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EDITORIAL

Chimeric antigen receptor macrophages: a new frontier in hepatocellular carcinoma treatment

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Hepatocellular carcinoma (HCC) is the fourth leading cause of cancer-related mortality worldwide[1]. The primary treatment options for this disease are surgical resection and liver transplantation. Unfortunately, most HCC cases are diagnosed in advanced stages and are inoperable. Even after surgery, the long-term prognosis remains unsatisfactory, because of a high recurrence rate. The HCC treatment landscape has recently evolved with the development of multikinase inhibitors[2]. Sorafenib has been found to be beneficial for patients with advanced HCC. More recently, lenvatinib, another MKI that selectively inhibits VEGFR1–3, FGFR1–4, PDGFRα, RET, and KIT, was approved by the Food and Drug Administration (FDA) as a first-line treatment for unresectable advanced HCC. In a phase III clinical trial, lenvatinib has been found to be non-inferior to sorafenib in terms of overall survival among treated patients with advanced HCC[3]. However, the survival benefits of both sorafenib and lenvatinib remain limited, because of intrinsic and extrinsic drug resistance.

Recent advancements in immunotherapy have opened new avenues for the treatment of advanced HCC. Immune checkpoint inhibitors, such as nivolumab and atezolizumab, have received FDA approval for use in patients with advanced HCC. However, the response rates to these therapies remain suboptimal, and their reported efficacy is below 20%[4]. Combination therapies enhance treatment efficacy, according to phase III clinical trial data. For instance, the IMbrave150 study (NCT03434379) demonstrated that atezolizumab in combination with bevacizumab has superior therapeutic efficacy to that of sorafenib alone[5]. Additionally, in the phase III HIMALAYA study (NCT03298451), the single tremelimumab regular interval durvalumab (STRIDE) regimen, compared with sorafenib, significantly improved overall survival[6]. On the basis of compelling data, these treatment regimens have been approved by the FDA for the treatment of advanced HCC. However, despite these advancements, the efficacy of immunotherapy in HCC continues to be limited, thus underscoring an urgent need to understand resistance mechanisms and identify predictive biomarkers of efficacy. Consequently, new therapeutic options must be explored for this challenging disease.

Chimeric antigen receptor (CAR) therapy has provided a groundbreaking cancer immunotherapy approach. CAR-T cell therapy, the primary cell therapy-based option currently available, involves genetically modifying a patient's T cells to express a synthetic receptor known as a CAR, which consists of an extracellular antigen-binding domain, a hinge region, a transmembrane domain, and an intracellular signaling domain[7]. After being modified, these CAR-T cells are infused back into the patient, in whom they recognize and bind cancer cells, and trigger an immune response that ultimately leads to tumor cell destruction. CAR-T cell therapy has demonstrated significant therapeutic efficacy in patients with hematological malignancies. Considerable progress has recently been made in clinical trials and preclinical animal models using CAR-T cells in solid tumors, including HCC. CAR-T cells have been designed to target a range of antigens preferentially expressed in HCC cells, such as glypican-3 (GPC3)[8], alpha-fetoprotein[9], and CD147[10]. Phase I data have shown promising antitumor activity of CT017 targeting GPC3 in patients with advanced HCC, accompanied by tolerable adverse effects

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(NCT03980288). Additionally, multiple clinical trials are currently recruiting participants to assess the efficacy and safety profile of epithelial cell adhesion molecule (EpCAM) CAR-T cells (NCT02729493) and MUC1 CAR-T cells for patients with advanced HCC (NCT02587689)[7]. Despite these advancements, the application of CAR-T therapy in HCC presents several challenges, including off-target effects, poor infiltration efficiency, induction of CAR-T cell exhaustion within the immunosuppressive tumor microenvironment (TME), and loss or reduction of antigen presentation in solid tumor cells[11]. Additionally, the high production costs of CAR-T cells limit their widespread clinical application[12]. Consequently, optimizing CAR-T cells for specific and efficient cancer treatment remains an ongoing challenge, thus highlighting the urgent need for alternative cell therapy approaches[13].

CAR macrophages (CAR-Ms) have emerged as a promising alternative cell therapy approach. Tumor-associated macrophages, the most abundant innate immune cells, constitute approximately 50% of the tumor mass within the TME in many solid tumors. These cells play critical roles in tumor progression by modulating various behaviors, including cell proliferation, angiogenesis, metastasis, immune escape, and therapeutic resistance. As professional antigen-presenting cells, activated macrophages also contribute to promoting an adaptive antitumor immune response[14]. CAR-Ms' intrinsic functional properties have enabled a new immunotherapy for solid cancers with promise in overcoming the limitations of CAR-T therapy. CAR-Ms offer several advantages over CAR-T cells[11]: first, macrophages can substantially penetrate the TME; second, they decrease the ratio of tumor-associated macrophages that promote tumor growth; third, they enhance antigen presentation and prime T cells; and fourth, they have limited circulation times and diminished tumor toxicity, particularly with respect to cytokine release syndrome. Because of its potential advantages over CAR-T approaches, several preclinical and clinical studies have investigated the use of CAR-M therapy in various cancer types.

A clinical trial based on the CAR-M strategy has led to FDA approval. The encouraging data suggest that CAR-Ms may provide a safe and feasible approach to targeting solid tumors. A first-in-human phase 1 clinical trial of CT-0508 (NCT04660929), an anti-human epidermal growth factor receptor 2 (HER2) CAR-M, has demonstrated preliminary safety and tolerability in patients with advanced HER2-overexpressing tumors (scored as 3+ according to immunohistochemistry or 2+ according to immunohistochemistry and positivity according to in situ hybridization) after their most recent systemic therapy[15]. No patients developed grade 3 or 4 cytokine release syndrome, and none experienced immune effector cell-associated neurotoxicity syndrome. Additionally, no prolonged immunosuppression or cytopenia was observed. Researchers have generated human CAR-Ms bearing a CD3 ζ signaling domain through adenoviral transfection of the CAR gene[15]. In that trial, only 44% of patients achieved stable disease, which was observed exclusively in HER2 3+ patients, thereby underscoring the need to confirm robust target expression before CAR-M treatment. Unlike conventional CAR-T therapies, CT-0508 quickly exited the peripheral blood and was found in 92% of post-treatment biopsies. This treatment activated myeloid cells in the TME and led to global upregulation of antigen processing, inflammation, and chemokine signatures, thus indicating immune-mediated TME remodeling. However, CT-0508's efficacy might be limited by its short persistence in the TME. Transitioning from terminally differentiated macrophages to CAR monocytes, which have a tenfold longer half-life in mice, might enhance persistence and allow for as many as 10 billion cells per apheresis. A phase 1 trial of CT-0525, anti-HER2 CAR monocytes, is currently ongoing (NCT06254807). In addition, CT-0508 has been demonstrated to suppress the growth of colorectal cancer tumors derived from CT26-hHER2 in a syngeneic mouse model[16]. The therapeutic efficacy of CT-0508 relies on T cell action, given that treatment with anti-CD8 and/or anti-CD4 depleting antibodies has been found to abolish CAR-M-induced anti-tumor responses in mice. Additionally, CT-0508-treated tumors exhibit an inflamed TME characterized by the influx and activation of T cells, pro-inflammatory macrophages, and cytokines. On the basis of these findings, the effects of combining CT-0508 with antibodies to PD-1 have been evaluated in the CT26-hHER2 model. This combination did not induce severe adverse effects and achieved maximal tumor suppression and prolonged survival by complementing PD-1 inhibition, thereby enhancing tumor-infiltrating lymphocyte activation. However, the therapeutic efficacy of CT-0508 was limited by its low persistence in the TME, similarly to observations in a clinical trial conducted by the same group. Therefore, using CAR monocytes for combination therapy might be more advantageous. Specifically in HCC, several preclinical studies have explored the therapeutic potential of CAR-Ms for treatment. In 2023, Yang et al[17]. used lipid nanoparticle-mediated dual mRNA co-delivery to generate Siglec-GΔITIM-expressing GPC3-specific CAR macrophages

in situ. lipid nanoparticle (LNP)-engineered Siglec-GΔITIM-expressing GPC3-specific CAR-Ms demonstrated significantly enhanced HCC-specific engulfment by macrophages, thereby stimulating an adaptive anti-tumor immune response and ultimately suppressing HCC tumor growth. Similarly, Zhang et al[18]. have developed quadrivalent CAR macrophages incorporating GPC3-CAR and Super IL-2 by using the same lipid nanoparticle method. These CAR macrophages have been found to effectively infiltrate tumor sites and stimulate durable T cell memory against both antigen-positive and antigen-negative cells[18]. Another research group has developed CAR-Ms by transfecting a CAR construct containing a single-chain variable fragment (scFv) targeting GPC3 and the CD3ζ intracellular domain through an adenoviral approach. This method has demonstrated efficient engulfment of HCC cells in a three-dimensional spheroid model[19]. Collectively, these encouraging findings underscore the therapeutic potential of CAR-Ms in cancer therapy, including HCC.

Perspectives

Despite encouraging current *in vitro*, *in vivo* animal, and clinical data, several limitations in the current CAR-M approach to HCC treatment require further optimization and improvement. First, many CAR-M therapies rely on viral transfection to introduce CAR components into macrophages. Whether the use of adenoviruses for engineering CAR macrophages poses immunogenicity concerns, which could potentially result in their rapid rejection, must be determined[13]. Second, because of the non-proliferative nature of macrophages, patients are administered a finite number of these cells through *in vitro* or *in vivo* methods, thus potentially influencing treatment efficacy. Consequently, ongoing administration of CAR-Ms might be necessary to maintain therapeutic effectiveness. Third, a major challenge lies in maintaining CAR-Ms in a pro-inflammatory M1 state after they migrate to the TME. Transitioning to a less inflammatory M2 state might create an immunosuppressive TME that diminishes the efficacy of treatments administered alone or in conjunction with immune checkpoint therapies.

Several new avenues for optimizing CAR-M therapy for HCC are emerging. As previously described[17,18], researchers have used lipid nanoparticle-based methods to synthesize CAR-Ms, thereby effectively overcoming the inherent limitations of viral-based approaches. Furthermore, to enhance the phagocytic potential of CAR macrophages, genetic manipulation of key phagocytosis-associated genes, such as NLRP6, might be beneficial, given that deletion of this gene leads to increased phagocytosis[20]. Further augmentation of the phagocytic activity of CAR-Ms may be achieved by inhibiting the CD47-SIRPα pathway. Moreover, to increase therapeutic efficacy, combining CAR-M therapy with PD-L1/PD-1 treatment might facilitate the synergistic activation of CD4 and CD8 T cells. As with CAR-T, the production of CAR-M can be costly, involves complex viral transfection procedures, and prompts long-term safety concerns. To mitigate these drawbacks, alternative strategies are urgently needed to emulate the genetic approach while offering greater reliability, shorter production times, minimized batch-to-batch variation, and reversible effects. Recently, our group has pioneered a bifunctional linker approach, conjugating it to the amines of cell surface proteins by attaching the arginine-glycine-aspartic acid (RGD) integrin-targeting peptide ($\alpha_v\beta_3$ integrin) to mouse red blood cells. This modification enables red blood cells to gain tumor-targeting abilities and tumor-killing effects through the use of photosensitizers, thus facilitating strong interactions between red blood cells and cancer cells[21] (**Figure 1**). This novel chemical modification strategy on cell surfaces might enable the creation of CAR-Ms tailored to target specific receptors on cancer cells. Moreover, various peptides that selectively bind receptors uniquely expressed on HCC cells could be designed, synthesized, and linked to the macrophage surface through the bifunctional linker. This approach enables CAR-Ms to be developed through peptide-based methods, thus yielding peptidic CAR-Ms.

The therapeutic efficacy of CAR-Ms has been demonstrated through various methods, including viral transfection, lipid nanoparticle delivery, and peptide-mediated modification. Although the viral method offers higher efficacy, it is costly and prompts long-term safety concerns. In contrast, lipid nanoparticle and peptide-mediated approaches are more cost-effective, scalable, and faster to produce, and pose fewer uncertainties and batch-to-batch variations. Among them, the peptide-mediated method is notable for its flexibility, by enabling rapid enhancement of efficacy and specificity by linking 1 or 2 peptides simultaneously. However, rigorous investigations are imperative to evaluate the application of this method in HCC therapy, and ensure the future safety, effectiveness, and overall viability of CAR-Ms in clinical settings.

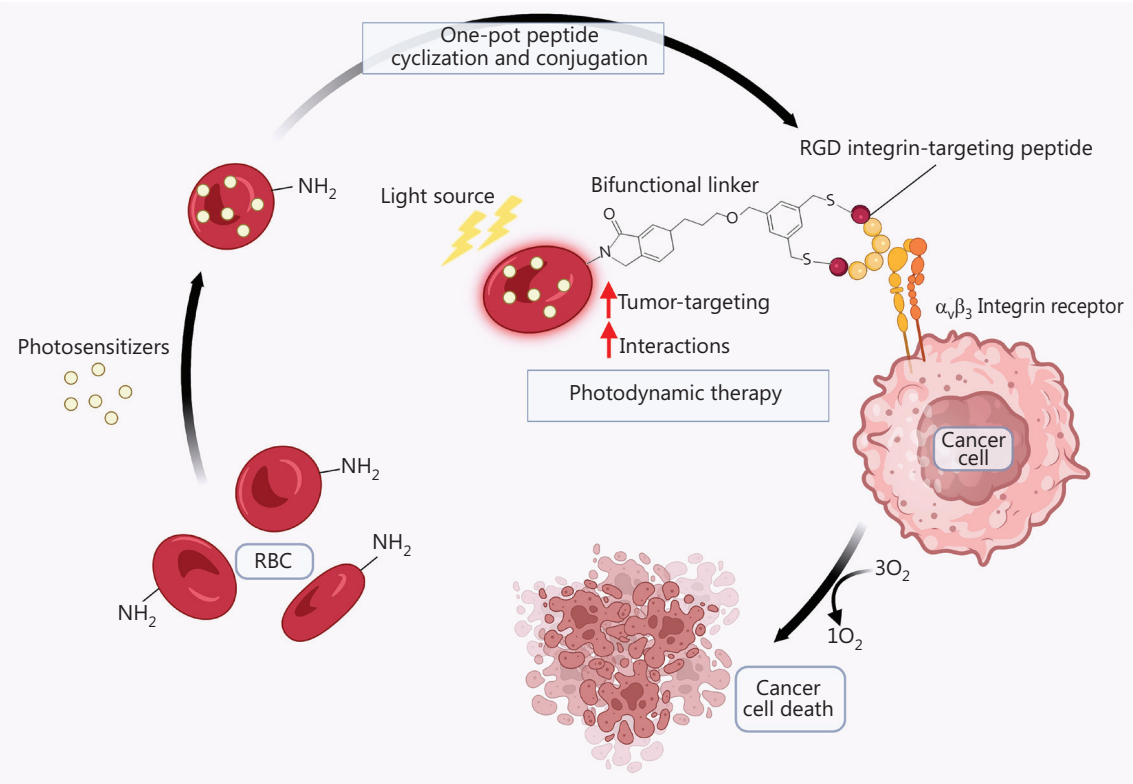

Figure 1 Schematic representation of the synthesis of peptide-linked RBCs targeting $\alpha_v\beta_3$ integrin for the specific killing of tumor cells *via* photodynamic therapy. RBC, red blood cell; RGD, arginine-glycine-aspartic acid.

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Conflict of interest statement

No potential conflicts of interest are disclosed.

Author contributions

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