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Armoring Strategies and Non-viral Engineering in Clinical Trials of CAR-T Cell Therapy

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Abstract. Chimeric antigen receptor (CAR)-T cell therapy has shown significant results in treating blood cancers, but it is not widely used for other reasons: high production costs, long-term manufacturing cycles, severe toxicity, and relatively poor efficacy against solid tumors. The two main directions of improvement have developed in parallel: "armoring" strategies that equip CAR-T cells with additional functional modules to counteract the immunosuppressive tumor microenvironment (TME), and non-viral engineering platforms that eliminate viral vectors to enhance safety and shorten production. Each approach has shown good clinical results but faced different translational barriers. This paper critically examines the current evidence for both strategies, identifies the remaining problems, and puts forward that non-viral platforms may serve as the enabling framework for the safe and effective clinical application of armored CAR-T products. The following are the main problems discussed in this paper: efficacy in solid tumors, compounded toxicity risks and regulatory standardization; corresponding countermeasures are proposed to provide a path for their eventual application in daily clinical practice.

Keywords: Armoring Strategies, Non-viral Engineering, CAR-T Cell Therapy

1. Introduction

With the development of CAR-T cell therapy, new treatment options have been introduced for relapsed or refractory B-cell acute lymphoblastic leukemia, diffuse large B-cell lymphoma and multiple myeloma [1]. However, long-term remissions are still suboptimal for a considerable proportion of patients; for example, the 4-year progression-free survival of BCMA CAR-T in multiple myeloma is only 37.4%, and the 6-year progression-free survival in relapsed/refractory CLL is 17.8% [2, 3]. The therapeutic index is also relatively small; the incidence of any-grade cytokine release syndrome (CRS) is about 83% across all products and disease settings, and immune effector cell-associated neurotoxicity syndrome (ICANS) occurs in about 28.3% of patients overall [4, 5]. In addition, on-target/off-tumor effects, that is to say, CAR-T cells attack non-malignant tissues expressing the target antigen, are still serious safety problems, and the extent of the damage depends on the expression pattern of the target antigen in healthy tissues. The previous CAR-T production method randomly inserted the transgene into the host genome via lentiviral or retroviral transduction. This way has an inherent risk of insertional mutagenesis, requires extensive quality control, and has a turnaround time of several weeks; this is too long for patients with aggressive

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diseases. Viral vector production is relatively expensive; thus, it has not been widely used abroad. The above problems have motivated continuous improvements in CAR-T function and manufacturing.

To solve the above problems, two general strategies have been proposed. The first type, generally referred to as "armoring", is a genetic modification that allows CAR-T cells to secrete cytokines, express membrane-bound costimulatory molecules, or convert inhibitory signals into activating signals to overcome TME-mediated suppression. The second method is not a viral carrier; instead, high-precision, shorter-production-cycle non-viral delivery systems are used, such as mRNA electroporation, transposon-based integration and CRISPR-mediated site-specific knock-in, to reduce the risk of genomic damage. Historically, the two approaches have developed independently. New technologies have recently emerged and can be combined with the previous ones. A serious deficiency in the current studies is that, although individual studies on each of the two strategies have been conducted, there has been no systematic review and analysis of their combined application.

Therefore, this paper will introduce the current status of each field, evaluate the strengths and weaknesses of these fields, and explore the feasibility and challenges of integrating them into a unified clinical platform to provide a theoretical reference basis.

2. Armoring strategies for CAR-T cells

Functionality Armored CAR-T cells are a fourth-generation design that goes beyond simple antigen recognition and cytotoxicity [6]. The idea is to give the engineered T cell some extra tools for continuous division, resistance to exhaustion, and modification of the local immune environment. Common armoring modules include cytokine secretion cassettes (e.g., IL-12, IL-15, IL-18), constitutively active costimulatory receptors, and "switch" receptors that repolarize inhibitory checkpoint signals into activating stimuli.

Clinical verification of this idea is accumulating. In a phase I study of huCART19-IL18, an IL-18-secreting armored CAR-T directed against CD19, the B-ALL cohort enrolled 10 patients, and all 7 evaluable for safety and efficacy achieved minimal residual disease-negative complete responses, including those with CNS involvement. CRS was mainly low-grade (≤2), and neurotoxicity was reversible; there were no relapses at a median follow-up of 15 months [7]. In a separate cohort of patients with CLL/SLL and Richter's transformation who had failed prior BTK inhibitor and venetoclax therapy, 10 patients were enrolled, and of these, 7 were evaluable for safety and efficacy (6 with CLL/SLL and 1 with Richter's transformation). All 7 patients achieved successful manufacturing at the protocol-assigned target dose level. Although the sample size is small, the first efficacy data are promising; among the 7 evaluable patients, long-term remission has been achieved. At 3, 6, 9 and 12 months after infusion, the responses were assessed according to the iwCLL criteria for CLL/SLL patients and the Lugano criteria for Richter's transformation patients [8]. The above results indicate that armor may reduce the immunosuppressive effect of the previous treatment, and the researchers have observed that the data support further expansion of this group.

However, the increased function of armored CAR-T cells also brings more safety problems. The same cytokines that improve T-cell fitness can also cause severe systemic inflammation when produced excessively. CRS and ICANS are more difficult to handle, and the manufacturing process is further complicated by the demand to co-express additional transgenes while maintaining product homogeneity and stability. Although early-stage trials have been conducted for solid tumors, the objective response rates are relatively low due to the tumor microenvironment (TME).

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3. Non-viral engineering platforms: precision and safety

Non-viral engineering refers to the ways that do not use viral vectors to avoid the risk of random integration and significantly reduce production cycle. The simplest mode is mRNA electroporation; it delivers transient CAR expression without genomic integration and has a good safety profile but lacks durability.

For long-term expression that is not viral, transposon-based systems such as Sleeping Beauty and piggyBac are available. These systems have a more favorable distribution of the CAR gene in the genome compared with viral vectors. The TcBuster platform has shown a small vector copy number and a safe integration profile in clinical-grade manufacturing. CRISPR-Cas9 and transposon technology can be used together to achieve site-specific insertion at safe-harbor loci for stable expression and gene disruption simultaneously.

Transposon systems are safer than viral vectors, but there is still a residual risk of insertional mutagenesis and prolonged follow-up is required.

CRISPR-mediated site-specific knock-in is a relatively advanced non-viral method clinically. BRL-201, a CD19-targeted CAR precisely integrated into the PD-1 locus, has been evaluated in a first-in-human phase I investigator-initiated trial (NCT04213469). From May 2020 to August 2021, 25 patients with relapsed/refractory B-cell non-Hodgkin's lymphoma were included, and 21 of them received BRL-201 infusion. The median age was 56 years (range: 34-70), the median number of prior therapy lines was 4 (range: 1-9), 17 patients (81%) had stage III/IV disease, and 13 (62%) were classified as high-intermediate to high risk according to IPI/aaIPI. The median follow-up time was 37.0 months, the total response rate reached 100%, and 18 of the 21 patients (85.7%) achieved complete remission. The median progression-free survival was 19.5 months (95% CI: 5.15-33.86) and the 12-month overall survival rate was 76.2%. Notably, there was no severe cytokine release syndrome (grade ≥3) or severe ICANS; mild (grade 1-2) CRS occurred in 66.7% of the patients (14/21), and grade 1-2 ICANS occurred in 19.0% (4/21). The production time was only 3 days. Four-year follow-up data (median 55.2 months) further confirmed prolonged responses, and the estimated 5-year PFS and OS rates were 40.6% and 42.9%, respectively [9].

Another preclinical study targeting the CISH locus for a FAPα-directed CAR in glioblastoma models used a non-viral cssDNA-based CRISPR-Cas9-mediated site-specific integration technology to achieve both CAR expression and CISH knockout [10]. FAPα expression was significantly elevated in glioblastoma compared to normal brain tissue and was associated with poor prognosis. In orthotopic glioblastoma xenograft models, these CISH locus-targeted FAPα CAR-T cells showed enhanced proliferative ability, significantly improved cytotoxicity against FAPα-positive targets in vitro, and extended survival with superior tumor control in vivo compared to controls. Modified CAR-T cells in patient-derived glioblastoma organoids rapidly caused structural damage to the tumor tissue. The above results show that multiplexed non-viral engineering is feasible; that is, CAR integration and checkpoint gene knockout can be achieved simultaneously at a single locus, and this strategy warrants further investigation in solid tumors.

In vivo CAR-T generation is the most advanced stage. Rather than ex vivo modification, the CAR gene or mRNA is directly added to the patient's T cells by a virus or lipid nanoparticle (LNP) vector. KLN-1010, a lentiviral-based in vivo CAR-T for multiple myeloma, demonstrated CAR-T expansion without lymphodepleting chemotherapy [11]. Legend Biotech's LB2501, an in vivo CD19/CD20 dual CAR-T for B-NHL, has shown a 100% objective response rate and an 83.3% complete response at the higher dose level [12]. LNP-based mRNA delivery systems are also being developed for repeated administration and prolonged CAR expression.

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In vivo delivery is also not highly specific, and off-target uptake by hepatocytes or other organs may lead to unexpected toxicity. Crucially, for solid tumors, the objective response rates achieved by non-viral CAR-T products have been significantly lower than those in the blood, indicating that safety and speed alone are insufficient to overcome the TME barrier.

4. Discussion: towards a unified approach

4.1. Structure rationale for convergence

Armoring and non-viral engineering address the deficiencies of conventional CAR-T therapy in tandem. Armored CAR-Ts have excellent functional performance, such as prolonged persistence, resistance to exhaustion and modification of the TME, but they are more complex to manufacture and may have toxicity. Non-viral platforms offer a safer, faster and more economical manufacturing route; however, they have not yet been able to eliminate random integration risks and lack the prolonged efficacy required for solid tumors.

Their convergence offers an attractive value proposition: by using non-viral platforms as the delivery backbone for armored constructs, a product that is both functionally enhanced and genomically safer can be rapidly realized. CRISPR-mediated site-specific integration, such as PD-1 or CISH locus targeting, can simultaneously insert the CAR gene and disrupt a checkpoint in a single step. Extending this to include cytokine-secreting cassettes or other armoring modules is technically feasible, and the resulting product would still have a non-viral integration profile. A short production cycle (e.g., 3 days for BRL-201) can be used to treat rapidly progressing diseases promptly, and armoring components may retain activity in the immunosuppressive TME.

4.2. Challenges and countermeasures

Nevertheless, some problems need to be solved before the joint application of these methods.

First, clinical data on the efficacy of non-viral armored CAR-T in solid tumors are still lacking. Current non-viral trials have not shown good objective response rates in solid tumors, and it is unknown whether armoring modules that address TME immunosuppression will have a positive clinical effect; this needs to be studied. Dedicated Phase I/II trials that incorporate TME-based biomarkers (e.g., infiltration status, PD-L1 expression, cytokine profiles) should identify responsive subgroups. Select Armoring modules based on the immunosuppressive mechanism of the tumor; use IL-15 for tumors with poor baseline T-cell infiltration, or IL-18 where myeloid-derived suppressor cells predominate.

Second, safety may be cumulative rather than merely additive. Armor already increases the risk of CRS and ICANS by continuously releasing cytokines; non-viral integration reduces insertion risk but does not directly address cytokine-mediated toxicity. Therefore, the combined product will have a double safety risk of being both genotoxic and immunotoxic. Good Design of a dose-finding experiment with a modified toxicity probability interval can be employed for safety management to determine the maximum tolerated dose and reduce toxicity. Inducible safety switches—such as iCasp9 or truncated EGFR—could provide fail-safe ablation in severe toxicity. Based on the initial inflammatory indicators (CRP, ferritin, IL-6), stratify the risk of the patients.

Third, the selection of transient (mRNA-based) or persistent (integrating) non-viral delivery affects the duration of effect. For chronic solid tumors that require continuous CAR-T presence, transient expression may be insufficient; for autoimmune diseases where a short-term immune reset is sufficient, mRNA-based armored CAR-T could be a safer option. Thus, the optimal platform will

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likely be disease- and indication-dependent. A modular approach can match the platform to the indication: integrate systems (transposon- or CRISPR-based) for solid tumors requiring prolonged activity, mRNA-based methods for short-term intervention, or hybrid inducible designs that separate CAR expression from armoring function.

Fourth, in vivo generation of armored CAR-T cells is still a difficult technical problem. Delivery of multiple genetic components (CAR + armor) specifically to T cells via targeted LNPs or engineered viral particles still has unsolved problems in targeting efficiency, payload capacity and expression durability. Iterative Optimization of LNP Formulation and T-cell-targeting Ligands is Advancing. Combinatorial Targeting Strategies: Dual-ligand LNPs that recognize both CD3 and tissue-specific markers could enhance specificity, and tissue-restricted promoters may limit expression to T cells. Although there are still some problems, the speed of innovation is relatively high; therefore, it may be achieved in the next ten years and will no longer require ex vivo manufacturing.

Finally, the regulatory and manufacturing systems for combined non-viral armored products have not been standardized. Characterization of insertion sites, off-target editing and functional stability for multiple transgenes will require extensive analytical validation, and the complexity of co-expressing CAR and armoring modules from a single construct adds uncertainty to product consistency. For regulatory standardization, the successful regulatory review of BRL-201 provides a reference; in cooperation with the industry, academia and regulatory authorities, consensus guidelines will be established for insertion site analysis, off-target assessment and potency testing to streamline the approval process for more complex variants.

In short, the convergence of armoring and non-viral engineering is both theoretically feasible and practically achievable if the problems mentioned above can be systematically solved by cooperative and well-designed research and development.

5. Conclusion

Armoring and non-viral engineering are complementary innovation paths that address the main deficiencies of traditional CAR-T cell therapy systematically. Armor expands the functional lifespan of cells through proliferation, avoids exhaustion, and actively modifies the immunosuppressive tumor microenvironment; non-viral delivery systems, such as transposon-based integration, CRISPR-mediated site-specific knock-in and mRNA electroporation, are relatively safer, faster and more scalable manufacturing routes that do not carry the risk of insertional mutagenesis from viral vectors. The merger of the two branches, promoted by programmable nucleases and advanced lipid-nanoparticle technology, has created a technical foundation for developing next-generation CAR-T products that are highly potent, genomically stable and logistically feasible.

Clinical translation of this unified approach, however, faces several interconnected problems. Given the combined toxicity of prolonged cytokine release and strong T-cell activation, a more precise dose-finding strategy and the addition of fail-safe devices, such as an inducible suicide switch, are required. Manufacturing consistency for constructs co-expressing multiple transgenes requires strict process validation to ensure batch-to-batch reproducibility. Regulatory systems for combined non-viral armor products are still developing, and unified rules for insertion-site analysis, off-target effects and potency tests are needed. Good clinical trials that add adaptive biomarker-driven stratification and extended follow-up will need to be conducted to confirm the long-term effectiveness of the response and optimize patient selection for both blood and solid tumors.

In the future, good armoring modules will be attached to the non-viral core to create a new kind of drug delivery system. Reduce the manufacturing cycle to a few days, eliminate the risk of viral

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transmission, enhance the robustness of CAR-T cells against the tumor microenvironment, and expand access to underserved populations and curative-intent therapy for solid tumors that are currently difficult to treat. Although there are still problems, rapid development in technology and extensive clinical data have given people some hope; through joint efforts, armoring and non-viral engineering are expected to be applied to next-generation cellular immunotherapy.

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