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EMERGING INSIGHTS INTO CAR-T THERAPY FOR SOLID MALIGNANCIES

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Abstract

Background:

Chimeric antigen receptor (CAR) engineered T cells have achieved great success in combating certain hematologic malignancies; however, translating these breakthroughs to solid tumors remains a formidable challenge. This article explores key insights from recent clinical studies, elucidates the mechanisms by which solid tumors evade immune surveillance, and highlights innovative engineering strategies designed to overcome these barriers and enhance the therapeutic potential of CAR T cell treatments.

Summary:

Although early evidence suggested that CAR-T cells could effectively treat various diseases, their achievement in eradicating solid cancer has been limited. A major obstacle lies in the absence of unique tumor-specific markers, coupled with the inability of conventional CAR T therapies to overcome the complex challenges posed by solid tumor environments. Current research is actively exploring numerous plans to boost the potency and persistence of CAR T cells, though many remain in the preclinical stage. Looking ahead, innovative approaches such as engineering next generation CAR T cells, targeting structural barriers and immunosuppressive cells within the tumor microenvironment, and employing advanced methods to shield immune cells from hostile tumor conditions—hold significant promise. These cutting-edge strategies aim to report existing limitations and substantially advance the efficacy of CAR T cell treatments against solid cancer.

Introduction:

Cell based immunotherapy using T cells engineered with chimeric antigen receptors (CARs) has renovated the treatment of specific blood cancers resistant to conventional chemotherapy.(1) Given that solid tumors comprise around 93% of all tumour cases, there is growing enthusiasm to spread the transformative potential of CAR T cell treatment to these far more prevalent and challenging malignancies.(2) But repeatedly, cell-based products for solid tumors have unsuccessful to provide lasting results and clear monitoring criteria. in spite of early indications of therapeutic

effectiveness. [3] Upon receiving the 1st clinical data on CAR T cells in solid tumors, it became seeming that traditional CER-TS cells, while effective in treating hematologic malignancies, may not be capable of managing the complexity of solid tumours.[4]

Current Status of CAR-Based Immune Cell Clinical Trials for Solid Tumors:

Though there are more and more clinical studies using CAR-T cells, The trials concentrate on solid tumors and the clinical effectiveness of CAR T treatment.

The majority of solid malignancies continue to lag behind or remain hard to treat. Due to little baseline expression on non-malignant bystander cells, the availability of prototypic tumor antigens that are overexpressed on tumor tissue and safe to target remains a significant barrier to immunotherapy today.[5] Although this aspect holds true for both hematologic and solid malignancies, solid tumors are primarily heterogenous and do not exhibit tumor-specific antigen, with a few exceptions. Because solid tumors lack cell surface antigens, initial clinical trials have concentrated on evaluating the protection of numerous cancers related antigens.[6] The majority of the 1st or 2nd generation CAR-T cell products are used in early clinical studies, and there are just a rare next generation cell products that have documented for CAR T cell trials in solid cancer.[5]

The majority of CAR T cell studies are geographically recorded in the USA (n = 124), and in China (n = 221), with just a small number of trials being conducted in other country, such as Australia and Europe (n= 24). Now, there are 46 distinct target antigens undergoing clinical trials, with the top 10 being the EGFRvIII (epidermal growth factor variant III), CEA (carcinoembryonic antigen) , PSMA/PCMA (prostate-specific membrane antigen), CD70, HER2, Claudin18.2 (CLDN18.2), disialoganglioside (GD2), B7-H3, glypican-3 (GPC3), mesothelin (MSLN), and NKG2D.Intriguingly, the majority of these studies have early phase basket studies that have concentrated on diverse solid tumors groups defined by antigen positivity, as opposed to particular tumors entities. There are few trials that target specific entities, and the majority of them focus on diseases like liver cancer, gastrointestinal malignancies, and brain cancer.[7,8] In various trials, encouraging outcomes have been achieved, which highlight important aspects to consider while investigating the safety and effectiveness of CAR-T cells for solid tumor, but long-term therapeutic responses for CAR T cells in solid cancer are still being developed. Trials that targeted GD2 in the neuroblastoma setting have yielded some of the most encouraging data for the application of CAR-T cells in solid tumors, with total response rates ranging from 57 - 63%. This is mostly because neuroblastoma lesions prefer to build up in the bone marrow as a lymphoid predilection site, but also because GD2 is a rather steadily expressed antigen, which enhances the CAR recognition of numerous tumor cells. In term of antigen expression level, homogeneity, and tumor burden, the clinical trials, interestingly, used rigorous inclusion criteria, and it is fair to conclude that these variables contributed favourably to the observed antitumor responses.[9] In addition, early indications of clinical effectiveness in treating brain malignancies have been seen in CAR-T cell experiments that target EGFRvIII or IL13Rα2. In several of these studies, local delivery has been chosen in order to bypass the function of the blood-brain barrier. In a similar way, MSLN-specific CAR T cells have been described for the treatment of pleural mesothelioma, where loco-regional administration was paired with turnpike blockade to produce an average overall survival of 23.9 months.[10] In a recent clinical trial, CLDN18.2-specific CAR T cells were utilized in 98 patients with gastrointestinal cancers, resulting in a disease control rate of 91.8% and a total response rate of 38.8%. The strict eligibility requirements are probably responsible for these findings, as only individual with gastrointestinal cancer, lesion lesser than 4 cm, and significant CLDN18.2 expression (over 40% of the tumor mass, mean H-score greater than 2) were included in this particular clinical trial. When taking into account the best potential therapeutic outcome in the treatment cohort of interest, such an approach is ideal.[11]

Based on multiple studies, it is understood that CAR T targeting solid tumors are predictable to inflate in a manner similar to those used for haematological cancers, typically reaching peak levels in the bloodstream between 7 and 14 days after infusion. Nevertheless, a deeper investigation into

tumor infiltration, T-cell proliferation and persistence, as well as the duration of their functional activity within tumor tissues, is necessary to fully comprehend their clinical effectiveness.[12] To address these questions, it is crucial to elucidate the trafficking pathways of CAR-T cells following administration. Although biopsies provide high-quality information about the tumor, its microenvironment, and immune cell infiltration, their scientific application is mostly restricted to baseline and endpoint evaluations due to moral concerns, therapeutic risks, patient discomfort, and associated budgets.[13] This challenge is further complicated by the limited sensitivity of traditional imaging techniques and selection bias in cases involving multifocal lesions. Consequently, our knowledge of CAR T cell migration to solid tumor sites remains incomplete.[14] Early clinical trial data suggest that CAR T cells primarily gather in the lungs before migrating to subsequent lymphoid organs.[15] Their therapeutic efficacy is also influenced by tumor-specific factors, including location, size, and antigen density. While basket trials have broadened patient access to CAR T cell therapies across numerous cancer types, several studies utilize relatively lenient eligibility criteria, particularly concerning antigen expression levels and uniformity. Conditions of specific relevance because activation of T cells over CAR mediated signalling generally needs greater expression levels of antigen compare to its native T cell receptor.[16]

A major obstacle in CAR T cell therapy is the treatment of bulky tumors. Despite adequate vascularization, achieving effective tumor infiltration is difficult, and the unfavourable effector to tumor cell ratio inside the tumor mass often leads to reduced anticancer activity.[10] To enhance therapeutic outcomes in such cases, several approaches have been explored. These include combining locoregional administration with intravenous delivery, using CAR-T cells as a neoadjuvant treatment to shrink large tumors and simplify subsequent surgeries, and applying CAR-T cells locally through fibrin glue to eradicate residual tumor cells after partial tumor removal.[17] Consequently, CAR-T therapy holds promise for managing metastatic and micro metastatic disease. However, interpatient tumor heterogeneity and variations in the expression of tumour associated antigens present significant hurdles in these scenarios. Glandular tumors offer another potential application, as they express distinct cell surface antigens that are minimally present in other tissues. Targeting these tissue-specific antigens carries the risk of damaging healthy antigen positive tissues (on-target, off-tumor toxicity), potentially resulting in the loss of the affected gland.[18,19] Assessing the impact of such adverse effects on a patient's quality of life requires individualized evaluation and is beyond the scope of this review. Furthermore, glandular tumor heterogeneity and metastatic lesions with changed surface-protein expression remain considerable barriers to effective treatment.[20]

Given the scarcity of reports of curative responses for CAR T cell treatment in the setting of solid malignancies, significant study has been conducted to either boost the effectiveness of these cell products or shield them from the negative effects of the tumor microenvironment (TME).

Tumor Immune Evasion: Core Mechanisms and Implications:

CAR T cell therapy faces significant obstacles in treating solid cancer. Its success mainly depends on the presence of tumour associated antigens (TAAs), which are abundant in hematologic cancers but difficult to identify in solid tumors due to pronounced inter- and intra-patient antigen heterogeneity. This variability reduces the ability to find effective targets and limits therapeutic efficacy.[21] Additionally, the tumor microenvironment (TME) in solid cancers is highly immunosuppressive. It consists of regulatory T cells, tumor-associated macrophages, cancer-associated fibroblasts, and myeloid-derived suppressor cells that inhibit immune responses through receptor signalling (LAG-3, TIM-3, PD-1), secretion of suppressive cytokines (IL-1, IL-4, TGF-β), and formation of extracellular matrix barriers.[22] The TME is also deprived of oxygen and essential nutrients, further impairing CAR-T cell survival and function. Moreover, factors such as T cell exhaustion, poor functional perseverance, and suboptimal CAR design contribute to reduced antitumor activity.[23,24] These challenges collectively hinder CAR-T therapy, emphasizing the need for novel approaches to enhance its efficacy against solid tumors.

Therapeutic Interventions for Antigen Heterogeneity in Solid Malignancies

Antigen homogeneity and expression levels show a vital role in determining the efficacy of CAR T cell therapies. To counter the challenge of tumor heterogeneity, several advanced strategies have been introduced that enable CAR-T cells to target multiple antigens simultaneously.[25] These include bispecific, tandem, split, and adapter CAR approaches. For example, trivalent CAR T cells targeting HER2, EphA2, and IL13Rα2 have demonstrated enhanced antitumor activity and improved survival outcomes in glioblastoma models.[26] These cells not only retain activation dynamics similar to conventional CAR T cells but also display greater activity and reduced exhaustion associated to cells expressing only IL13Rα2 and HER2 CARs.[27]

Adapter CAR technologies further broaden the therapeutic potential by employing biorthogonal compound, such as fluorescein isothiocyanate-derived adapters or diketone, allowing flexible and safer tumor targeting. The combination of common CAR receptors with these adapters offers a highly adaptable strategy for addressing heterogeneous tumors. Nevertheless, these multi-specific designs pose additional challenges in safety evaluation, as they may increase the risk of on-target off tumor toxicities, reported in cases targeting HER2 and carbonic anhydrase IX (CAIX).[28] Moreover, toxicities can also arise from factors unrelated to the tumor itself, including preconditioning regimens or concurrent inflammatory conditions. To further improve efficacy against tumors with variable antigen expression, researchers are also investigating highly sensitive receptor systems, such as optimized CARs, STAR (synthetic TCR and antigen receptors), HLA independent T cell receptors (HIT), and TRuCs.[29,30] Together, these methods represent promising advancements aimed at overcoming antigen heterogeneity and improving clinical outcomes in solid tumors.

Strategies to Enhance T-Cell Fitness:

Solid tumors present a highly challenging tumor microenvironment (TME) that contains numerous obstacles, including physical barriers, immunosuppressive signals, and metabolic restrictions. These factors collectively interfere with the infiltration, persistence, and antitumor efficiency of CAR-T cells. To overcome these hurdles, significant research efforts have focused on engineering novel approaches to enhance CAR-T cell function and reprogram the hostile TME. One promising approach involves the development of “armored” CAR-T cells, particularly T cells redirected for universal cytokine killing (TRUCKs).[31] These specialized CAR-T cells are designed not only to target tumor cells directly but also to secrete immune-stimulating cytokines locally within the TME upon activation. This dual functionality enhances CAR-T cell cytotoxicity while simultaneously recruiting and activating other immune effector cells, thereby amplifying the overall antitumor response.[32] Several interleukins have been explored in this context, including IL-23, IL-18, IL-21, IL-15, IL-7, and IL-12. Among these, IL-12 has attracted considerable attention due to its potent ability to stimulate immune responses.[33] Studies have demonstrated that IL-12-secreting CEA-specific CAR-T cells can increase tumor infiltration by M1-polarized macrophages. These macrophages enhance CAR-T cell efficacy by promoting phagocytosis and antigen cross-presentation. However, despite its strong therapeutic potential, systemic administration of IL-12 has been associated with severe toxicities.[34] To address this limitation, researchers have developed controlled delivery methods, including inducible transgene cassettes, membrane-bound IL-12, and attenuated IL-12 variants. The importance of these optimizations is highlighted by a recent clinical trial (NCT02498912) investigating mucin-16 (MUC16)-specific IL-12 TRUCKs in patients with ovarian, primary peritoneal, and fallopian tube cancers, where dose-limiting toxicities related to IL-12 were observed.[35]

IL-18 is another cytokine with significant potential in TRUCK-based therapies. It has been publicised to enhance the infiltration of solid tumors by CD8+ T cells and natural killer (NK) cells, induce M1 polarization in tumor-associated macrophages, and reduce populations of immunosuppressive dendritic cells, M2 macrophages, and regulatory T cells (Tregs).[36] These effects collectively reshape the TME into a more immune-permissive state, improving the overall effectiveness of CAR-T therapy. Furthermore, preclinical studies have demonstrated that

recombinant human IL-18 is well tolerated in both mice and monkeys, supporting its potential for clinical application.[37]

In summary, armored CAR-T cells, particularly TRUCKs, represent a promising advancement in adoptive cell therapy for solid tumors. By integrating cytokine release with targeted cytotoxicity, these engineered cells can overcome multiple immunosuppressive mechanisms within the TME. Ongoing research into cytokines such as IL-12 and IL-18 continues to refine their safety and efficacy profiles, paving the way for more effective CAR-T therapies against solid malignancies.

Overcoming Structural Barriers in the TME

The TME (Tumor microenvironment) in solid cancer features a dense extracellular matrix (ECM) composed of heparan sulphate proteoglycans, collagen, glycoproteins, hyaluronic acid, and other proteins, differing markedly from normal tissues. This dense structure disrupts intertumoral signalling, alters metabolism, and limits CAR-T cell infiltration and activity.[38] To overcome these barriers, CAR-T cells have been engineered to produce ECM-degrading enzymes, such as heparinase or hyaluronidase, enhancing tissue penetration. Another strategy targets cancer-associated fibroblasts (CAF) that drive ECM deposition, using fibroblast activation protein (FAP)-specific CAR-T cells. Sequential use of FAP- and tumor-targeted CAR-T cells has been shown to remodel the stroma, reduce CAF and myeloid-derived suppressor cells, and improve antitumor efficacy.[39,40] However, FAP-targeting raises safety concerns, as FAP expression in healthy stromal cells of bone marrow and skeletal muscle can lead to severe toxicity and cachexia, requiring further safety evaluations. Additionally, overexpressing chemokine receptors like CXCR2, CXCR4, and CCR2 enhances CAR-T recruitment to the TME.[41]

Shielding CAR-T Cells from Tumor-Mediated Immune Suppression:

Solid tumors often overexpress inhibitory ligands that suppress immune responses, creating a major obstacle for CAR-T cell therapy. To counteract these signals, multiple shielding strategies have been developed, including immune checkpoint blockade with antibodies, gene editing, use of dominant negative receptors (DNRs), and modulation of signalling through switch receptors (SRs). While antibody-based checkpoint inhibitors enhance tumor immune control, direct genome editing of CAR-T cells offers superior antitumor efficacy and potentially a better safety profile. For example, PD-1 disruption in T cells increases IFNγ production and prolongs antitumor activity. Similarly, CRISPR/Cas9-mediated knockout of TGF-β receptor II (TGFBR2) reduces T-cell exhaustion, limits regulatory T cell conversion, and improves tumor clearance in xenograft models, with enhanced central and effector memory CAR-T subsets.[42,43]

DNRs act as decoys that bind inhibitory ligands without signaling, shielding CAR-T cells from immunosuppressive factors. TGF-β DNRs, for instance, preserve CAR-T cell function in TGF-β–rich environments. In contrast, SRs not only block inhibitory signals but convert them into stimulatory ones. PD-1/CD28-SR, which fuses PD-1's extracellular domain with CD28's signaling domain, transforms PD-1 inhibition into activation, boosting CAR-T proliferation and cytokine secretion. This approach has shown success in several models, including cMet-targeted CAR-T cells, highlighting its therapeutic potential.[44,45]

Alternative CAR Immune Cell:

Recent research has highlighted that other immune cell types, beyond T cells, also play important roles in tumor control. Consequently, the application of CARs (chimeric antigen receptors) has expanded to NK (Natural killer) cells and macrophages (CAR-M). NK cells, as part of the innate immune system, rapidly attack non-self-cells without requiring antigen presentation via MHC molecules.[46] Clinical studies using HLA mismatched CAR NK cells from cord blood reported no graft-versus-host disease, supporting the feasibility of "off-the-shelf" therapies using NK92 cell lines, donor-derived cord blood, or induced pluripotent stem cells. CAR-NK cells produce a cytokine profile dominated by GM-CSF, which lowers the risk of CRS (Cytokine release syndrome) and neurotoxicity. They also eliminate tumor cells through antibody-dependent cytotoxicity and

activation of death receptors such as Fas and TRAIL. However, their short lifespan limits sustained efficacy, prompting research into modifications that enhance persistence, such as expressing IL-12, IL-15, and IL-18.[47,48]

Macrophages, abundant in tumor microenvironments (TME), infiltrate tissues efficiently and can polarize into pro-inflammatory M1 or immunosuppressive M2 phenotypes. CAR-M technology, first introduced by Klichinsky et al. in 2020, uses adenoviral vectors to engineer macrophages, inducing an M1 phenotype and facilitating antigen-specific phagocytosis and neoantigen presentation.[49] CAR-M can also degrade the extracellular matrix (ECM) and promote immune infiltration, especially when modified with alternative signaling domains like CD147. Challenges include their short lifespan, high vector requirements, and inhibitory "do not eat me" signals such as CD47/SIRPα. Blocking this pathway, via gene editing or anti-CD47 antibodies, enhances phagocytosis but carries safety concerns, as seen in clinical setbacks with magrolimab. Alternative delivery methods, including lipid nanoparticles and intratumorally gene delivery, are under investigation to improve CAR-M efficacy.[50]

Next-Gen CAR-T Strategies in Solid Tumor Immunotherapy:

Despite the rapid development of innovative approaches to enhance immune cell therapies for solid cancer, only a limited number have progressed to clinical trials. A primary focus of current investigations is overcoming tumor heterogeneity. Most advanced products target no more than two antigens, as demonstrated in ongoing studies. For instance, early findings from a trial using EGFR/IL13Rα2 bispecific CAR-T cells in six patients indicated acceptable safety profiles.[7]

New techniques aimed at dropping the antigen recognition threshold of CAR immune cells are also under evaluation. Although these approaches show potential for tumors with low antigen expression, their safety remains uncertain. STAR and TRuC platforms, targeting mesothelin-positive tumors, are in phase I testing. Similarly, receptor-optimized CAR products with IL-8R and IL-7RA modifications, and CXCR5-modified CAR-T cells for EGFR-positive non-small cell lung cancer, aim to improve cell recruitment to tumor sites.[51,52]

Many trials emphasize improving T-cell fitness and persistence. Notably, ROR1-specific CAR-T cells with constitutive c-Jun expression achieved a 37.5% overall response rate (6/16 patients).[53] Additional strategies employ genome editing to resist TME suppression, including PD-1, CTLA-4, and TGF-βR knockouts. Moreover, several "armored" CAR-T products, such as MSLN-specific CAR-T cells secreting PD-1/CTLA-4 nanobodies, IL-15 or IL-21-enhanced variants, and those expressing constitutively active IL-7 receptors, are under active investigation.[54,55]

Conclusion:

Even though CAR T cell therapy has faced significant hurdles in demonstrating effectiveness against solid tumors, recent research provides encouraging evidence of its potential. Advances in understanding both solid cancer biology and T cell function have driven the development of numerous plans to enhance CAR T efficacy, including overcoming tumor heterogeneity, remodelling the tumor microenvironment, and engineering cells with superior persistence and resistance to immunosuppression. One of the primary obstacles remains the limited availability of specific tumor-associated antigens; however, emerging diagnostic technologies are expected to facilitate the identification of novel targets and improve patient selection.

In the coming years, data from first-in-class, next-generation CAR-T products will likely offer critical insights into the complex interactions between tumors, immune cells, and the tumor microenvironment. These findings will not only validate new therapeutic approaches but also guide iterative refinements in design and delivery. Furthermore, integrating complementary strategies—such as combination therapies, gene-editing innovations, and armored cell constructs—holds promise for overcoming current barriers. Ultimately, continued progress in clinical and translational research is expected to pave the way toward the shared objective in the field: achieving safe, effective, and potentially curative CAR-T cell therapies for patients with solid tumors.

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