enhance immune priming and lysis	[110]
CAR-T + Cytokine Support	GPC3	Hepatocellular carcinoma	Ph I – NCT05003895	Promote proliferation and cytotoxicity	[111]

CAR-T + Chemotherapy	Claudin 18.2	Gastric, Pancreatic	Ph I – NCT04581473	Improve penetration and reduce suppression	[112]
CAR-T + Radiotherapy	EGFRvIII	Glioblastoma	Ph I – NCT04099797	Enhance antigen release and homing	[113]
CAR-T + STING Agonist	MUC1	Triple-negative breast cancer	Ph I – NCT05239143	Stimulate innate-adaptive synergy	[114]
CAR-T (PD-1–/– Gene Edited)	EGFR	NSCLC	Ph I – NCT03545815	Intrinsic checkpoint resistance	[115]

8. Clinical Translation and Current Trial Landscape

8.1 Phase I/II Trial Results

Translational development of engineered cellular treatments and combinatory immunotherapeutic approach has evolved quickly with the demonstration of Phase I and II clinical trials, which stands out as important insight into safety, efficacy, and biomarker-directed optimization [116]. The main focus of preclinical research is on reducing adverse events, such as cytokine release syndrome (CRS), immune effector cell-associated neurotoxicity syndrome, immune control syndrome (ICANS), and off-target activity. The management of CRS using tocilizumab and corticosteroids with approaches that include regimens of stepwise dosing, engineered safety switches, and stringently standardized protocols of CRS management have been effective in the reduction of adverse events and maintenance of therapeutic efficacy. These breakthroughs in safety have enabled increased clinical testing of advanced modalities- such as the checkpoint resistant CAR-T cells, CAR- NK therapies, and multiplex engineered T cells- that result in promising tumor regression and long lasting outcomes in instances of relapse or therapeutic resistance [117]. The efficacy is also increased by its combining with checkpoint blockade, cytokine armoring, and dual-targeting constructs. Meanwhile, development of biomarkers, including immune phenotypes, cytokine signature, tumor mutational burden, antigen expression, and circulating tumor DNA, refinishes response prediction and stratification of patients [118]. High-throughput sequencing and single-cell transcriptomics are the state of the art methodologies that support biomarker discovery and can monitor the immune in real time. Together, the accumulated evidence of each of these studies creates a platform of safer, more personalized and effective next generation immunotherapies, effectively setting the stage of larger randomized studies, regulatory streamlining, and the wide-scale application of precision oncology [119].

8.2 Manufacturing and Scalability Challenges

Meeting the manufacturability and scalability challenges will form one of the conditions to the large-scale clinical adoption of engineered cellular therapies and sophisticated immunotherapeutics, including economic factors, quality control, regulatory controls, and fair global accessibility. The first obstacle is cost-efficiency, since autologous therapeutic means that require personalization involve complex and labour-intensive cell-harvesting, genetic engineering, cell proliferation, and reinfusion procedures that result in costly treatments that are prohibitively expensive [120]. The mitigation strategies targeting the reduction of such costs include enhancement of culture systems, automation of production through bioreactors, allogeneic universal platform development, and use of high-throughput gene-editing technology to help reduce the number of manual operations without compromising functional potency. It is also of critical significance that standardization of quality-control procedures be in place to ensure parenthood in cell identity, viability, functional activity, and safety between batches of production

and ensured by rigorous in-process monitoring and aligned potency, sterility, and genomic assays [121]. The regulatory systems need to be adjusted to embrace new gene edits and multivalent constructs, which have international guidelines harmonized, a rapid review process, and a strong longitudinal monitoring system. Moreover, mitigation of access discrepancies is also a priority, where high prices and infrastructural facilities limit accessibility in the low- and middle-income locations [122]. The expansion of scalable allogeneic platforms, worldwide collaboration development, and the establishment of the affordability programs is necessary to guarantee the equitable distribution. Eventually, these manufacturing and scalability challenges will be overcome to allow the implementation of safe, cost-effective, and globally available next-generation immunotherapies [123].

9. Biomarkers and Predictive Indicators

9.1 Response Prediction Strategies

Biomarkers and predictive markers have become key factors to optimize the immunotherapy process since they allow clinicians to predict the effect of treatment, tailor therapy, and reduce side effects. Tumor-encoded biomarkers such as mutational load, neoantigen repertoire, antigen expression patterns, and oncogenic signaling pathways give the much-needed information on tumor vulnerability to the immunologically-oriented treatments. High tumor mutational / neoantigen load often is associated with increased T-cell recognition and better response to checkpoint blockade or adoptive cell therapy [124]. Host immune parameters including baseline immune composition, cytokine profiles, T-cell clonality and immunosuppressive populations (Tregs and MDSCs) are equally important and measure patient immunocompromisement and outcome of therapy. Real-time therapeutic activity is provided through pharmacokinetic markers, which include the CAR-T cell expansion, persistence and infiltration of tumors. The accomplishment of tumor, host, and pharmacokinetic data is a multidimensional predictive model, which cleans up the selection of therapeutics, dosage, and timing. Together these biomarkers are changing immunotherapy into a more focused, effective and safer clinical paradigm [125].

9.2 Resistance Mechanisms and Monitoring

The understanding and suppression of resistance effects in immunotherapeutic settings require a comprehensive, temporally dynamic study of tumour dynamics, immune reactions and treatment effectiveness. The combination of conventional high-throughput sequencing, single-cell transcriptomic analysis, and functional immunological analysis is useful in the early detection of resistance mechanisms, such as the loss of antigens and the induction of inhibitory signalling pathways [126]. Circulating biomarkers, including cell-free tumour DNA, circulating tumour cells, cytokines, and specific immune cell subsets, minimally invasive, would give useful data on tumour burden, immune competency and emerging immune escape mechanisms, thus facilitating timely therapeutic adjustments. Spatially resolved and functional images of tumouric response dynamics and immune cell infiltration are provided by adjunctive imaging modalities such as positron emission tomography, magnetic resonance imaging and molecular imaging. All these molecular, cellular, and anatomical surveillance systems combine to provide a single system that is sufficient to capture the complexity of therapeutic resistance [127]. A multidimensional framework of this kind enables clinicians to dynamically tune therapeutic therapies, be it by modulating dose, implementing combinatorial therapeutic interventions, or by performing immune redirection, thus enhancing accuracy, efficacy, and safety in the next generation of immunotherapeutic, and eventually the quality of patient outcomes by active, data-driven decision-making [128].

10. Safety Considerations and Risk Management

10.1 On-Target Off-Tumor Toxicity

The issue of safety in engineered cell therapies also continues to be of paramount concern, especially when it comes to on-target off-tumor toxicity, where therapeutic cells attack normal tissues, which express homologous antigens, leading to a severe side effect. Extensive preclinical risk evaluation,

including antigen distribution mapping, in silico modeling, and in vitro testing is a crucial requirement prior to the prediction of the possibility of cross-reactivity. Risk mitigation approaches entail using very specific antigens, design of affinity-tuned receptors with affinity to high antigen densities on tumour cells and use of combinatorial antigen recognition systems whereby dual engagement is required to induce activation [129]. Also, safety switch systems, including inducible suicide genes, small-molecule-regulated receptors, and CRISPR-based off-switches may offer quick control or extinguishing of engineered cells, in case of toxicity. By combining the predictive modeling with the active control systems, the methods can improve the safety profile of the next-generation immunotherapies, allowing clinicians to maintain a high level of antitumor activity and, at the same time, protect patients and limit negative immune responses[130] .

10.2 Cytokine Release Syndrome Management

Cytokine release syndrome (CRS) management is a critical element in the maintenance of the safety profile of engineered cellular therapies since excessive immune response may result in systemic inflammation, dysfunction of multiple organs, and even life-threatening side effects. Prevention programs focus on careful selection of patients, dosages that are either controlled or fractionated and preconditioning programs that reduce immune over activation. In cases of CRS, the treatment involves use of immunomodulatory therapy like interleukin-6 receptor blockers (e.g., tocilizumab), corticosteroids, and supportive therapies like fluid balance and other hemodynamic stabilization which is adjustable to severity grading and constant observation [131]. In addition to the acute condition, chronic follow-up is necessary to identify the delayed or repeated CRS events, immune reconstitution or complications, such as cardiovascular and neurological aftereffects. CRS can be managed successfully through proactive avoidance, early intervention, and close follow-up to maintain the high antitumor efficacy of the immunotherapeutic treatments, saving the life of the patient and guaranteeing the success of the treatment [132].

11. Future Perspectives and Emerging Paradigms

The field of immunotherapy is undergoing revolutionary changes driven by artificial intelligence and artificial intelligence is making it possible to discover antigens faster, predict the resistance patterns, and tailor treatment plans to the molecular and clinical phenotype. Machine learning procedures take precision a step further by optimizing dosing schedules, time schedule, and also combinatorics, increasing clinical effectiveness and reducing toxicity. Synthetic biology also drives engineered cellular therapies by the creation of programmable genetic circuits that sense tumor specific cues and that will only become activated under specified contextual conditions. Logic-gated CAR designs, bifunctional armored cellular and integrated circuits with checkpoint resistance and cytokine augmentation have a positive effect on tumor specificity, safety kinetics, and therapeutic persistence. Globally, the intensive use of the advanced cellular therapies dictates the need to resolve the financial, scalability, and infrastructural barriers. Off-the-shelf allogeneic modalities, intergovernmental regulatory harmonies, skilled health care staff, and global collaborative activities will become invaluable in the process of access expansion, especially in low- and middle-income jurisdictions. By working together, AI-driven design thinking, innovations in synthetic biology, and global efforts in healthcare, the emergence of more specific and more accessible and more clinically effective immunotherapies is predicted.

12. Conclusion

CAR-T cell therapy has transformed the treatment landscape for hematologic malignancies, yet its translation to solid tumors remains constrained by antigen heterogeneity, immunosuppressive microenvironments, and barriers to trafficking and persistence. Rapid advancements—including armored CAR designs, CRISPR-enabled precision engineering, metabolic and epigenetic reprogramming, and novel antigen discovery—are redefining the potential of next-generation cellular therapies to overcome these obstacles. Combination strategies integrating CAR-T with checkpoint inhibitors, targeted therapies, radiotherapy, and oncolytic platforms further enhance therapeutic synergy and durability. Emerging delivery innovations and alternative immune cell

platforms broaden feasibility while addressing safety and scalability concerns. Collectively, these breakthroughs signal a pivotal shift toward more potent, programmable, and personalized CAR-T therapies capable of extending the success achieved in hematologic cancers to solid tumors. Continued integration of synthetic biology, artificial intelligence, and biomarker-driven personalization will be essential to fully realize the clinical impact of next-generation CAR-T immunotherapy.

In summary, CAR-T therapy has transformed outcomes in hematologic cancers, yet its translation to solid tumors remains limited by antigen heterogeneity, trafficking barriers, and a profoundly immunosuppressive microenvironment. Rapid advances including armored CAR-T cells, CRISPR-enabled precision editing, synthetic biology circuits, and combination regimens are redefining therapeutic potential. Emerging solid-tumor targets and novel delivery platforms further expand feasibility. Continued progress in manufacturing, safety engineering, and biomarker-guided personalization will be essential to achieving durable responses. These innovations position next-generation CAR-T therapy to overcome the solid-tumor frontier.

Acknowledgment

The authors would like to express their sincere gratitude to their respective institutions for providing the necessary support and resources for the successful completion of this review. Special thanks to the collaborative efforts and contributions of all co-authors for their valuable insights and dedication throughout the preparation of this manuscript.

Author Contribution

M.A.R Data Analysis and interpretation, **T.S.** Visualization**. P.C** writing, reviewing and editing, **S.Q** Methodology.

Conflict of Interest

The authors declare no conflict of interest.

Source of Funding

There is no funding available to conduct this study.

References:-

1. Ho, M., S. Zanwar, and J. Paludo, *Chimeric antigen receptor T-cell therapy in hematologic malignancies: Successes, challenges, and opportunities.* Eur J Haematol, 2024. **112**(2): p. 197-210.
2. Brudno, J.N., M.V. Maus, and C.S. Hinrichs, *CAR T Cells and T-Cell Therapies for Cancer: A Translational Science Review.* Jama, 2024. **332**(22): p. 1924-1935.
3. Srivastava, S. and S.R. Riddell, *Chimeric Antigen Receptor T Cell Therapy: Challenges to Bench-to-Bedside Efficacy.* J Immunol, 2018. **200**(2): p. 459-468.
4. Jogalekar, M.P., et al., *CAR T-Cell-Based gene therapy for cancers: new perspectives, challenges, and clinical developments.* Front Immunol, 2022. **13**: p. 925985.
5. Guzman, G., et al., *CAR-T Therapies in Solid Tumors: Opportunities and Challenges.* Curr Oncol Rep, 2023. **25**(5): p. 479-489.
6. Guerra, M., et al., *Relevance, Risks, and Benefits of Early-Phases Clinical Trials Participations for Patients With Hematological Malignancies From 2008 to 2023.* Eur J Haematol, 2025. **114**(1): p. 89-97.
7. Titov, A., et al., *Advancing CAR T-Cell Therapy for Solid Tumors: Lessons Learned from Lymphoma Treatment.* Cancers (Basel), 2020. **12**(1).
8. Sterner, R.C. and R.M. Sterner, *CAR-T cell therapy: current limitations and potential strategies.* Blood Cancer J, 2021. **11**(4): p. 69.
9. Luo, J., et al., *Neoleukin-2/15-armored CAR-NK cells sustain superior therapeutic efficacy in solid tumors via c-Myc/NRF1 activation.* Signal Transduct Target Ther, 2025. **10**(1): p. 78.
10. Zhu, J., et al., *Advancements and challenges in CAR-T cell therapy for solid tumors: A comprehensive review of antigen targets, strategies, and future directions.* Cancer Cell Int, 2025. **25**(1): p. 313.
11. Wang, Y., et al., *The combination therapy of oncolytic virotherapy.* Front Pharmacol, 2024. **15**: p. 1380313.
12. Kong, Y., et al., *CAR-T cell therapy: developments, challenges and expanded applications from cancer to autoimmunity.* Front Immunol, 2024. **15**: p. 1519671.

13. Rafii, S., et al., *Advancing CAR T-Cell Therapy in Solid Tumors: Current Landscape and Future Directions.* Cancers (Basel), 2025. **17**(17).
14. Milewska, S., et al., *Current Trends and Challenges in Pharmacoeconomic Aspects of Nanocarriers as Drug Delivery Systems for Cancer Treatment.* Int J Nanomedicine, 2021. **16**: p. 6593-6644.
15. Borkar, S., et al., *Revolutionizing Oncology: A Comprehensive Review of Digital Health Applications.* Cureus, 2024. **16**(4): p. e59203.
16. Huang, Z., et al., *CAR T cells: engineered immune cells to treat brain cancers and beyond.* Mol Cancer, 2023. **22**(1): p. 22.
17. Yoo, H.J. and B.N. Harapan, *Chimeric antigen receptor (CAR) immunotherapy: basic principles, current advances, and future prospects in neuro-oncology.* Immunol Res, 2021. **69**(6): p. 471-486.
18. Mezősi-Csaplár, M., Á. Szöőr, and G. Vereb, *CD28 and 41BB Costimulatory Domains Alone or in Combination Differentially Influence Cell Surface Dynamics and Organization of Chimeric Antigen Receptors and Early Activation of CAR T Cells.* Cancers (Basel), 2023. **15**(12).
19. Peña-Romero, A.C. and E. Orenes-Piñero, *Dual Effect of Immune Cells within Tumour Microenvironment: Pro- and Anti-Tumour Effects and Their Triggers.* Cancers (Basel), 2022. **14**(7).
20. Mina, A., et al., *Collection of peripheral blood mononucleated cells for chronic graft-versus-host disease immunology research: safety and effectiveness of leukapheresis in 132 patients.* J Transl Med, 2022. **20**(1): p. 519.
21. Labbé, R.P., S. Vessillier, and Q.A. Rafiq, *Lentiviral Vectors for T Cell Engineering: Clinical Applications, Bioprocessing and Future Perspectives.* Viruses, 2021. **13**(8).
22. Ayala Ceja, M., et al., *CAR-T cell manufacturing: Major process parameters and next-generation strategies.* J Exp Med, 2024. **221**(2).
23. Zhang, X., et al., *CAR-T Cell Therapy in Hematological Malignancies: Current Opportunities and Challenges.* Front Immunol, 2022. **13**: p. 927153.
24. Saleh, K., et al., *CAR T-Cells for the Treatment of B-Cell Acute Lymphoblastic Leukemia.* J Clin Med, 2023. **12**(21).
25. Gerardi, C., et al., *Long-Term Follow-Up of Classical Hodgkin Lymphoma and Diffuse Large B-Cell Lymphoma Survivors: Aims and Methodological Approach for Fondazione Italiana Linfomi Systematic Reviews.* Cancers (Basel), 2021. **13**(12).
26. Atilla, P.A. and E. Atilla, *Resistance against anti-CD19 and anti-BCMA CAR T cells: Recent advances and coping strategies.* Transl Oncol, 2022. **22**: p. 101459.
27. Crawford, J., et al., *The impact of myelosuppression on quality of life of patients treated with chemotherapy.* Future Oncol, 2024. **20**(21): p. 1515-1530.
28. Alsaieedi, A.A. and K.A. Zaher, *Tracing the development of CAR-T cell design: from concept to next-generation platforms.* Front Immunol, 2025. **16**: p. 1615212.
29. Li, S., et al., *Advances in CAR optimization strategies based on CD28.* Front Immunol, 2025. **16**: p. 1548772.
30. Zugasti, I., et al., *CAR-T cell therapy for cancer: current challenges and future directions.* Signal Transduct Target Ther, 2025. **10**(1): p. 210.
31. Depil, S., et al., *'Off-the-shelf' allogeneic CAR T cells: development and challenges.* Nat Rev Drug Discov, 2020. **19**(3): p. 185-199.
32. Zhang, B., et al., *Strategies to Overcome Antigen Heterogeneity in CAR-T Cell Therapy.* Cells, 2025. **14**(5).
33. Wang, L., et al., *The dilemmas and possible solutions for CAR-T cell therapy application in solid tumors.* Cancer Lett, 2024. **591**: p. 216871.
34. Zhang, G., et al., *Current Advances and Challenges in CAR-T Therapy for Hematological and Solid Tumors.* Immunotargets Ther, 2025. **14**: p. 655-680.
35. Rojas-Quintero, J., et al., *Car T Cells in Solid Tumors: Overcoming Obstacles.* Int J Mol Sci, 2024. **25**(8).
36. Wu, J., et al., *The tumor microenvironment in the postsurgical liver: Mechanisms and potential targets of postoperative recurrence in human hepatocellular carcinoma.* Med Res Rev, 2023. **43**(6): p. 1946-1973.
37. Yang, Y., et al., *Metabolic hallmarks of natural killer cells in the tumor microenvironment and implications in cancer immunotherapy.* Oncogene, 2023. **42**(1): p. 1-10.
38. Chen, T., et al., *Current challenges and therapeutic advances of CAR-T cell therapy for solid tumors.* Cancer Cell Int, 2024. **24**(1): p. 133.

39. Shin, S.H., et al., *An innovative strategy harnessing self-activating CAR-NK cells to mitigate TGF-β1-driven immune suppression.* Biomaterials, 2025. **314**: p. 122888.
40. Soni, A., et al., *Nano-biotechnology in tumour and cancerous disease: A perspective review.* J Cell Mol Med, 2023. **27**(6): p. 737-762.
41. Wang, Z., L. Zhou, and X. Wu, *Influencing factors and solution strategies of chimeric antigen receptor T-cell therapy (CAR-T) cell immunotherapy.* Oncol Res, 2024. **32**(9): p. 1479-1516.
42. Buruiană, A., et al., *The Tumor Stroma of Squamous Cell Carcinoma: A Complex Environment That Fuels Cancer Progression.* Cancers (Basel), 2024. **16**(9).
43. Aghlara-Fotovat, S., et al., *Targeting the extracellular matrix for immunomodulation: applications in drug delivery and cell therapies.* Drug Deliv Transl Res, 2021. **11**(6): p. 2394-2413.
44. Huang, M., et al., *Innovative strategies to advance CAR T cell therapy for solid tumors.* Am J Cancer Res, 2020. **10**(7): p. 1979-1992.
45. Roy, S.M., et al., *Kinetics of Nanomedicine in Tumor Spheroid as an In Vitro Model System for Efficient Tumor-Targeted Drug Delivery With Insights From Mathematical Models.* Front Bioeng Biotechnol, 2021. **9**: p. 785937.
46. Kim, S.J., D. Khadka, and J.H. Seo, *Interplay between Solid Tumors and Tumor Microenvironment.* Front Immunol, 2022. **13**: p. 882718.
47. Matuszewska, K., et al., *Normalizing Tumor Vasculature to Reduce Hypoxia, Enhance Perfusion, and Optimize Therapy Uptake.* Cancers (Basel), 2021. **13**(17).
48. Melssen, M.M., et al., *Barriers to immune cell infiltration in tumors.* J Immunother Cancer, 2023. **11**(4).
49. Escobar, G., T.R. Berger, and M.V. Maus, *CAR-T cells in solid tumors: Challenges and breakthroughs.* Cell Rep Med, 2025: p. 102353.
50. Haist, M., et al., *The Functional Crosstalk between Myeloid-Derived Suppressor Cells and Regulatory T Cells within the Immunosuppressive Tumor Microenvironment.* Cancers (Basel), 2021. **13**(2).
51. Lv, Y., et al., *Metabolic checkpoints in immune cell reprogramming: rewiring immunometabolism for cancer therapy.* Mol Cancer, 2025. **24**(1): p. 210.
52. Kgokolo, M.C.M., et al., *Elevated Levels of Soluble CTLA-4, PD-1, PD-L1, LAG-3 and TIM-3 and Systemic Inflammatory Stress as Potential Contributors to Immune Suppression and Generalized Tumorigenesis in a Cohort of South African Xeroderma Pigmentosum Patients.* Front Oncol, 2022. **12**: p. 819790.
53. Moreno, C., et al., *Alternative CAR Therapies: Recent Approaches in Engineering Chimeric Antigen Receptor Immune Cells to Combat Cancer.* Biomedicines, 2022. **10**(7).
54. Gómez-Melero, S., et al., *Tandem CAR-T cell therapy: recent advances and current challenges.* Front Immunol, 2025. **16**: p. 1546172.
55. Cassioli, C., et al., *Learning from TCR Signaling and Immunological Synapse Assembly to Build New Chimeric Antigen Receptors (CARs).* Int J Mol Sci, 2022. **23**(22).
56. Rafiq, S., C.S. Hackett, and R.J. Brentjens, *Engineering strategies to overcome the current roadblocks in CAR T cell therapy.* Nat Rev Clin Oncol, 2020. **17**(3): p. 147-167.
57. Smirnov, S., et al., *Advancing CAR-T Therapy for Solid Tumors: From Barriers to Clinical Progress.* Biomolecules, 2025. **15**(10).
58. Yeku, O.O., et al., *Armored CAR T cells enhance antitumor efficacy and overcome the tumor microenvironment.* Sci Rep, 2017. **7**(1): p. 10541.
59. Reighard, S.D., et al., *Immunomodulatory effects of cytokine-induced expansion of cytotoxic lymphocytes in a mouse model of lupus-like disease.* Cytotherapy, 2021. **23**(1): p. 37-45.
60. Dagar, G., et al., *Harnessing the potential of CAR-T cell therapy: progress, challenges, and future directions in hematological and solid tumor treatments.* J Transl Med, 2023. **21**(1): p. 449.
61. Kwon, N. and Y.Y. Chen, *Overcoming solid-tumor barriers: armored CAR-T cell therapy.* Trends Cancer, 2025. **11**(10): p. 1019-1029.
62. Alvanou, M., et al., *Empowering the Potential of CAR-T Cell Immunotherapies by Epigenetic Reprogramming.* Cancers (Basel), 2023. **15**(7).
63. Ganjoo, S., et al., *The role of tumor metabolism in modulating T-Cell activity and in optimizing immunotherapy.* Front Immunol, 2023. **14**: p. 1172931.
64. Costa, P., et al., *Epigenetic reprogramming in cancer: From diagnosis to treatment.* Front Cell Dev Biol, 2023. **11**: p. 1116805.
65. Gao, T.A. and Y.Y. Chen, *Engineering Next-Generation CAR-T Cells: Overcoming Tumor Hypoxia and Metabolism.* Annu Rev Chem Biomol Eng, 2022. **13**: p. 193-216.

66. Feng, X., et al., *CRISPR/Cas9 technology for advancements in cancer immunotherapy: from uncovering regulatory mechanisms to therapeutic applications.* Exp Hematol Oncol, 2024. **13**(1): p. 102.
67. Liu, B., et al., *Exploring treatment options in cancer: Tumor treatment strategies.* Signal Transduct Target Ther, 2024. **9**(1): p. 175.
68. Ercilla-Rodríguez, P., et al., *CAR-T lymphocyte-based cell therapies; mechanistic substantiation, applications and biosafety enhancement with suicide genes: new opportunities to melt side effects.* Front Immunol, 2024. **15**: p. 1333150.
69. Tao, R., et al., *Revolutionizing cancer treatment: enhancing CAR-T cell therapy with CRISPR/Cas9 gene editing technology.* Front Immunol, 2024. **15**: p. 1354825.
70. Ghaffari, S., N. Khalili, and N. Rezaei, *CRISPR/Cas9 revitalizes adoptive T-cell therapy for cancer immunotherapy.* J Exp Clin Cancer Res, 2021. **40**(1): p. 269.
71. Kolahi Azar, H., et al., *Mesenchymal stromal cells and CAR-T cells in regenerative medicine: The homing procedure and their effective parameters.* Eur J Haematol, 2024. **112**(2): p. 153-173.
72. Vasquez-Vivar, J., Z. Shi, and S. Tan, *Tetrahydrobiopterin in Cell Function and Death Mechanisms.* Antioxid Redox Signal, 2022. **37**(1-3): p. 171-183.
73. Martins, F. and M.H.L. Ribeiro, *Quality and Regulatory Requirements for the Manufacture of Master Cell Banks of Clinical Grade iPSCs: The EU and USA Perspectives.* Stem Cell Rev Rep, 2025. **21**(3): p. 645-679.
74. Chan, Y.T., et al., *CRISPR-Cas9 library screening approach for anti-cancer drug discovery: overview and perspectives.* Theranostics, 2022. **12**(7): p. 3329-3344.
75. Zhu, Z., et al., *Transforming cancer treatment: integrating patient-derived organoids and CRISPR screening for precision medicine.* Front Pharmacol, 2025. **16**: p. 1563198.
76. Șerban, M., C. Toader, and R.A. Covache-Busuioc, *CRISPR and Artificial Intelligence in Neuroregeneration: Closed-Loop Strategies for Precision Medicine, Spinal Cord Repair, and Adaptive Neuro-Oncology.* Int J Mol Sci, 2025. **26**(19).
77. Xu, Y., et al., *Effect of CRISPR/Cas9-Edited PD-1/PD-L1 on Tumor Immunity and Immunotherapy.* Front Immunol, 2022. **13**: p. 848327.
78. Pietrobon, V., et al., *Improving CAR T-Cell Persistence.* Int J Mol Sci, 2021. **22**(19).
79. Andrews, L.P., et al., *LAG-3 and PD-1 synergize on CD8(+) T cells to drive T cell exhaustion and hinder autocrine IFN-γ-dependent anti-tumor immunity.* Cell, 2024. **187**(16): p. 4355-4372.e22.
80. Jin, K., F. Zhou, and L. Zhang, *Cancer Environment Immunotherapy: targeting TGF-β finds its way towards tissue healing and vasculature remodeling.* Signal Transduct Target Ther, 2021. **6**(1): p. 41.
81. Yi, F., et al., *CAR-engineered lymphocyte persistence is governed by a FAS ligand/FAS auto-regulatory circuit.* bioRxiv, 2024.
82. Jiang, N., et al., *Recent advances in universal chimeric antigen receptor T cell therapy.* J Hematol Oncol, 2025. **18**(1): p. 82.
83. Han, X., et al., *The role of B2M in cancer immunotherapy resistance: function, resistance mechanism, and reversal strategies.* Front Immunol, 2025. **16**: p. 1512509.
84. Ciraolo, E., et al., *Simultaneous Genetic Ablation of PD-1, LAG-3, and TIM-3 in CD8 T Cells Delays Tumor Growth and Improves Survival Outcome.* Int J Mol Sci, 2022. **23**(6).
85. Ahn, T., E.A. Bae, and H. Seo, *Decoding and overcoming T cell exhaustion: Epigenetic and transcriptional dynamics in CAR-T cells against solid tumors.* Mol Ther, 2024. **32**(6): p. 1617-1627.
86. Kilgour, M.K., et al., *Advancements in CAR-NK therapy: lessons to be learned from CAR-T therapy.* Front Immunol, 2023. **14**: p. 1166038.
87. Liu, Y., et al., *Targeting Claudin18.2 for cancer theranostics: From molecular imaging to precision therapy.* iScience, 2025. **28**(10): p. 113491.
88. Chen, Q., Y. Sun, and H. Li, *Application of CAR-T cell therapy targeting mesothelin in solid tumor treatment.* Discov Oncol, 2024. **15**(1): p. 289.
89. Dutta, H. and N. Jain, *Post-translational modifications and their implications in cancer.* Front Oncol, 2023. **13**: p. 1240115.
90. Han, M., et al., *6-O-endosulfatases in tumor metastasis: heparan sulfate proteoglycans modification and potential therapeutic targets.* Am J Cancer Res, 2024. **14**(2): p. 897-916.
91. Balkhi, S., et al., *CAR-NK cell therapy: promise and challenges in solid tumors.* Front Immunol, 2025. **16**: p. 1574742.
92. Chettri, D., et al., *CAR-macrophages: tailoring cancer immunotherapy.* Front Immunol, 2024. **15**: p. 1532833.

93. Fang, Y., Y. Chen, and Y.R. Li, *Engineering the next generation of allogeneic CAR cells: iPSCs as a scalable and editable platform.* Stem Cell Reports, 2025. **20**(7): p. 102515.
94. Malik, S., K. Muhammad, and Y. Waheed, *Emerging Applications of Nanotechnology in Healthcare and Medicine.* Molecules, 2023. **28**(18).
95. Holtermann, A., et al., *Prospects of Synergy: Local Interventions and CAR T Cell Therapy in Solid Tumors.* BioDrugs, 2024. **38**(5): p. 611-637.
96. Zhao, B., et al., *Recent advances in nano-drug delivery systems for synergistic antitumor immunotherapy.* Front Bioeng Biotechnol, 2022. **10**: p. 1010724.
97. Siddiqui, M., C. Tous, and W.W. Wong, *Small molecule-inducible gene regulatory systems in mammalian cells: progress and design principles.* Curr Opin Biotechnol, 2022. **78**: p. 102823.
98. Zamani, M.R. and P. Šácha, *Immune checkpoint inhibitors in cancer therapy: what lies beyond monoclonal antibodies?* Med Oncol, 2025. **42**(7): p. 273.
99. Park, J. and B.S. Skålhegg, *Combination of PD-1/PD-L1 and CTLA-4 inhibitors in the treatment of cancer - a brief update.* Front Immunol, 2025. **16**: p. 1680838.
100. Mireștean, C.C., R.I. Iancu, and D.P.T. Iancu, *LAG3, TIM3 and TIGIT: New Targets for Immunotherapy and Potential Associations with Radiotherapy.* Curr Oncol, 2025. **32**(4).
101. Hang, Z.S., et al., *Radiotherapy elicits immunogenic cell death and metabolic shifts in the tumor microenvironment: implications for immunotherapy.* Int J Med Sci, 2025. **22**(13): p. 3277-3291.
102. Rodrigues, M.C., et al., *An Overview on Immunogenic Cell Death in Cancer Biology and Therapy.* Pharmaceutics, 2022. **14**(8).
103. Dolton, G., et al., *Targeting of multiple tumor-associated antigens by individual T cell receptors during successful cancer immunotherapy.* Cell, 2023. **186**(16): p. 3333-3349.e27.
104. Chianese, A., et al., *Oncolytic Viruses in Combination Therapeutic Approaches with Epigenetic Modulators: Past, Present, and Future Perspectives.* Cancers (Basel), 2021. **13**(11).
105. Mengie Ayele, T., et al., *Role of JAK2/STAT3 Signaling Pathway in the Tumorigenesis, Chemotherapy Resistance, and Treatment of Solid Tumors: A Systemic Review.* J Inflamm Res, 2022. **15**: p. 1349-1364.
106. Abhimanyu, et al., *Reversing Post-Infectious Epigenetic-Mediated Immune Suppression.* Front Immunol, 2021. **12**: p. 688132.
107. DePeaux, K. and G.M. Delgoffe, *Integrating innate and adaptive immunity in oncolytic virus therapy.* Trends Cancer, 2024. **10**(2): p. 135-146.
108. Castelletti, L., et al., *Anti-Mesothelin CAR T cell therapy for malignant mesothelioma.* Biomark Res, 2021. **9**(1): p. 11.
109. Koumarianou, A. and J. Duran-Moreno, *The Sarcoma Immune Landscape: Emerging Challenges, Prognostic Significance and Prospective Impact for Immunotherapy Approaches.* Cancers (Basel), 2021. **13**(3).
110. Jafari, M., et al., *Immunovirotherapy: The role of antibody based therapeutics combination with oncolytic viruses.* Front Immunol, 2022. **13**: p. 1012806.
111. Chen, S., et al., *IL-21- and CXCL9-engineered GPC3-specific CAR-T cells combined with PD-1 blockade enhance cytotoxic activities against hepatocellular carcinoma.* Clin Exp Med, 2024. **24**(1): p. 204.
112. Qi, C., et al., *Claudin18.2-specific CAR T cells in gastrointestinal cancers: phase 1 trial final results.* Nat Med, 2024. **30**(8): p. 2224-2234.
113. Maggs, L., et al., *CAR T Cell-Based Immunotherapy for the Treatment of Glioblastoma.* Front Neurosci, 2021. **15**: p. 662064.
114. Hovhannisyan, L., et al., *CAR T cell-based immunotherapy and radiation therapy: potential, promises and risks.* Mol Cancer, 2023. **22**(1): p. 82.
115. Daei Sorkhabi, A., et al., *The current landscape of CAR T-cell therapy for solid tumors: Mechanisms, research progress, challenges, and counterstrategies.* Front Immunol, 2023. **14**: p. 1113882.
116. Wu, Y., et al., *Advancing non-small cell lung cancer treatment: the power of combination immunotherapies.* Front Immunol, 2024. **15**: p. 1349502.
117. Morris, E.C., et al., *Cytokine release syndrome and associated neurotoxicity in cancer immunotherapy.* Nat Rev Immunol, 2022. **22**(2): p. 85-96.
118. Michaelidou, K., S. Agelaki, and K. Mavridis, *Molecular markers related to immunosurveillance as predictive and monitoring tools in non-small cell lung cancer: recent accomplishments and future promises.* Expert Rev Mol Diagn, 2020. **20**(3): p. 335-344.

119. Yang, X., et al., *High-Throughput Transcriptome Profiling in Drug and Biomarker Discovery.* Front Genet, 2020. **11**: p. 19.
120. Lee, N.K. and J.W. Chang, *Manufacturing Cell and Gene Therapies: Challenges in Clinical Translation.* Ann Lab Med, 2024. **44**(4): p. 314-323.
121. Abou-El-Enein, M., et al., *Scalable Manufacturing of CAR T cells for Cancer Immunotherapy.* Blood Cancer Discov, 2021. **2**(5): p. 408-422.
122. Jenkins, D., et al., *Impacts of the regulatory environment for gene editing on delivering beneficial products.* In Vitro Cell Dev Biol Plant, 2021. **57**(4): p. 609-626.
123. Shah, M., et al., *Promises and challenges of a decentralized CAR T-cell manufacturing model.* Front Transplant, 2023. **2**: p. 1238535.
124. Bai, R., et al., *Predictive biomarkers for cancer immunotherapy with immune checkpoint inhibitors.* Biomark Res, 2020. **8**: p. 34.
125. Zhang, M., et al., *Advances in cancer immunotherapy: historical perspectives, current developments, and future directions.* Mol Cancer, 2025. **24**(1): p. 136.
126. Jia, Q., et al., *High-throughput single-cell sequencing in cancer research.* Signal Transduct Target Ther, 2022. **7**(1): p. 145.
127. Mokhtari, R.B., et al., *A Comprehensive Oncological Biomarker Framework Guiding Precision Medicine.* Biomolecules, 2025. **15**(9).
128. Lakhan, S.E., *The Composite Digital Therapeutic Index (cDTI): A Multidimensional Framework and Proof-of-Concept Application to FDA-Authorized Treatments.* Cureus, 2025. **17**(5): p. e83886.
129. Lebrec, H., et al., *Nonclinical safety assessment of engineered T cell therapies.* Regul Toxicol Pharmacol, 2021. **127**: p. 105064.
130. Khajanchi, N. and K. Saha, *Controlling CRISPR with small molecule regulation for somatic cell genome editing.* Mol Ther, 2022. **30**(1): p. 17-31.
131. Cobb, D.A. and D.W. Lee, *Cytokine Release Syndrome Biology and Management.* Cancer J, 2021. **27**(2): p. 119-125.
132. Jain, M.D., M. Smith, and N.N. Shah, *How I treat refractory CRS and ICANS after CAR T-cell therapy.* Blood, 2023. **141**(20): p. 2430-2442.