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Research advances of nanoparticles for CAR-T therapy in solid tumors

Bohan Chen^{a,b,c}, Liming Gong^{a,b}, Jing Feng^{a,b}, Mingji Jin^{a,b}, Liqing Chen^{a,b},
Zhonggao Gao^{a,b}, Wei Huang^{a,b,*}

^a State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Department of Pharmaceuticals, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China

^b Beijing Key Laboratory of Drug Delivery Technology and Novel Formulations, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China

^c School of Pharmacy, Qingdao University, Qingdao 266071, China

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ABSTRACT

As one of the most promising adoptive T-cell therapies, chimeric antigen receptor T-cell (CAR-T) therapy has acquired Food and Drug Administration (FDA) approval for a variety of products and has been used successfully in the treatment of malignant hematological tumors. CAR-T therapy, on the other hand, faces a number of obstacles in the field of solid tumor therapy that limit its widespread clinical implementation. Significant advances in nanoparticle research in cancer therapy and immunotherapy have been made in recent years, providing novel strategies to address the challenges encountered by CAR-T therapy in the treatment of solid tumors. This review commences with a comprehensive explanation of the basic framework of CAR-T therapy as well as the challenges it faces in the treatment of solid tumors. Subsequently, we encapsulate a summary of the developmental research combining nanoparticles with CAR-T cells for the treatment of solid tumors, which includes gene transfection, cell activation and expansion, targeted infiltration, immune escape inhibition, and combination with other therapies. Coupled with the overview of the research progress, a discussion has been initiated on the challenges and perspectives of CAR-T based on nanoparticles.

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1. Introduction

Tumor immunotherapy has indisputably revolutionized the therapeutic landscape of malignancies. The advent of tumor immunotherapy can be traced back to 1936 with the Food and Drug Administration (FDA)'s approval of interferon α (IFN α), marking an officially inaugurated developmental phase for this field [1]. Subsequently, tumor immunotherapy has gradually expanded to include lysosomal virus therapy, tumor vaccines, cytokine therapy, immune checkpoint blockers and adoptive T-cell therapy [2]. Currently, adoptive T-cell therapy (ACT) exists as a swiftly maturing forefront in the arena of tumor immunotherapy that includes naturally-occurring tumor-infiltrating lymphocyte (TIL) therapy, T-cell receptors that targeting specific tumor antigen (TCR-T) therapy, and chimeric antigen receptor T-cells (CAR-T) therapy [3]. Preliminary experimental outcomes associated with *in vitro* infusion of autologously expanded TILs have indicated durable complete responses in a subgroup of patients. However, ACT of tumor-responsive TILs

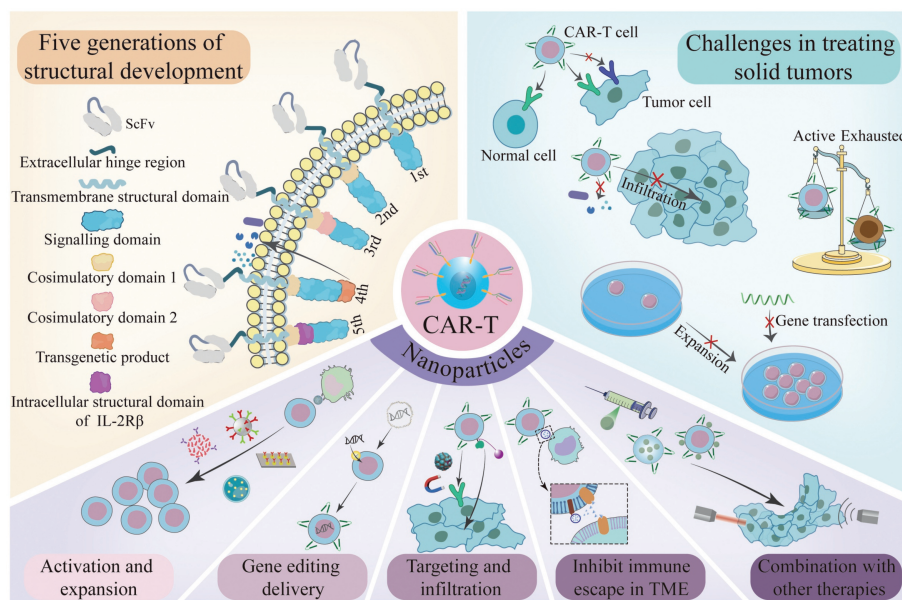
has not so far become a standard approach to cancer treatment, given the complexity, cost and technical difficulty of the therapy [4]. Since 2006, a series of clinical studies have elucidated the potential of TCR-modified lymphocytes for mediating tumor tissue regression [5], but this approach is limited by the need for major histocompatibility complex (MHC) complexes during treatment. In contrast, CAR-T therapy is able to specifically recognize targets on cancer cells, is not limited by the MHC complex and replaces the TCR-T functionally, thus becoming the latest option in the field of ACT [6]. At the moment, the FDA has authorized seven CAR-T therapy agents, all of which are used to treat B-cell lymphoma by targeting CD19, with the exception of Abecma and Carvykti, which are used to treat multiple myeloma by targeting B cell maturation antigen (BCMA) [7,8]. Despite its remarkable effectiveness in malignant hematological diseases, CAR-T treatments encounter a variety of challenges in solid tumors. These barriers include systemic toxicity caused by target antigen expression in normal tissues, tumor tissue heterogeneity limiting CAR-T efficacy against specific targets, vascular abnormalities in tumor tissues impeding CAR-T infiltration within the tumor, poor *ex vivo* activation and amplification of CAR-T, and issues with tumor microenvironment (TME) escape from the

* Corresponding author.

E-mail address: huangwei@imm.ac.cn (W. Huang).

Table 1
Clinical trials of CAR-T products for solid tumors.

Antigen	Cancer	Year	Trial registration No.	Ref.
CLDN18.2	Gastric cancer	2019	NCT03874897	[12]
MSLN	Malignant pleural mesothelioma	2015	NCT02414269	[13]
mCRPC	Prostate cancer	2018	NCT03089203	[14]
GD2	Diffuse pontine glioma	2019	NCT04196413	[15]
GPC3/MSLN/CD20	Hepatocellular carcinoma/prostate cancer/ovarian cancer	2017	NCT03198546	[16]
EGFR	Non-small cell lung cancer	2017	NCT03182816	[17]
cMET	Metastatic melanoma/triple negative breast cancer	2017	NCT03060356	[18]
HER2	CNS tumor	2018	NCT03500991	[19]
CD133	Hepatocellular carcinoma	2015	NCT02541370	[20]

**Fig. 1.** The framework for the research advances of nanoparticles for CAR-T therapy in solid tumors. This paper is divided into three main parts: (1) Introduction to the structure of CAR-T and its iterative development. (2) Challenges faced by CAR-T drugs in the treatment of solid tumors. (3) Application of nanoparticles in CAR-T therapy which are divided into five main categories based on the role they play.

immune response [9,10]. Despite the fact that clinical as well as preclinical trials of CAR-T therapies for solid tumors are now underway [11] (Table 1), there are no FDA-approved CAR-T medications on the market that can be used to treat solid tumors due to the limitations of the aforementioned issues.

Nanoparticle, owing to its excellent capabilities, has found broad applications in oncology, notably in the collaborative realm of immunotherapy [21]. For example, one study used iron oxide nanoparticles and dual-targeted doxorubicin liposomes to achieve a combination of chemotherapy and immunotherapy that reversed tumor resistance [22]. Nanoparticle introduces innovative strategies to tackle the obstacles faced during CAR-T therapy for solid tumors. Within this review, we summarize the latest research progress on nanoparticles in improving CAR-T therapy for the treatment of solid tumors. In particular, we highlight the application of nanoparticles in T-cell gene editing delivery, the potential of nanoparticles in enhancing CAR-T activation and expansion, the role of nanoparticles in facilitating CAR-T targeting and infiltration into tumor tissues, the role of nanoparticles in inhibiting the TME in response to the escape of immune response, and the prospect of nanoparticles in combination with other therapies (Fig. 1).

2. CAR-T therapy

CAR-T therapy is an emerging ACT with chimeric antigen receptors capable of directly targeting antigens on the surface of tumor cells, independent of the MHC molecular restriction of the

TCR. This asserts that CAR-T cells possess the capability to attack and destroy tumor cells, independent of a single HLA haplotype, thereby rendering them universally applicable for all patients [4]. Commencing from the inception of the CAR concept in 1989, CAR-T therapy have evolved through five generations. From the original first-generation CAR, which consisted of a single-chain fragment variable (scFv) originated from an antibody and an intracellular signaling structural domain with a CD3 ζ -chain, to the second-generation CAR, which introduces co-stimulatory structural domains to increase the activity of CAR-T cells [23]. Subsequently, to extend the capabilities of CAR-T cells, cytokines were added to the therapy based on the three-signaling principle of T-cell activation, including CAR-T cell release, anchoring of cytokines to the exterior of CAR-T cells, construction of activated cytokine receptors and so on [24]. The latest fifth-generation CAR integrates binding sites for signal transducer and activator of transcription 3 (STAT3) transcription factor and interleukin 2 (IL-2) receptor to the second-generation CAR, which triggers the activation of JAK-STAT and thus promotes CAR-T proliferation [25].

2.1. Structure

In the structure of CAR-T cells, they exhibit the expression of genetically engineered receptor, which complements the pre-existing TCR, thus providing novel abilities for targeting specific antigens. The receptor is chimeric and consists of four modules: (1) antigenic domain; (2) extracellular hinge region; (3) transmem-

brane structural domain; (4) intracellular T-cell signaling domain [26].

2.1.1. Antigenic domain

Within the structure of CAR, the antigen recognition domain is embodied by a scFv, usually derived from monoclonal antibodies. It is comprised of variable light chain (V_L) and variable heavy chain (V_H) regions joined together by the linker segment [27]. Ligands and CARs for new cancer-related receptors are currently being actively investigated and characterized, mostly based on the extracellular structural domains of natural ligands, mimicking the natural binding of ligands to receptors. For instance, an experimental model of CAR-T, derived from the extracellular structural domain of CD6, showed potential cytotoxic effects on CD166-positive cells (CD6-CAR-T), thereby suggesting its efficacy in colorectal cancer (CRC) treatment [28]. Given that the heterogeneous expression of antigens can influence CAR-T effectiveness in solid tumors, designing CARs for co-targeting two tumor-associated antigens is a popular research direction. For example, researchers constructed a bivalently linked CAR (TanCAR) that utilizes CD70 and B7-H3 scFv as external binding domains for targeting tumor cells. This TanCAR was able to induce more potent cytotoxicity and cytokine release in tumor cells co-expressing both target antigens [29].

2.1.2. Extracellular hinge region

The extracellular hinge region serves as a vital connector between the antigen recognition domain and the trans-structural domain, which is a dynamic portion of the CAR and can be related to CAR-T cell function regulation [30]. For instance, scholarly investigations have led to the development of two hinges, namely N3 and N4, that are based on the human nerve growth factor receptor NGFR/CD271, which contribute significantly to the efficiency of enrichment of transgenic CAR-T cells. The introduction of N3 and N4 hinges allowed the detection of CAR-positive cells *in vitro* and *in vivo* by direct-coupled antibody staining and flow cytometry, and compared to CAR-T cells with CD8 hinges, they proved efficacious on hematopoietic stem cells *in vitro*, and echoed similar effects on monocytic leukaemia within the context of an immunodeficient mouse xenograft model [31].

2.1.3. Transmembrane structural domain

Transmembrane structural domains, situated between the hinge region and the intracellular signaling structural domains, are derived from natural proteins such as CD3 ζ , CD4, CD8, and CD28. The critical role of the transmembrane structural domains lies in anchoring the CAR to the T-cell surface and correlating with the effector activity of CAR-T cells [32]. For instance, a VHH nanobody (D4)-based CAR-T was designed to target glypican-1 (GPC1) membrane-distal epitopes. The rigid IgG4 hinge and CD28 transmembrane structural domains facilitated the close approximation of two D4 fragments, thereby inducing the dimerisation of CAR and enhancing T cell signaling, which resulted in tumor regression in pancreatic cancer models [33].

2.1.4. Intracellular T-cell signaling domains

Intracellular T-cell signaling domains, also known as endodomains, function through the coordination and recruitment of signaling elements by immune receptor tyrosine-based activation motifs (ITAMs) [27]. Conventional first-generation CAR-T endodomains contain only one signaling domain, usually the CD3 ζ chain. Subsequent development of second-generation CAR-T internal domains introduced co-stimulatory domains, mainly from CD28, 4-1BB, OX40, or CD27, whereas third-generation CAR-Ts integrate several co-stimulatory domains based on the second generation [23]. The relative efficacies among CAR variants remain undefined and warrant further investigations. Researchers constructed a

novel CAR-T targeting mesothelin (MSLN) by mutating two ITAMs in the inner domain of the CD3 ζ chain to a CD3 ζ chain and CD28 co-stimulatory domain containing only one ITAM (M1xx). Compared to conventional second-generation CAR-Ts targeting MSLN (encoding CD28 co-stimulatory domains or encoding 4-1BB co-stimulatory domains), they showed higher anti-tumor potency and durability in breast cancer models [34].

2.2. Difficulties faced in the treatment of solid tumors with CAR-T therapy

2.2.1. On-target off-tumor (OTOT) toxicity due to target antigen expression in normal tissues

Tumor-associated antigens (TAA), which are over-expressed in malignant tumors or exclusively at certain phases of differentiation, constitute a common target for therapies such as CAR-T therapy and immunotherapy [35]. Nevertheless, expression of these antigens is not restricted to tumor tissues, as they can also appear in normal tissues. Currently, clinical trials of CAR-T therapies against solid tumors mainly target MSLN, glypican-3 (GPC3), gangliosides-2 (GD2), epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor-2 (HER2) [36]. Immunotherapies targeting MSLN, for instance, could potentially initiate inflammatory assaults on MSLN(+) mesothelial cells surrounding vital visceral organs. This risk has been corroborated by studies investigating the toxicity of systemically administered CAR-T that targets MSLN [37]. Similarly, pruritus and severe upper gastrointestinal bleeding have been observed in clinical studies of CAR-T targeting HER2 and EGFR [38,39]. Currently, studies have made some progress in regionally delivered CAR-T therapies [13], such as regionally delivered CAR-T targeting MSLN, which has led to an improvement in the safety of CAR-T therapies. Nevertheless, OTOT toxicity engendered by CAR-T cells targeting normal tissues presents a substantial limitation to their broader implementation.

2.2.2. Tumor heterogeneity limits CAR-T therapy

Tumor heterogeneity refers to the fact that tumors undergo numerous divisions and ascend in value, resulting in progeny cells exhibiting modifications in molecular biology or genetic structure. Such heterogeneity can manifest in various forms including molecular divergences among patients harboring the same type of tumor, discrepancies at distinct locations or temporal variations within the same patient, and even differentiation into other cellular lineages *via* genetic mutations [40]. When cancer cells mutate, potential loss of antigens recognized by CAR-T cells may happen culminating to antigen escape [41], which induces tumor tissue resistance to treatment. In turn, increasing the administered dose against tumor resistance creates the risk of inducing OTOT toxicity. Currently, the main approaches to address this aspect by designing CARs are to enhance the sensitivity of CARs to tumor antigens and TanCARs that can target both antigens as mentioned earlier [42].

2.2.3. Insufficient targeting and infiltration of tumor tissue by CAR-T

There are two key explanations for the better efficacy of CAR-T therapy against malignant hematological tumors. First, as CAR-T cells are usually administered intravenously into the systemic circulation, they can directly interact with hematological tumor cells [41]. Secondly, hematological tumor cells usually express a tumor-specific antigen (TSA) on their surface, such as CD19, which has been targeted by marketed CAR-T products. However, solid tumors are typically characterized by the expression of TAA, and these antigens are also present in normal tissues, which prevents CAR-T targeting TAA from accurately identifying somatic tumors [10]. For T cells to be sufficient to migrate from blood to peripheral tissues, this process necessitates three stages: the initial transient

attachment coupled with selectin-mediated endothelial rolling, integrin activation mediated by chemokines, and finally, integrin-dependent migration coupled with infiltration [43]. However, CAR-T cells' ability to extravasate and infiltrate in tumor tissues is insufficient due to the complex and poor vascular system and the down-regulated expression of chemokines and adhesion molecules in tumor tissues [44]. Also the mismatch of chemokine receptors on CAR-T cells and the aberrant chemokine expression in tumors might lead to a reduction in T cell migration to the tumor site [45].

2.2.4. Inefficiencies and risks of the CAR-T production process

The entire cycle of CAR-T production usually includes the following steps: initially, a blood sample is collected from the patient and leukocyte isolation is performed to procure T cells. In this regard, microfluidic chip technology may have a whole new role to play [46]. Presently, some researchers are exploring the use of unisolated peripheral blood as the material [25]. Subsequently, the T cells must be activated, commonly by using immunomagnetic beads covered with anti-CD3/anti-CD28 monoclonal antibodies to provide a co-stimulatory signal to the T cells [47], or by using paramagnetic beads conjugated with antibodies targeting CD14/CD16/CD19/CD56 to remove non-T cells, such as B cells, natural killer (NK) cells and monocytes, and then T-cell activation reagents are added to stimulate T-cell proliferation [48]. Following activation, CAR genes need to be introduced into T cells, which are usually transduced using viral vectors, including lentivirus (LV) and retrovirus (GRV), while non-viral transduction methods also exist [49]. Subsequent to this phase, the transduced CAR-T cells are cultured and undergone expansion in a medium enveloping recombinant IL-2 amongst other cytokines. However, CAR-T production is a personalized process with complex procedures and limited expansion efficiency. One review reported that NKG2D CAR-T was amplified up to a maximum of only 400-fold [50], while utilizing viral vectors carries the risk of residual viral and oncogenic elements [51]. This obstacle prevails not solely in CAR-T therapies targeting solid tumors, but also extends to the production of CAR-T targeting malignant hematological tumors.

2.2.5. Immunosuppression of CAR-T by TME

TME is understood as an umbrella term encapsulating non-malignant cellular constituents, metabolites and secretory products extant within the tumor, including cellular components such as extracellular matrix (ECM), fibroblasts, macrophages, lymphocytes, endothelial cells. The secretions and cytokines of these cells are also important components of the TME [52]. It is now believed that these TME components participate in cross-talk with tumor cells to support tumor growth and metastatic dissemination [53]. The tumor immune environment (TIME), on the other hand, could be defined as the environment where interactions between the TME and an array of immune cells, along with their respective products, take place. A dysfunctional TIME may lead to immune evasion of the TME through immunosuppression [54]. Immunosuppressive cell populations (e.g. tumor-associated macrophages, regulatory cells (Tregs), and cancer-associated fibroblasts), secreted factors (e.g. cytokines and chemokines), and low levels of glucose, hypoxia, and acidity due to the Warburg effect are the main inhibitory components in TME [41]. Alterations in collagen density and tissue rigidity caused by non-coding RNA derived from tumor-associated macrophages (TAM)-derived exosomes [55] and ECM remodeling [56] have been identified to enhance immunosuppression. Immunosuppression produces inhibitory molecules/receptors as well as sustained antigenic stimulation in the TME leading to T-cell depletion [57]. CAR-T cells, originating from modified T-cells, necessitating activation, signal transduction, and proliferation mechanisms to ensure effectiveness, are consequently also susceptible to TME triggered immunosuppression.

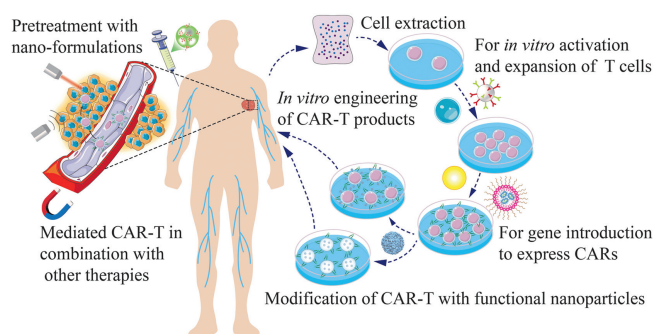


Fig. 2. Nanoparticles demonstrate applicability across the entire cycle of CAR-T therapy: (1) T-cell expansion, e.g. aAPCs and AuNPs-modified hydrogels. (2) Transfection of CAR genes, e.g. LNPs and AuNPs. (3) Functionalised modification of CAR-T, e.g. coupling and membrane encapsulation. (4) Pretreatment with nano formulations to improve CAR-T efficacy.

3. Nanoparticles in CAR-T therapy for the treatment of solid tumors

Over the past few decades, the field of cancer research has witnessed substantial advancement focused on the application of nanoparticle. Various nanoparticles such as lipid nanoparticles, polymer nanoparticles and inorganic nanoparticles have been developed to deliver chemotherapeutic drugs, nucleic acid drugs and immunotherapeutic drugs, etc. targeted to the tumor site in order to exert therapeutic effects and to improve therapeutic efficacy while reducing off-target toxicity [58]. Immunotherapy, evolving rapidly, has seen widespread application of nano-delivery technologies [59]. Recently, an array of nano-agents has been incorporated to augment anti-cancer immunity or work in synergy with existing immunotherapies. Nevertheless, CAR-T therapy continues to encounter multiple challenges in treating solid tumors. This article aims at delving deeper into the potential of nanotechnology to address these issues (Fig. 2). All the nanoparticles we summarized are shown in Table S1 (Supporting information) for comparison and contrast.

3.1. Nanoparticles for T-cell gene editing delivery

The initial stage in adoptive T-cell therapy necessitates the extraction of an ample quantity of host T-cells for *in vitro* manipulation. The successful integration of CAR-encoding genes is the decisive factor in the transformation of T-cells into CAR-T cells. Gene delivery methods can be divided into two categories: viral-mediated and non-viral-mediated. Viral-mediated delivery utilizes enveloped RNA viruses from the retroviral family, including gamma GRV and LV vectors. The primary distinction between these vectors lies in the fact that while GRV vectors are restricted to infecting dividing cells, LV vectors possess the capability to infect both dividing and quiescent cells [60]. Nonetheless, the viral-mediated methodology is associated with certain perils as the insertional mutations instituted to prompt an immune reaction may heighten the probability of tumorigenesis [61]. Clinical studies have shown that patients may experience serious adverse reactions, such as cytokine release syndrome (CRS), after treatment with CAR-T cells modified by viral gene transduction [62]. As contrasted with viral mediation, non-viral mediated methods include transposon transfection, electroporation and nanodelivery. Transposon transfection utilizes transposon DNA and transposases for stable gene delivery, with commonly used vectors such as piggyBac (PB) and Sleeping Beauty (SB), which are transferred from one gene location to another by a cut-and-paste mechanism [63]. The dual plasmid setup is commonly used to deliver transposons and transposases as it is

the simplest and most cost-effective method. However, the presence of unmethylated cytoplasmic DNA of bacterial DNA origin, especially unmethylated CpG dinucleotides, may trigger an immune response by activating intracellular DNA sensors, thus reducing the efficiency and safety of genetic engineering strategies [64]. Electroporation involves the process of subjecting a target cell to an electric field, thereby transiently disrupting the cell membrane and permitting the ingress of charged molecules. However, current electroporation devices have transfection efficiencies of about 40% or less, significant and irreversible damage to cell membranes leading to cell death if the resulting transmembrane potential is larger than 1 V [65]. Consequently, the *in vitro* programming mechanism of T cells is portrayed as complicated, inefficient, and lacking optimal safety standards.

In contrast, nanodelivery systems are highly customized methods for delivering nucleic acid molecules such as DNA, mRNA and siRNA, as well as their mixtures [66]. Because of their lower immunogenicity and improved safety profile as compared to other conventional delivery methods, nanoparticles have been generally recognized as a viable tool for T-cell gene transfer. For cationic nanoparticles, they attach to sulfated proteoglycans on the T cell membrane and are subsequently endocytosed into the cell. The nucleic acid cargo is then released upon escape from the endosomes and enters the host nuclear membrane *via* nuclear uptake [67]. Fan *et al.* then designed a three-band amphiphilic copolymer methoxypolyethylene glycol-branched polyethyleneimine-poly(2-ethylbutylphosphorane) (mPEG-bPEI-PEBP) with cationic PEI as a carrier, and delivered a plasmid that expresses the CAR by co-culturing it with *in vitro* cells [68]. However, PEGylated modification suffers from poor cell membrane affinity and low gene uptake, and the incorporation of peptide bonds between block copolymers that can be degraded by metallo-matrix proteases is a solution [69]. Recent research has revealed that using nanoparticle-loaded DNA enables *in vivo* programming of T cells to produce CAR, reducing the time necessary for generating CAR-T cells *in vitro*. The researchers utilized peptides carrying microtubule-associated sequences (MTAS) and nucleus localization signals (NLS) to modify biodegradable poly(β -amino acid) nanoparticles to facilitate the delivery of genetic material *via* a microtubule transport mechanism. Plasmid vectors encoding 194-1BBz CAR and highly active iPB7 transposase were then loaded into these nanoparticles to improve gene integration efficiency. Subsequently, an anti-CD3e-F(ab')₂ fragment was modified on the surface of the nanoparticles to enable them to target T cells' receptors [70].

Contrastingly to DNA, mRNA transfection permits instantaneous CAR expression, as it bypasses the necessity for a gene integration process inherent in DNA. However, the susceptibility of mRNA to degradation limits its application for *in vivo* delivery, and lipid nanoparticles (LNPs) have been used to engineer encapsulated mRNAs to improve their stability and mediate intracellular delivery [71]. Rurik *et al.* succeeded in programming T cells into CAR-T cells *in vivo* by loading mRNA using LNPs that can target CD5. Such CAR-T cells were able to effectively target fibroblast activation protein (FAP) to exert an anti-fibrotic effect, demonstrated trophocytosis, and successfully improved cardiac function in a mouse heart failure model [72]. Although the study did not address the field of CAR-T treatment of solid tumors, it certainly provides important guidance in terms of *in vivo* programming of nanoparticles in combination with CAR-T. Circular mRNA is a natural or synthetic closed mRNA without 3' and 5' ends, and its unique covalent closed structure avoids degradation by nucleic acid endonucleases. In this way, the *in vivo* stability of circular mRNAs is enhanced compared to linear mRNAs [73]. A study verified that circular mRNA expresses proteins with better stability and duration than linear mRNAs and constructed a circular mRNA-LNP system that showed strong *in vivo* editing capabilities [74]. Based on the advantages of LNP-

loaded mRNA for delivery, a study established a LNP, specifically designed for mRNA transfection aimed at the genetic modification of T cells. The researchers prepared 24 LNPs using different ionisable lipids with cholesterol, phospholipids and PEG-C14, and introduced LNPs carrying luciferase-encoding mRNA into Jurkat cells for screening, and ultimately selected the best C14-4 nanoparticles. In addition, through the isolation and purification method, the researchers prepared pure and fully saturated C14-4 LNP, and the transfection efficiency of the nanoparticles on primary T cells as well as the killing ability of transfected CAR-T cells on cancer cells were similar to that of the electroporation-mediated method [75]. Currently, a study developed an inorganic gold nanoparticle that enables mRNA transfection by vapor nanobubble (VNB) photoporation. This gold nanoparticle was 60 nm in size and covered with the cationic polymer poly(diallylammmonium dimethyl chloride) (PDDAC), which formed AuNP. mRNA was incubated with the cells during loading of mRNA into these nanoparticles. For HeLa cells adhering to the culture dish, the nanoparticles were endocytosed and brought close to the cell membrane, whereas for Jurkat cells suspended in the culture medium, the nanoparticles were adsorbed to the cell membrane. When irradiated with nanosecond laser pulses, the gold nanoparticles generate VNB. When the thermal energy is consumed, the VNB collapses, forming membrane pores that allow the mRNA within the nanoparticles to be transfected into the target cells. However, to avoid the effect of nuclease on the naked mRNA, co-incubation with the cells for 10 min using reduced serum medium (Opti-MEM) was required prior to performing a single pulse of laser irradiation. The outcomes of this study demonstrated a quintupling in the number of successful live cell transfections post-nanosecond laser pulse irradiation, relative to the standard electroporation method (Fig. S1 in Supporting information) [76]. Hence, the utilization of nanoparticles is speculated to supersede conventional viral transfection and non-viral electroporation techniques, providing enhanced transfection efficiency while diminishing toxicity.

3.2. Nanoparticles for T-cell activation and expansion

Subsequent to the segregation of T cells from human peripheral blood, a successful infusion requires efficacious activation and expansion of an adequate quota of tumor-specific cells. Currently, the use of magnetic beads containing anti-CD3/anti-CD28 co-cultured with T cells is the standard method for *in vitro* T cells expansion. Yet, this method encounters several complications such as an impaired antibody function at the surface and the lack of precision in the modulation of stimulatory signals [77,78].

To address these issues, Jovana Matic's team proposed for the first time a novel stimulation platform based on nano-arrays. They employed the block copolymer micellar nanolithography (BCML) technique for the systematic deposition of AuNPs onto a glass substrate, with flexibility in adjusting the interparticle spacing, and modified the surface of the AuNPs with immobilized anti-CD3, while adding free anti-CD28. This platform is capable of delivering antibodies with higher activity, and is able to precisely modulate the stimulation signal [79]. On this basis, a study used hydrogels instead of glass substrates and crosslinked cRGD peptides. cRGD promotes the $\alpha 5 \beta 1$ -associated signaling pathway, which is known to have a facilitating effect on T cell proliferation. In addition, the peptide-containing PEG hydrogel is superior to coated glass and avoids non-specific cell adhesion [80]. Another study chose to employ a TiO₂ rigid matrix as a platform, which also crosslinked the cRGD peptide, and was designed to be pretreated with phorbol ionomycin, 12-myristate 13-acetate (PMA) and protein translocation inhibitors. The findings indicated a further enhancement in the activation and expansion of T cells *in vitro* using the nanoarray stimulation platform under

the implemented pretreatment conditions (Fig. S2 in Supporting information) [81].

In the absence of a newly engineered stimulation platform, T cell stimulation can be accomplished by adding cytokines, thereby augmenting the expansion of anti-CD3/anti-CD28 magnetic beads. A more superior alternative involves the packing of protein nanogels on the T cell surface instead of the direct infusion of free cytokines. Tang *et al.* designed a disulfide bond-containing bis-*n*-hydroxysuccinimide crosslinker (NHS-SS-NHS), which can be cleaved under reducing conditions, according to the higher reducing circumstances on the surface of T cells. This crosslinker was crosslinked with protein drugs (IL-2Fc, IL-15sa) to form protein nanogels (NGs). A small proportion of anti-CD45 was modified on the surface of NGs, thereby prolonging the persistence of these gels on cell surfaces. Meanwhile, a small amount of poly(ethylene glycol)-poly(lysine) (PEG-*b*-PLL) was modified to form a covalent linkage with the residual NHS on the surface of NGs, which resulted in uniform positive ionic charge of NGs, thus achieving a uniform loading of NGs on the surface of the T cells. Compared with free cytokine cultures, this nanogel system showed enhanced *in vitro* value-added T cell value-added in the conditions of anti-CD3/anti-CD28 magnetic beads stimulation activity [82].

Apart from the nanoplatfroms articulated previously, researchers in the field are actively probing into alternative nanoplatfrom schemes. As a novel nanoparticle platform, nano-aAPC can mimic T cell recognition and *in vitro* stimulation for *in vitro* proliferation of T cells to improve their activation degree specificity and functional level [83]. Jonathan's team prepared iron-dextran aAPC by chemically coupling MHC-immunoglobulin dimer (MHC-Ig) and anti-CD28 on the surface of biodegradable particles (Mitenyi Biotec) to prepare iron-dextran aAPC. This nanoparticle has a diameter in the range of 50–100 nm and was found to be more functional in enriching and expanding *in vitro* T-cells compared to conventional particulate aAPC [84]. Subsequently, they improved the platform by changing the diameter of the nanoparticles to approximately 300 nm, which allowed the iron-dextran aAPC to be magnetically isolated by weaker magnetic fields (*e.g.* conventional neodymium magnets), thus adapting to the 96-well plate format and improving its parallel processing capability [85]. Previous aAPCs have mainly been used to expand CD8⁺ T cells, which play an significant role in direct tumor cell killing, but CD4⁺ T cells also have an essential function. Jonathan's team devised a novel MHC II aAPC, which involved the use of iron-dextran nanoparticles encapsulated with MHC II and co-stimulatory proteins. This aAPC can expand CD4⁺ T cells and delivers relevant signals from CD4⁺ T cells to CD8⁺ T cells, which helps CD8⁺ T cells to produce cytokines and improve anti-tumor activity [86].

Following the *in vitro* activation, proliferation, and CAR gene introduction of T cells, the recognition of target antigens becomes a prerequisite for their additional activation and expansion. One research utilized the innate targeting capability of albumin towards lymph nodes and accordingly conceptualized an amph-ligand-based chimeric receptor-enhanced vaccine. The ligand binds to albumin upon injection, enters the lymph nodes, and eventually binds to the surface of dendritic cells (DCs). When the DCs come into interaction with CAR-T cells, the amph-ligand binds to the CAR while the DCs provide co-stimulatory signals to the CAR-T cells, activating the *in vivo* proliferation of CAR-T cells [87]. This represents a significant advancement in the realm of CAR-T cell therapy strategies for solid tumors. Meanwhile, albumin has a tendency to enrich to tumor tissues, some studies then constructed lipid siRNAs with high affinity to albumin for transporting siRNAs to tumor tissues [88]. Nevertheless, albumin-mediated chimeric receptor-enhanced vaccines suffer from insufficient active targeting ability and reduced ligand residence time on the surface of APCs attributed to lipid transport and endocytosis. A novel strat-

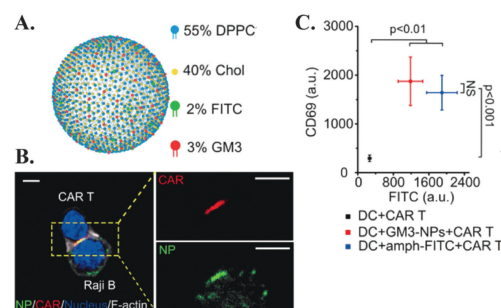


Fig. 3. (A) Scheme of GM3-FITC-NPs. (B) Confocal slice regarding FITC CAR-T cell and the conjunction of Raji B/CD169 cell. (C) Expression of CD69 in CAR-T cells as a function of FITC signal intensity of GM3-FITC-NPs, amph-FITC, and mixture of DCs and CAR-T cells devoid of FITC (control) ($n = 4$). Reproduced with permission [89]. Copyright 2022, American Chemical Society.

egy is to change the medium to nanoparticles, which can maintain some mobility between CAR-T cells and APCs while being able to avoid being cleared by endothelial capillaries. The study by Zang *et al.* employed a lipid layer for encapsulating biodegradable polylactic acid (PLA) cores, which were subsequently modified with monosialodihosylganglioside (GM3) capable of targeting DCs, as well as ligands that CAR-T cells can identify. Then the GM3-fluorescein isothiocyanate-nanoparticles (GM3-FITC-NPs) were finally made. Experimental results demonstrated that the employment of nanoparticle-ligand-mediated activation resulted in elevated CD69 expression in T cells, surpassing the levels achieved by amph-ligand-mediated activation under equivalent ligand concentration conditions (Fig. 3) [89]. If GM3 is modified appropriately, it might also function as an anti-cancer vaccine at the same time [90]. Hence, nanotechnology assumes a crucial role in facilitating T cell activation and proliferation *in vitro*, along with CAR-T cell activation and growth *in vivo*, thereby augmenting therapeutic effectiveness.

3.3. Nanoparticles promote CAR-T targeting and infiltration of tumor tissue

Post activation, gene transfection, and *in vitro* expansion of T cells, the principal challenge for successful treatment of solid tumors within the body, resides in accurately targeting and penetrating the tumor tissue. This is a critical issue that does not need to be considered when treating malignant hematological tumors, but it significantly attenuates the efficacy of CAR-T in the treatment of solid tumors. And the binding of CAR-T and TAA on normal tissues predisposes to OTOT toxicity, such as CRS. Research indicates that the delivery of MSLN-targeting CAR-T in a malignant thoracic tumor model was considerably more effective *via* local thoracic delivery compared to systemic intravenous infusion. Even though the concentration of CAR-T at the tumor's location during systemic injection was equal to the local delivery, it still showed lower activation and amplification [91]. This strongly suggests that complex circulatory processes and multiple layers of physiological barriers block cell entry into the tumor. Although genetically modified ACT cells to express tumor chemokines or combination strategies with anti-angiogenic drugs and lysosomal viruses have been employed in an attempt to direct cells to target solid tumors, these approaches are often accompanied by lower tumor infiltration efficiency and side effects [92].

Nanotechnology, magnifying the potential for solution, confers two distinct advantages upon nanoparticles: their diminutive size facilitates access to specific regions and their adaptability allows for active targeting or drug coupling. Magnetic nanoparticles (MNPs) can offer one potential strategy. Taking lymphocytes as an

example, Jang *et al.* utilized fluorescently labelled MNPs to significantly enhance the capacity of NK cells to aggregate near tumors, demonstrating that the use of external magnetic fields (EMFs) can effectively enrich metastatic cells [93]. Nevertheless, it has also been noted that while EMF was able to promote the retention and persistence of tumor-specific T cells modified by APS-MNP in tumor-draining lymph nodes, it inhibited the percentage of cells targeted to infiltrate the tumor [94]. Consequently, it is hypothesized that co-implementation of local EMFs and MNPs might potentially augment T-cell targeting towards tumors. An exemplar case can be extracted from the research conducted by Nie *et al.* [95], where the team utilized $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, PEI, EG, and PEG to fabricate magnetic nanoclusters (NCs), which demonstrated both magnetic responsiveness and superparamagnetism. The NCs were coupled with anti-programmed death receptor 1 (PD-1) (aP) through a counter-electron demanding Diels-Alder cycloaddition reaction creating benzoic acid-imine bonds to complete the binding. Upon entry into the body, successful recruitment of T cells and NCs couples to the tumor site after magnetic guidance under magnetic resonance imaging (MRI) guidance was observed. Given the low pH conditions within the TME, a fruitful separation of aP from NCs occurred, owing to the cleavage of the benzoic acid-imine bond. Thereafter, free aP binds to PD-1 on T cells, preventing PD-1 from connecting to its ligand PD-L1, which in turn inhibits the immune escape from the tumor and facilitates the release of its immunotoxic effects by T cells, and the dissociated CAR-T can also act by directly targeting the tumor antigen (Fig. S3 in Supporting information). With the help of nanoparticle, it is also possible to direct the action of CAR-T cells only at the tumor's site by local light response, which also enhances targeting, avoids systemic toxicity and reduces the risk of, for instance, CRS. An earlier study utilized a photosensitive dimer of the photooxygenated voltage structural domain (LOV2-ssrA/sspB) as a photoresponsive dimerisation tool to design light-convertible CAR-T cells (LiCAR-T). Under the action of visible blue light, the internally isolated LOV2-ssrA and sspB formed dimers, which stimulated the immunotoxicity of CAR-T. This avoided the traditional inherent toxicity of CAR-T to a certain extent, and improved the therapeutic specificity. However, attributable to the suboptimal penetration capabilities of visible blue light, it lacks the ability to reach the in-depth tumor tissues for the activation of LiCAR-T. Consequently, researchers employed up-conversion nanoparticles (UCNPs) acting as nanoconverters, which upon stimulation by near-infrared (NIR) light, emit visible blue light, leveraging the abundant substable intermediate energy levels of lanthanides, thus solving the problem of the failure to reach deep tumor tissues to activate LiCAR-T by directly using visible blue light [96]. This study has important implications for avoiding side-effects of CAR-T.

After CAR-T targeting reaches the tumor tissue, TME acts as a barrier to cell infiltration [97]. One approach to overcoming this barrier involves nanoparticle-mediated coupling of cytokines, for instance, IL-12 which, according to prior research, amplifies the therapeutic effect of CAR-T cells, especially in terms of tumor tissue infiltration [98]. Liu *et al.* devised an unnatural azide sugar nanoparticle (G400NP), which upon cellular uptake, metabolically translates into azide monomers and manifests on the cellular membrane, functioning as a cell marker. Researchers proceeded to label the cytokine using diphenylcyclooctane (DBCO), and co-cultured the same with the marked T cells, resulting in a coupling process mediated by a click reaction between DBCO and the azide present on the membrane. This attributed to the cytokine's activity was effectively maintained while reducing its toxicity to the T cells. When coupled with IL-12, it was found that T-cells infiltrated in increased numbers for solid tumors and enhanced their ability to inhibit tumor growth (Fig. 4) [99]. To conclude, the functionalized modification of nanoparticles provide a promising route for

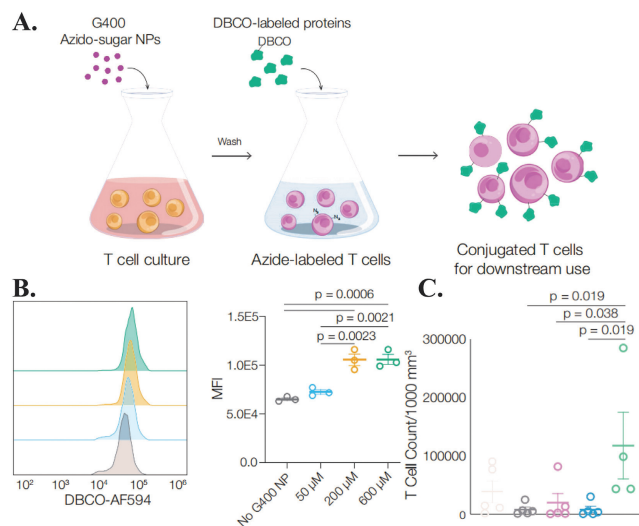


Fig. 4. (A) Scheme of T cells metabolic labeling and cytokine coupling with azido-sugar G400 NPs. (B) Median fluorescence intensity (MFI) of azide signals on the surface of T cells after treatment with different concentrations of G400 NPs for 3 d. (C) T cells count per 1000 mm² of tumor ($n=5$). Reproduced with permission [99], Copyright 2023, National Academy of Science.

augmenting the suboptimal targeting and infiltration capabilities of CAR-T cells towards tumorous sites.

3.4. Nanoparticles inhibit immune escape from TME

Following specific infiltration into tumor tissue, CAR-T cells might encounter TME resistance to the immune-toxicological action of CAR-T cells. As mentioned earlier, the inhibitory elements in TME may negatively regulate T cell responses, leading to T cell senescence or even depletion. T cells in this depleted state typically exhibit oligoreactivity, high expression of inhibitory receptors, reduced types of potent cytokines, and diminished cytotoxic activity. In addition, when cytotoxic T cells (CTLs) enter the TME, they are exposed to a complex network of cells and cytokines that may further inhibit their effectiveness, leading to T cell depletion [100]. Nevertheless, studies have demonstrated the influence of TME-induced immunosuppression is reversible upon their withdrawal from TME [101].

To overcome immune response challenges, the implementation of immune checkpoint blockade therapy emerged as a novel strategy. PD-1, a vital inhibitory receptor on T cells, selectively binds to its ligands PD-L1 and PD-L2. These ligands, expressed by tumor or stromal cells, contribute to the suppression of T-cell function, impeding an effective immune response. Blocking the interaction of PD-1 in T cells and PD-L1 in tumor cells has the potential to boost the immune response and promote anti-tumor activity of T cells [102]. However, clinical studies have found that at the case of CAR-T cells targeting ganglioside GD2 in neuroblastoma (NB) combined with the PD-1 inhibitor pembrolizumab, although this approach proved to be safe and feasible, there was no noteworthy impact of pembrolizumab on CAR-T cell expansion, persistence, or cytokine secretion [103]. This phenomenon may be due to the fact that the monoclonal antibodies required for immune checkpoint blockade therapy are a class of macromolecular biologics and therefore lack sufficient targeting *in vivo* to achieve effective concentrations within tumor tissue.

In this situation, the integration of nanomaterials offers a promising solution in tackling the challenge of targeted drug delivery. For instance, the magnetic NCs, as previously described, are conjugated with T cells through pH-sensitive benzoic acid-imine bonds linking to aP. With the aid of *in vitro* MRI, these complexes

are directed to the TME, consequently leading aP to the same destination, where it is subsequently liberated under low pH conditions. Subsequently, aP binds PD-1 on T cells at the TME site to inhibit immune escape [95]. However, blocking a single immune checkpoint such as PD-1, cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) or lymphocyte activation gene-3 (LAG-3) results in compensatory up-regulation of additional checkpoint pathways, further enhancing the ability to inhibit localized T cells, but combinatorial blockade strategies may become an effective means of addressing this issue [104]. In related studies, Zhang *et al.* formulated liposomes with lecithin acylcholine, cholesterol, and DSPE-PEG-maleimide, further modified by iRGD peptides to impart tumor-tissue targeting to the liposomal construct. In addition, the P110 γ PI3K inhibitor PI-3065 and the natural killer T-cell (NKT) agonist 7DW8-5 were loaded into the liposome bilayer as hydrophobic drugs. Experimental data revealed that a liposomal pretreatment strategy paired with subsequent CAR-T cell infusion therapy yielded a substantial increase in T-cell counts relative to conventional CAR-T cell therapy, as well as a significant enhancement survival rates in mice. This demonstrated the remodeling effect of liposomes in the TME environment, which could be maintained for about 2 weeks [105]. In addition, studies have also utilized nanoparticles to mediate a strategy that combines *in situ* vaccination with gene-mediated anti-PD therapy [106], and such a similar strategy may have unintended effects if combined with CAR-T therapy.

Fas ligand (FasL), a surface component of cytotoxic T cells, serves as the natural ligand for the death receptor Fas, playing a pivotal role in the apoptotic signaling pathways responsible for CTL-mediated cell killing. The distinction in the signaling pathways between CAR-T cells and endogenous T cells necessitates a reassessment of the significance and implications of the FAS-FASL pathway in CAR-T therapies. In a study exploring the CAR-T cell therapy targeting CD30 for the treatment of embryonal carcinomas (ECs), researchers have demonstrated that antigen-dependent targeting of CD30⁺ EC tumor cells by CAR-T cells has been proven. Moreover, the CAR-T cells exhibited antigen-independent elimination of surrounding CD30⁻ EC tumor cells by engaging in cell-cell contact-dependent Fas-FasL interactions [107]. These findings imply that Fas-FasL interactions between CAR-T cells and tumor cells have the potential to mitigate tumor escape and alleviate the problem of tumor heterogeneity. The role of FAS in tumors has sparked controversy, yet historical records and empirical studies predominantly support Fas as a potential target for eradicating tumor cells [108]. One study provided evidence that FAS-mediated targeted and paracrine killing is critical for T-cell immunotherapy effectiveness, and also indicated that FAS-deficient tumor cells affect the CAR-T cells killing activity against tumor cells both *ex vivo* and *in vivo* [109]. However, a feature of immune escape manifestation in the TME is the frequent down-regulation of FAS in solid tumors, thereby potentially reducing FAS-FASL-mediated apoptosis of tumor cells [110], which would consequently trigger resistance to CAR-T therapy. A recent study employed DOTAP-cholesterol encapsulation of human FAS-encoding plasmid (hCOFAS01) to produce LNP. By delivering this LNP to tumor cells, the level of FAS expression on tumor cells could be significantly increased, further overcoming the resistance of melanoma cells to FAS-mediated apoptosis *in vitro*. This novel strategy, when combined with CAR-T cell therapy, has the potential to provide new insights and possibilities for CAR-T treatment of solid malignancies via the FAS-FASL pathway (Fig. 5) [111].

Contemporary years have witnessed burgeoning interest in the production and role of adenosine as an immunosuppressive pathway, presenting it as a potential therapeutic target for antagonizing tumor-induced immunosuppression. Adenosine, which is converted from ATP in the TME catalyzed by the exoenzymes CD73

and CD39, binds to the A2a adenosine receptor (A2aR) on the surface of T cells, which in turn blocks T-cell signaling and down-regulates the secretion of interferon-gamma (IFN- γ), thereby inhibiting the effects of CAR-T cell therapy [112]. In alignment with this concept, Siriwon *et al.* synthesized multilayered liposomes (cMLVs) incorporating SCH, an antagonist to A2aR, which disrupts adenosine's inhibitory potency on T cells. By covalently coupling the maleimide end-groups of cMLVs to thiols on the surface of CAR-T cells and exploiting the tendency of CAR-T cells to be tumor-orientated to function in the TME, the decompensation of CAR-T cells in adenosine-enriched TME was effectively prevented [113]. These evidence points towards the potential of nanoparticles as a strategy to surmount immune evasion in TME, either through initial pre-treatment or association with CAR-T cells, by inhibiting immunosuppressive elements and manifesting an immune checkpoint blockade effect.

3.5. Nanoparticles for mediating CAR-T therapy in combination with other therapies

The TME of solid tumors presents numerous obstacles that are not conducive to CAR-T therapy, including accumulation of immunosuppressive cells (*e.g.*, M2-type macrophages and Tregs) due to persistent inflammation, an abnormally dense vascular network, and a high stiffness of the ECM [114]. Currently, research is attempting to overcome the challenges posed by TME by combining CAR-T therapy with other therapeutic strategies, such as photothermal therapy (PTT) that can remodel TME, in which nanoparticle plays a key bridging role [115]. In their work, Chen *et al.* manipulated cellular metabolism to induce the expression of surface N3 groups on CAR-T cells, which they subsequently conjugated with indocyanine green nanoparticles (INPs) to synthesize CTINPs. Under NIR irradiation, the degree of penetration of CTINPs into tumor tissues is enhanced, and the local temperature of the tumor rises, substantially inhibiting the rate of tumor growth. This can largely be attributed to the ability of INPs coupled with CAR-T cells to mediate mild photothermal stimulation, disrupt the ECM, dilate blood vessels, increase blood perfusion, which in turn promotes the infiltration of immune cells, as well as stimulate the production of more tumor chemokines and anti-tumor cytokines [116].

Apart from the conjugation of CAR-T cells with nanoparticles capable of mediating PTT, another viable approach involves prior tumor pretreatment with PTT nanoparticles prior to administering CAR-T cells, which is also an effective combination strategy. Photodynamic therapy (PDT) in the presence of oxygen and PDT drugs generates ROS, mediates tumor cell damage, and promotes the immunocidal effects of T cells [117]. Zhu *et al.* created nano-enzymes targeted towards CD44 present on the surface of tumor cells through the employment of hyaluronic acid (HA), CuCl₂, and Na₂S. By modifying the surface of PEG, they enhanced its biocompatibility, thus obtaining a nanocomplex referred to as PHCN. Upon administration of NIR irradiation *in vitro*, PHCN could trigger a photothermal catalytic effect, leading to the killing of tumor cells by PTT and PDT. In the tumor model, perfusion of TME pre-treated with PHCN was significantly increased, contributing to more CAR-T cells for infiltration [118]. Transforming growth factor-beta (TGF- β), instrumental in the proliferation of tumor growth and depletes CAR-T cells [119,120], can be considered as a potential target to enhance CAR-T efficacy. Incorporating nanoparticles as a means of pretreatment, a study created an amphiphilic Hydroxyethyl Starch-Polycaprolactone (HES-PCL) nanodelivery system. The hydrophobic TGF- β receptor inhibitor LY and the photosensitizer ICG can be co-loaded into HES-PCL nanoparticles to form LY/ICG@HES-PCL nanoparticles for the purpose of combining anti-TGF- β and photothermal effects. Through the photothermal effect of ICG and the TGF- β blocking effect of LY, it can synergistically induce changes

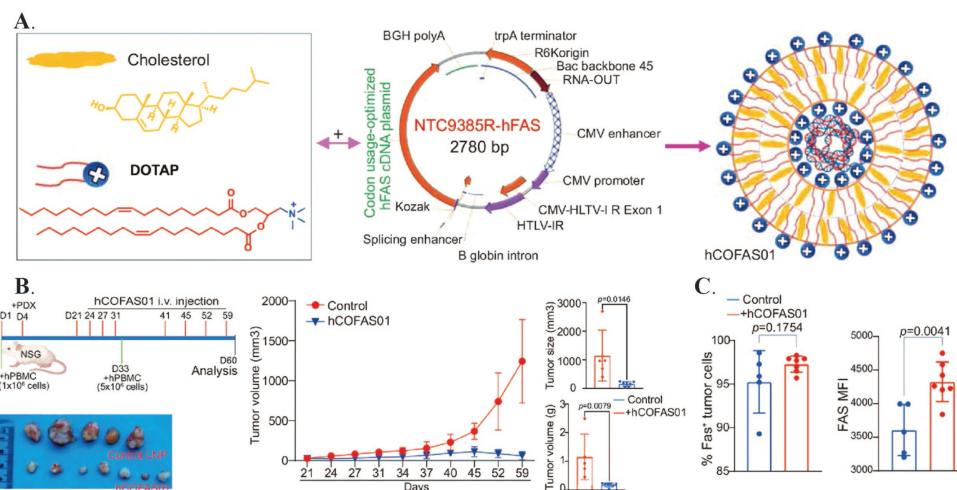


Fig. 5. (A) Cationic LNPs and FAS-encoding plasmids were prepared and assembled into hCOFAS01. (B) Changes in tumor volume after treatment. (C) Expression of FAS on tumor cells after administration of hCOFAS01. Reproduced with permission [111]. Copyright 2022, American Chemical Society.

in the physicochemical properties of the tumor, which in turn promotes the penetration and activity of CD19-CAR-T and up-regulates the expression of IFN- γ [121].

Beyond merely conjugating CAR-T cells to nanoparticles capable of mediating PTT, another strategy to combine CAR-T therapy with PTT is to encapsulate nanoparticles with CAR-T cell membranes. A study conducted in 2020 generated CAR-T cell membranes that recognize hepatic glycoprotein 3, and monodisperse silica (IMs) loaded with photothermal effect mediator IR780 nanoparticles were encapsulated in order to target lung cancer cells with high GPC3 expression for the treatment of lung cancer [122].

Beyond the combination of PTT, the amalgamation of sonodynamic therapy (SDT) can enhance treatment efficacy. Li *et al.* accomplished this combination by encasing nanoparticles in CAR-T cell membranes. They constructed CAR-T targeting CD19 and isolated cell membranes with which they wrapped horseradish peroxidase (HRP)-loaded gold nanoparticles/polydopamine (PDA) NPs and silver sulfide quantum dots to form APHA@CM nanocomplexes. Among them, AuNPs could be used as acoustic sensitizer and photothermal reagent to mediate SDT and PTT. The PDA encapsulation on the outside of the AuNPs expanded the excitation wavelength of the AuNPs and increased the depth of the laser penetration into the tissue, and the HRP was used to convert H₂O₂ to O₂ in order to improve the TME hypoxic state, thus enhancing the effect of SDT. On the other hand, silver sulfide quantum dots act as near-infrared two-region fluorescents, which can provide precise *in vivo* imaging and assist CAR-T therapy (Fig. S4 in Supporting information) [123].

4. Challenges and perspectives

In this review, we present a comprehensive overview of CAR-T therapy, outlining the structure of CAR-T cells along with the current problems that CAR-T therapy still faces in treating solid tumors. Most importantly, we summarize the relevant studies on the application of nanotechnology to CAR-T in recent years, which provide enlightenment for further combining and clinically translating CAR-T with nanoparticles and thus treating solid tumors more effectively.

Clinical and preclinical studies have begun to explore the possibility of CAR-T for other solid malignancies. Nevertheless, a review compiling clinical studies of CAR-T treatments for four prevalent solid tumors observed that several substantial hurdles remain in using CAR-T cell therapy effectively for the management of

solid tumors, including colorectal cancer, hepatocellular carcinoma, prostate cancer and intrathoracic malignancies [124].

For solid tumors, the therapeutic landscape pragmatically presents several challenges compared to hematological malignancies. Regarding the production process of CAR-T products itself, several pervasive challenges exist in the field as well. These have been fully discussed in the preceding paragraphs.

Numerous investigators have strived to refine therapeutics by enhancing the structural components of CAR-T cells (including extracellular antigen-binding domains, hinge regions, transmembrane structural domains, and intracellular signaling domains), but have still been unable to fully overcome the limitations of solid tumors to CAR-T [125]. Though the advent of aAPCs has improved the efficiency of *in vitro* expansion, the relative size of these particles does not significantly contribute to the enhancement of efficacy [126], coupled with a complex production process rendering the benefits of minor commercial significance. The genomic modification efforts of the transfected viruses do not completely alleviate the potential safety concerns with viral genes.

Simultaneously, the employment of nanotechnology, specifically nanoparticles, introduces novel therapeutic prospects. This review concentrates on the synergistic application of nanoparticles and CAR-T cells in addressing solid tumors, highlighting their potential superiority. Although nanoparticle offers us numerous possibilities, its challenges in the design, preparation, functional modification of nanoparticles and also in the assessment of safety and efficacy should not be underestimated.

Primary consideration is the safety implications of utilizing smart nanoparticles in human subjects. This is the key to determining whether they can be practically applied in clinical treatment. While nanoparticles might improve the inherent side effects of CAR-T, such as CRS, they would likewise introduce new chemicals. Although the biocompatibility and pharmacokinetics of each nanoparticle are examined during the research process, the issue of safety in actual human applications cannot be ignored.

An additional issue arises when the incorporation of nanoparticle has the potential to increase the complexity of the producing procedure, thereby lengthening the production cycle and escalating prices. The discussion here is divided into two main aspects: (1) CAR-T drug preparation is already complex enough and the production cost is high, which is also the reason for the high price of marketed CAR-T drugs. The introduction of nanoparticles makes the efficiency of the CAR-T production process improved, including both cell expansion and gene transfection, thus

reducing the production cost accordingly. (2) Nevertheless, in order to enhance the efficacy of CAR-T drugs by combining its combination with nanoparticles, including coupling, encapsulation, pre-treatment, etc., such a complicated design is bound to increase the cost. At the same time, whether the functional combinations generated through nanoparticle surface modification can truly enhance the therapeutic effectiveness of CAR-T therapy on cancers is an issue that deserves to be extensively investigated. If the anti-tumor effect of the nanoparticles is not significant to compare with that of direct intravenous or intratumoral injection of CAR-T, this may also lead to the problem of putting the cart before the horse and further delaying the conduct of clinical trials.

Taken together, the most problematic contradiction at present lies in whether the combination of nanoparticle can achieve substantial enhancement of CAR-T function while concurrently minimal increase in process complexity and cost. Only when these issues are further deciphered will CAR-T therapies relying on nanos-trategies have the potential to be widely used for the curative purposes of solid malignancies.

5. Conclusion

CAR-T therapy has revolutionized the field of tumor immunotherapy, representing a novel and promising approach. However, the application of CAR-T therapy in treating solid tumors is faced with numerous challenges, resulting in a scarcity of available products in the market. Recognizing the remarkable properties of nanoparticles, several immunotherapies employing nanoparticle technology have progressed to the stage of clinical translation. Notably, nanoparticles are anticipated to play a pivotal role in every stage of CAR-T therapy, generating optimism that they could overcome the barriers associated with treating solid tumors. Nevertheless, it is worth noting that no nanoparticles in combination with CAR-T have yet entered clinical trials, highlighting the necessity for comprehensive and extensive research before clinical translation can be achieved. Encouragingly, recent advancements in this field have unveiled substantial functional improvements attributed to nanoparticle-mediated enhancements in CAR-T immunotherapy. In light of these advancements, nanoparticles are expected to assume a crucial and multifaceted role throughout the entirety of CAR-T therapy while ensuring production efficiency, anti-tumor efficacy and safety.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ccl.2023.109432.

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