

Research and progress of CAR-macrophage cell therapy for solid tumors

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ABSTRACT

Though chimeric antigen receptor T cell (CAR-T) technology has emerged as an effective breakthrough against hematological malignancies, its application to solid tumors remains challenging and is restricted by the high complicity of the tumor microenvironment (TME). Macrophages are innate immune cells that are inherently equipped with a wide range of therapeutic effects, including elevated infiltration rate, enhanced phagocytosis and cytotoxicity, mediation of immune suppression, and antigen presentation. In light of these unique biological functions and their ability to penetrate tumors, macrophages have emerged as a promising approach for the treatment of solid tumors. This review initially clarifies the biological characteristics of macrophages and tumor-associated macrophages (TAMs), then reviews macrophage sources and the CAR design structure, outlines the ways to deliver CAR to macrophages and the preparation of CAR-macrophages (CAR-Ms), and finally summarizes the application and prospects for the treatment of solid tumors by CAR-Ms in recent years.

Keywords: chimeric antigen receptor, CAR-macrophage, tumor-associated macrophage, cell therapy, solid tumor

INTRODUCTION

Tumor immunotherapy has revolutionized cancer treatment by modulating the immune system to suppress tumor progression, invasion, and metastasis^[1]. Immunotherapy mainly includes adoptive cell transfer therapy (ACT), immune checkpoint inhibitors (ICIs), and cancer vaccines^[2]. Chimeric antigen receptor T cell (CAR-T) technology has emerged as a predominant ACT against tumors, which applies genetically engineered immune cells to specifically target antigens expressed on the surface of cancer cells^[3]. This approach has shown remarkable efficacy in treating hematological malignancies, especially in recurrent and refractory B-lymphocytic leukemia^[4]. However, the effectiveness of CAR-T is

still inconclusive in the context of solid tumors and is restricted by the high complexity of the tumor microenvironment (TME).

Different from hematological tumors in which cancer cells can easily spread to peripheral blood, solid tumors demand active transportation and extravasation, often penetrating into immunologically cold and dense fibrotic masses. Therefore, how to promote the homing and penetration efficiency of CAR-T cells in solid tumors is a tough problem^[5]. An additional challenge for CAR-T is how to overcome various barriers to eventually reach the tumor site^[6]. Due to the complex TME composition, CAR-T is likely to be restrained to varying degrees and presents a state of exhaustion^[7].

Macrophages, innate immune cells with a high

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infiltration rate, are the major component of TME and take effects at all stages of tumor progression. Due to their powerfully phagocytic function and high infiltration efficacy, macrophages are crucial for the elimination of foreign pathogens, and can easily locate and persist in tumors^[8-9]. Additionally, due to their high plasticity and polarization, macrophages undoubtedly have an impact on the growth and development of tumors^[10]. According to their phagocytic capabilities, antigen presentation, activation of other immune cells, and penetration in the TME, macrophages can be considered an ideal therapy for solid tumors^[11]. The macrophages infiltrating tumor tissues, also known as tumor-associated macrophages (TAMs), are typically classified into two types: the "classically activated" M1 phenotype, which exhibits a proinflammatory effect to increase tumoricidal activities^[12], and the "alternatively activated" M2 phenotype, which features an anti-inflammatory property to promote angiogenesis and increase vascular density, thereby supporting tumorigenesis^[13].

Compared with CAR-T cells, CAR-macrophages (CAR-Ms) exhibit the following superior qualities. Firstly, they have a higher macrophage infiltration efficiency in tumors, being capable of immersing in the TME, while T cells have difficulty entering the TME, inhibited by the physical barriers formed by the matrix around tumors^[14]. More importantly, TAMs play a vital role in tumor invasion, metastasis, immunosuppression, and angiogenesis^[15]. Moreover, CAR-Ms are able to influence the cellular TAM phenotype and increase the ratio of M1 to M2, which have positive effects on solid tumor treatment. Secondly, in addition to the phagocytosis of macrophages, CAR-Ms also improve the capability of antigen presentation and enhances the cytotoxic effects of other immune cells within the TME. Thirdly, the main advantage of CAR-Ms is safety. Due to the limited time of CAR-Ms in circulation, less non-tumor toxicity is generated, such as cytokine release syndrome (CRS), caused by several cytokines being released into the blood stream by CAR-Ms. Additionally, contrary to the limited survival and persistence of CAR-T in the immunosuppressive TME, CAR-Ms exhibit longer survival time and persistence, ensuring the long-time therapeutic duration.

This review highlights the biological characteristics of macrophages and TAMs, displays the preparation methods of CAR-M cells, and summarizes the latest research progress and prospects of CAR-M cell therapy for solid tumors at home and abroad.

BIOLOGICAL CHARACTERISTICS OF MACROPHAGES AND TAMs

Macrophages are phagocytes that exist in the innate immune system and are capable of engulfing pathogens, bacteria, cancer cells, and coordinating an effective immune response to protect the host and maintain homeostasis^[11]. Moreover, macrophages, regarded as the first line of defense, are also proficient antigen-presenting cells (APCs), which generate a specific antitumor immune response and activate T cells^[16]. In its early stage, a tumor's volume is relatively small; due to their broad infiltration ability, macrophages slowly aggregate around the tumor and inside the tissues. At the tumor progression stage, along with the increase in tumor volume, an intratumor vascular network is gradually formed. Monocytes in the blood penetrate into the interior of the tumor tissue, and then develop and mature into TAMs, eventually forming the TME^[17].

TAMs exhibit dual effects in tumor progression due to their high plasticity and polarization. On the one hand, macrophages generate important immunogenic cytokines that inhibit tumors. On the other hand, they can promote the growth, invasion, and metastasis of tumor cells. These two entirely opposite effects may be affected by different macrophage phenotypes which are influenced by certain inducible factors in the TME, such as specific signals and differing stimuli. M1 phenotypes represent antitumor activity, which exhibits proinflammatory features, such as generating immunogenic cytokines and participating in tumor suppression^[18]. M1 macrophages are typically induced by IFN- γ from Type 1 T helper cells (Th1) and are characterized by secreting cytokines such as TNF- α , IL-8, IL-6, IL-12, and IL-1 β , which promote proinflammatory responses^[19-20]. These cytokines are capable of coordinating immune responses and generating reactive oxygen species and reactive nitrogen species to promote the killing of pathogens^[21]. M1 macrophages act as APCs and ligate the innate and adaptive immune systems to exert anti-tumorigenic functions^[22-23]. M2 phenotypes usually evolve from TAMs, which are typically regarded as anti-inflammatory phenotypes that play biological roles in mediating tissue repair and increasing vascular density to stimulate angiogenesis^[24-25]. M2 macrophages promote tumor formation through secreting immunoregulatory cytokines such as IL-4, IL-10, IL-13, and TGF- β to support tumor cell growth, invasion, and metastasis^[12,21]. In addition, M2 macrophages create an immunosuppressive environment and greatly inhibit T-cell-mediated

adaptive immune responses, allowing tumor cells to evade immune surveillance and leading to the further development of cancer^[17].

In general, macrophages have an inseparable relationship with the TME. Therefore, for anti-tumor immunotherapy, utilizing the high plasticity and polarization of macrophages is of vital importance.

SOURCES OF MACROPHAGES TO FABRICATE CAR-Ms

The sources of macrophages to fabricate CAR-Ms are extensive and can be obtained through multiple production routes. Various sources of macrophages are presented in [Table 1](#).

Table 1 Summary of the common CAR construction applied in CAR-Ms

Target antigens	Target cells	CAR constructions	Macrophage sources
CD19	Cancerous Raji B cells	CD19 + Megf10 CD19 + FcR γ CD19 + tandem connection of FcR γ and p85 subunit of PI3K	J774A.1 murine macrophages
HER2	SKOV3 (HER2 ⁺ ovarian cancer cell line) 4T1 cells overexpressing human HER2-RGFP (HER2-4T1)	HER2 + CD3 ζ HER2 + CD147	Primary human macrophages, THP-1 RAW 264.7
CD19	CD19-expressing K562n leukemia cells and mesothelin-expressing OVCAR3/ASPC1 ovarian/pancreatic cancer cells	CD19 + CD3 ζ + FcR γ	iPSCs -derived macrophages
CCL19	4T1 cells	CCL19 + TLR2 CCL19 + TLR4 CCL19 + TLR6 CCL19 + MerTK CCL19 + 4-1BB + CD3 ζ	RAW 264.7

CAR: chimeric antigen receptor; CAR-Ms: CAR-macrophages; iPSCs: induced pluripotent stem cells.

Currently, conceptual verification research can be carried out in cell lines such as THP1 and RAW 264.7 or from primary/immortalized mouse bone marrow-derived macrophages (BMDM). Zhang *et al.* demonstrated that CAR-147, based on the murine RAW 264.7 cell line, effectively generated more matrix metalloproteinases (MMPs) through recognizing the antigen HER2 and triggering the CD147 intracellular domain^[26]. Besides, Niu *et al.* also applied RAW 264.7 to structure CAR-Ms, utilizing CCR7 as the antigen-recognition domain and Mer receptor tyrosine kinase (MerTK) as the intracellular domain, which displays splendid tumor cell phagocytic activity^[27]. Klichinsky *et al.* transduced the THP-1 cell line with CAR targeting mesothelin or HER2, demonstrating the phagocytosis of CAR-Ms on antigen-positive targeted cells^[28]. Morrissey *et al.* introduced CAR-P into J774A.1 murine macrophages through lentiviral infection to achieve cancer antigen-dependent engulfment^[29].

Although current research can be carried out in human and mouse model cell lines as listed above, clinical translation acquires scalable primary human cell sources. Compared with T cells, macrophages may become attractive cell therapies because of their splendid capability to avoid graft-versus-host disease (GVHD). Thus, macrophages derived from induced pluripotent stem cells (iPSCs) are ideal sources

for myeloid cell therapy. In the circumstances of identifying tumor-associated antigens, CAR-expressing iPSC-derived macrophage (CAR-iMac) cells represent antigen-dependent macrophage functions and anti-tumor effects *in vitro* and *in vivo*. Zhang *et al.* utilized iPSCs to engineer CAR-Ms with the antigen-recognition domain CD19, and found that they were able to induce macrophages to polarize into the M1 phenotype to exhibit anti-tumor activities *in vivo*^[26].

In fact, naive monocyte cell lines are unsuitable clinical sources for CAR-Ms as they have a high risk of tumorigenesis and development. To ensure security, genetic manipulation to modify monocyte cell lines is necessary^[30]. Researchers applied leukapheresis to obtain large numbers of peripheral blood monocytes and utilized granulocyte-macrophage colony-stimulating factor (GM-CSF) to further increase the number of available monocytes to 2–4 times^[31–32]. Generated from peripheral blood CD14⁺ monocytes with GM-CSF, primary human macrophages were cultured and differentiated into M1^[28].

STRATEGIES AND TACKS FOR CONSTRUCTING CAR: CURRENT ATTEMPTS

Recent attempts to engineer CARs for macrophages

have shown that the basic design principles of CARs for T cells' structures are also suitable for macrophage biology. Traditional CARs are transmembrane proteins, which comprise an extracellular antigen-recognition domain, a hinge domain, and an intracellular domain. Additionally, the intracellular domain contains a costimulatory domain and a signaling domain^[33].

Researchers have demonstrated that the antigen-recognition domain of CAR-Ms mainly contains widely representative targets, such as CD19, HER2, mesothelin, *etc.* Additionally, CD8 is the main hinge domain of CAR-Ms, which together with the intracellular domain CD3 ζ , can effectively redirects macrophages and induces antigen-dependent phagocytosis, cytokine release, and antitumor activity^[28]. The expression of CAR-Ms with CD3 ζ is capable of killing and engulfing tumor cells in an antigen-specific manner. Another activating domain of CAR-Ms is Fc receptor common gamma chain (FcR γ), which has a cytosolic domain that shares significant homology with CD3 ζ . In addition, a study has demonstrated that the activation domain of CAR-Ms constructed with either CD3 ζ or FcR γ has functional similarities in phagocytosis, confirming that CD3 ζ and FcR γ based CARs have comparable capabilities to activate T cells^[34]. Different CAR constructions are presented in [Table 1](#).

The choice of signaling domains has broadened markedly for designing CAR-Ms, and alternative domains have been explored. The first-generation CAR-Ms are modified with genetically programmed CARs to enhance the ability to target specific antigens and phagocytosis. However, the structure of CAR-Ms simply utilizes the properties of macrophages, mainly the phagocytic ability.

One of the most representative structures from the first generation is chimeric antigen receptor-phagocytosis (CAR-P). Researchers engineered a series of CAR-P which were able to direct macrophages to phagocytose specific antigens, especially cancer cells. However, the interaction between CAR-P macrophages and targeted cells was insufficient to induce the whole cell engulfment. Thus, experts discovered that the cytosolic domain of CAR-Ms, which expressed Megf10 and FcR γ , together with the addition of tandem PI3K recruitment domains, greatly improved the efficiency of macrophage phagocytosis on tumor cells^[29]. Furthermore, researchers designed a tandem CAR (CAR-Ptandem), whose structure was formed by connecting the PI3K p85 subunit with CAR-P-FcR γ . CAR-Ptandem performed better whole-cell

phagocytosis, implicating that the addition of phagocytic effectors can increase macrophage engulfment^[29].

The reason why CAR-T cell therapy is ineffective for solid tumors is that T cells are difficult to infiltrate into tumor tissues^[35]. Certain distinctive histopathological features of solid tumors, such as densely covered angiogenesis, tumor associated fibroblasts, as well as the extracellular matrix (ECM), keep T cells away from infiltrating into tumor parenchyma^[36]. Although these characteristics are beneficial to the growth of tumor cells, they create physical barriers for T cell infiltration in tumor sites. Thus, these characteristics prevent continuous engagement between T cells and tumor cells, impeding T cells from exerting cytotoxic antitumor effects. The synthesis and degradation of ECMs are mainly monitored by MMPs which originate from macrophages. Zhang *et al.* modified macrophages with an activation domain for CD147 (CAR-147), a membrane molecule that plays a significant part in MMPs expression and remodeling ECM^[37–38]. Therefore, after recognizing the tumor antigen HER2, the internal signaling of CD147 is triggered. Besides activating phagocytosis, CAR-147 targets tumor ECM by increasing the expression of MMPs^[26]. It was also found that CAR-147 macrophages could break ECMs and thus promote T cell infiltration into tumors. Furthermore, CAR-147 significantly increases the levels of IL-12 and IFN- γ in the TME, which plays an important role in anti-tumor activities^[28].

The second-generation CAR-Ms are under-developed. Besides maintaining the properties of the first-generation CAR-Ms, the aims of the second-generation CAR-M technologies comprise the improvement of tumor-associated antigen presentation and T cell activation. According to CAR-T therapy research, adding an intracellular cytoplasmic domain in CAR structure appears effective. Additionally, overcoming the plasticity of TAMs and inducing and maintaining anticancer phenotypes are of vital importance. Moreover, CAR-engineered macrophages are expected to expand *in vitro* and last a relatively long period to greatly achieve therapeutic effects. Based on these concepts, iPSCs can be utilized to deliver the CAR structure and later differentiate into macrophage-like cells, known as CAR-iMac^[39]. In the absence of antigens, CAR-iMac inclines to polarize into the M2 phenotype. Contrarily, in the presence of antigens, CAR-iMac prefers to transform into the inflammatory M1 phenotype and promotes tumor cell engulfment. Besides, the phagocytosis and immunologic activity of CAR-iMac are also enhanced

by the stimulation of solid tumor cell antigen expression. Therefore, to keep CAR-iMac closer to the M1 stage and further promote efficiency, it is of vital significance to design a more effective CAR structure and gene modification. Niu *et al.* applied CCL19, the natural ligand of CCR7, as the antigen-recognition domain, instead of scFv. They proved that MerTK, as an intracellular domain, exhibited splendid tumor cell cytotoxicity by CAR-Ms^[27]. In conclusion, the future field of iPSC-derived macrophages in myeloid-based cancer immunotherapy is bright.

The third-generation CAR-Ms are expected to greatly promote anticancer efficacy. This new strategy of reprogramming CAR-Ms *in vivo* through a nonviral vector is favored due to its highly cost-effective and simple manufacturing procedures. Moreover, the adaptation of cytokine receptor domains is also essential for further improving the immune regulation and anti-tumor ability of CAR-M products. Kang *et al.* attempted to utilize macrophage-targeting polymer nanocarriers to deliver gene-edited CARs and CAR-interferon- γ -encoding plasmid DNA into macrophages^[23]. The antitumor potency was further increased through IFN- γ , which induced CAR-Ms from M2 phenotypes into M1 phenotypes. However, the amplification ability of macrophages and transduction efficiency of the non-viral vector ought to further improve therapeutic efficacy.

These studies jointly illustrated that macrophages are able to utilize modular CARs to customize the responses of target tumor antigens. Future efforts of designing CAR-Ms can take the sophisticated structures of CAR-T into account, which include tandem activation domains, multi-antigen logic gates, drug-sensitive modules, and construction of secretory structure^[40–46]. In addition, it is of great significance to carefully optimize functions when generating new CAR-M constructions.

METHODS OF DELIVERING GENETICALLY MODIFIED CARs TO MACROPHAGES

Delivering CARs to macrophages remains a challenging task for researchers. However, owing to recent advances in gene delivery, it can be achieved through multiple viral-mediated and nonviral-mediated strategies.

The superior ability of myeloid cells to detect and respond to foreign nucleic acids leads macrophages and monocytes to become resistant to genetic modification^[47]. Thus, seeking out a delivery medium is of vital significance. Modified lentiviral virions

containing Vpx, a virion-packaged accessory protein, were confirmed capable of delivering genetically modified CARs to myeloid cells efficiently^[48]. Indeed, Vpx plays an important role in inducing the degradation of SAMHD1, an antiretroviral protein expressed in myeloid cells that inhibits the early stage of the viral life cycle through restricting the deoxynucleotide and preventing reverse transcription^[49]. Considering macrophages' restricted proliferation capacity, researchers speculated that non-integrating, replication deficient adenoviral vectors may reverse this difficulty by enhancing efficient and long-range transduction. Therefore, utilizing chimeric adenovirus 5-fiber 35 vector (Ad5f35) is an ideal option for macrophage transduction. Ad5f35 equipped with strong transduction ability, can effectively induce gene transference into primary human macrophages^[28,50]. Moreover, Ad5f35-infected macrophages stimulated inflammasome, which was involved in sustaining the M1 phenotype produced by pro-inflammatory activation signals^[51].

The third-generation CAR-Ms are mainly based on non-viral strategies. As for non-viral strategies, mRNA transfection, transposon systems, and bacterial plasmid DNA have been applied to macrophage bioengineering^[52–54]. In recent research, polymer nanocarriers (mannose-conjugated polyethyleneimine, MPEI) have been shown capable of transferring genes encoding CAR and IFN- γ into macrophages to potentially promote their anti-tumor ability^[23]. The main role of IFN- γ further increases their anti-tumor ability by inducing CAR-M1 macrophages^[55].

CLINICAL APPLICATION OF CAR-Ms

As of June 2023, three clinical trials based on CAR-M cell therapy were ratified by the US Food and Drug Administration (FDA) ([Table 2](#)). The first study to undergo a Phase I clinical trial was MaxCyte's MCY-M11, and patients were under recruiting. In this study, mRNA-targeted peripheral blood mononuclear cells (PBMCs) were utilized to construct mesothelin-CAR for the purpose of dealing with relapsed/refractory ovarian cancer and peritoneal mesothelioma. The second study involved a drug candidate from CARISMA Therapeutics, CT-0508. The first patient dosing was completed in the Phase 1 clinical trial, and relapsed/refractory solid-tumor patients who inclined to over-express HER2 were treated with anti-HER2 CAR-Ms. Here, CAR-Ms were modified with a chimeric adenoviral vector Ad5f35 carrying scFv targeting HER2 and went through recruiting status, and over 28 patients were involved to investigate the

therapeutic efficacy in humans^[28]. The third study was approved to collect tumor samples to develop breast cancer patients' derived organoids to test the antitumor

efficacy of CAR-Ms, and patients were being recruited for Phase I clinical trial. However, CAR-Ms have not entered clinical trials in China yet.

Table 2 Clinical trials of CAR-Ms

No. NCT	Start year	Phase	Diseases	Macrophage sources	Targets	Gene transfer
03608618	2018	I	Advanced ovarian cancer and peritoneal mesothelioma	PBMCs	Mesothelin	mRNA transfer
04660929	2020	I	HER2 overexpressing solid tumors	Primary human macrophages	HER2	Adenovirus transfection
05007379	2021	I	Breast cancer	No records	No records	No records

PBMCs: peripheral blood mononuclear cells

LIMITATIONS OF CAR-Ms

Though CAR-Ms have great immunotherapy potential against solid tumors, many difficulties still need to be conquered in order to achieve optimal results.

The first restriction is resulted from macrophage characteristics. Owing to their strong antiviral capabilities, transfection of CAR-viral vectors is an enormous challenge. The second restriction is the maintenance of M1 phenotypes *in vivo*. Due to the high plasticity and polarizability of macrophages, macrophage functions change dynamically in the TME, so CAR-Ms may in some cases promote tumor occurrence and progression. The third limitation is the expansion potential of CAR-Ms *in vivo*. The life cycle of macrophages can last for several months, so the therapeutic duration can last for a long time. Conversely, this may cause potential adverse effects, such as CRS, neurotoxicity, and so on. Furthermore, the fourth restriction has a correlation with migration characteristics of macrophages *in vivo*. During the treatment process, most exogenous macrophages are intercepted by the liver, which may produce adverse effects on cancer treatment^[55]. The last restriction is the loss of tumor antigens. A prominent issue in CAR-T therapy is insufficient target antigens owing to the high heterogeneity of tumor cells, which may also act as the main obstacle to the development of CAR-M treatment.

Urgent and effective measures are required to develop more credible transfection systems, control potential life-threatening adverse reactions, and avoid the catastrophic tumor promoting effects of CAR-Ms. In terms of clinical application, importance should be attached to multiple aspects. First and foremost, the safety and effectiveness of CAR-Ms needs to be considered, which is affected by the limited ability of macrophages in proliferation^[56]. Although this has been verified by animal experiments, further investigation is still needed to elucidate the capability

and potential risks of CAR-Ms in the human body. Ongoing research has demonstrated that the combination of CAR-Ms and antibody drugs can further improve the efficacy of CAR-Ms, and furthermore, that the effectiveness of CAR-Ms can be enhanced *via* antibody-based immunotherapy, which promotes macrophage phagocytosis to stimulate the immune response^[57]. For example, T cell checkpoint inhibitors which block PD1 signaling, were also shown to improve macrophage phagocytosis *in vivo*, indicating that future iterations of CAR-Ms may work synergistically with gene editing or assisted transgene overexpression^[58]. Secondly, reliable resources and amplification methods will provide favorable conditions for the clinical application of CAR-Ms. Thirdly, the application of virus-transfected CAR-M gene transfer may induce insertion mutations, which cannot be ignored.

SUMMARY AND PROSPECTS

The extraordinary biological functions of macrophages, such as engulfment, high plasticity, and polarization, have led macrophages to become a promising novel antitumor cellular immunotherapy. Moreover, macrophages can be generated from reliable sources including peripheral blood, THP-1, and iPSCs. Compared with CAR-T, the lower risk of developing GVHD is an additional advantage of CAR-Ms. There are numerous strategies available to construct CAR-Ms, mainly centering around different varieties of antigen-recognition domains and signaling domains. However, the splendid ability of macrophages to detect and respond to foreign nucleic acids makes them resistant to genetic modification. Thus, multiple strategies based on viral-mediated or nonviral-mediated have been developed, which include Vpx, Ad5f35, mRNA transfection, polymer nanocarriers, and so on.

CAR-Ms have high potential as an effective immunotherapy against cancer, but their effectiveness

and safety still need to be verified in clinical treatments in the future. Researchers should further explore the mechanism and regulatory factors of macrophage phagocytosis and antigen presentation, which may result in CAR-Ms exhibiting stronger anti-tumor effects against solid tumors and provide a promising method for adoptive immune cell therapies.

References

- [1] Kennedy LB, Salama AKS. A review of cancer immunotherapy toxicity[J]. *CA Cancer J Clin*, 2020, 70(2): 86–104.
- [2] Zhang Y, Zhang Z. The history and advances in cancer immunotherapy: understanding the characteristics of tumor-infiltrating immune cells and their therapeutic implications[J]. *Cell Mol Immunol*, 2020, 17(8): 807–821.
- [3] Hong M, Clubb JD, Chen YY. Engineering CAR-T cells for next-generation cancer therapy[J]. *Cancer Cell*, 2020, 38(4): 473–488.
- [4] Dai H, Wu Z, Jia H, et al. Bispecific CAR-T cells targeting both CD19 and CD22 for therapy of adults with relapsed or refractory B cell acute lymphoblastic leukemia[J]. *J Hematol Oncol*, 2020, 13(1): 30.
- [5] Martinez M, Moon EK. CAR T cells for solid tumors: new strategies for finding, infiltrating, and surviving in the tumor microenvironment[J]. *Front Immunol*, 2019, 10: 128.
- [6] Hanahan D, Coussens LM. Accessories to the crime: functions of cells recruited to the tumor microenvironment[J]. *Cancer Cell*, 2012, 21(3): 309–322.
- [7] Renner K, Singer K, Koehl GE, et al. Metabolic hallmarks of tumor and immune cells in the tumor microenvironment[J]. *Front Immunol*, 2017, 8: 248.
- [8] Franken L, Schiwon M, Kurts C. Macrophages: sentinels and regulators of the immune system[J]. *Cell Microbiol*, 2016, 18(4): 475–487.
- [9] Yang L, Zhang Y. Tumor-associated macrophages: from basic research to clinical application[J]. *J Hematol Oncol*, 2017, 10(1): 58.
- [10] Gunasekaran GR, Poongkavithai Vadevoo SM, Baek MC, et al. M1 macrophage exosomes engineered to foster M1 polarization and target the IL-4 receptor inhibit tumor growth by reprogramming tumor-associated macrophages into M1-like macrophages[J]. *Biomaterials*, 2021, 278: 121137.
- [11] Guerriero JL. Macrophages: the road less traveled, changing anticancer therapy[J]. *Trends Mol Med*, 2018, 24(5): 472–489.
- [12] Arango Duque G, Descoteaux A. Macrophage cytokines: involvement in immunity and infectious diseases[J]. *Front Immunol*, 2014, 5: 491.
- [13] Biswas SK, Allavena P, Mantovani A. Tumor-associated macrophages: functional diversity, clinical significance, and open questions[J]. *Semin Immunopathol*, 2013, 35(5): 585–600.
- [14] Yoon DH, Osborn MJ, Tolar J, et al. Incorporation of immune checkpoint blockade into chimeric antigen receptor T cells (CAR-Ts): combination or built-in CAR-T[J]. *Int J Mol Sci*, 2018, 19(2): 340.
- [15] Netea-Maier RT, Smit JWA, Netea MG. Metabolic changes in tumor cells and tumor-associated macrophages: a mutual relationship[J]. *Cancer Lett*, 2018, 413: 102–109.
- [16] Smith-Garvin JE, Koretzky GA, Jordan MS. T cell activation[J]. *Annu Rev Immunol*, 2009, 27: 591–619.
- [17] Noy R, Pollard JW. Tumor-associated macrophages: from mechanisms to therapy[J]. *Immunity*, 2014, 41(1): 49–61.
- [18] De Palma M, Lewis CE. Macrophage regulation of tumor responses to anticancer therapies[J]. *Cancer Cell*, 2013, 23(3): 277–286.
- [19] Schildberger A, Rossmanith E, Eichhorn T, et al. Monocytes, peripheral blood mononuclear cells, and THP-1 cells exhibit different cytokine expression patterns following stimulation with lipopoly-saccharide[J]. *Mediators Inflamm*, 2013, 2013: 697972.
- [20] Shapouri-Moghaddam A, Mohammadian S, Vazini H, et al. Macrophage plasticity, polarization, and function in health and disease[J]. *J Cell Physiol*, 2018, 233(9): 6425–6440.
- [21] Fang FC. Antimicrobial actions of reactive oxygen species[J]. *mbio*, 2011, 2(5): e00141–11.
- [22] Le J, Prenskey W, Yip YK, et al. Activation of human monocyte cytotoxicity by natural and recombinant immune interferon[J]. *J Immunol*, 1983, 131(6): 2821–2826.
- [23] Kang M, Lee SH, Kwon M, et al. Nanocomplex-mediated *in vivo* programming to chimeric antigen receptor-M1 macrophages for cancer therapy[J]. *Adv Mater*, 2021, 33(43): e2103258.
- [24] Zhang F, Parayath NN, Ene CI, et al. Genetic programming of macrophages to perform anti-tumor functions using targeted mRNA nanocarriers[J]. *Nat Commun*, 2019, 10(1): 3974.
- [25] Shan H, Dou W, Zhang Y, et al. Targeted ferritin nanoparticle encapsulating CpG oligodeoxynucleotides induces tumor-associated macrophage M2 phenotype polarization into M1 phenotype and inhibits tumor growth[J]. *Nanoscale*, 2020, 12(43): 22268–22280.
- [26] Zhang W, Liu L, Su H, et al. Chimeric antigen receptor macrophage therapy for breast tumours mediated by targeting the tumour extracellular matrix[J]. *Br J Cancer*, 2019, 121(10): 837–845.
- [27] Niu Z, Chen G, Chang W, et al. Chimeric antigen receptor-modified macrophages trigger systemic anti-tumour immunity[J]. *J Pathol*, 2021, 253(3): 247–257.
- [28] Klichinsky M, Ruella M, Shestova O, et al. Human chimeric antigen receptor macrophages for cancer immunotherapy[J]. *Nat Biotechnol*, 2020, 38(8): 947–953.
- [29] Morrissey MA, Williamson AP, Steinbach AM, et al. Chimeric antigen receptors that trigger phagocytosis[J]. *Elife*, 2018, 7: e36688.
- [30] Liu Q, Li J, Zheng H, et al. Adoptive cellular immunotherapy for solid neoplasms beyond CAR-T[J]. *Mol Cancer*, 2023, 22(1): 28.
- [31] Hennemann B, Rehm A, Kottke A, et al. Adoptive

- immunotherapy with tumor-cytotoxic macrophages derived from recombinant human granulocyte-macrophage colony-stimulating factor (rhuGM-CSF) mobilized peripheral blood monocytes[J]. *J Immunother*, 1997, 20(5): 365–371.
- [32] Stevenson HC, Miller P, Akiyama Y, et al. A system for obtaining large numbers of cryopreserved human monocytes purified by leukapheresis and counter-current centrifugation elutriation (CCE)[J]. *J Immunol Methods*, 1983, 62(3): 353–363.
- [33] June CH, Sadelain M. Chimeric antigen receptor therapy[J]. *N Engl J Med*, 2018, 379(1): 64–73.
- [34] Fu W, Lei C, Ma Z, et al. CAR macrophages for SARS-CoV-2 immunotherapy[J]. *Front Immunol*, 2021, 12: 669103.
- [35] Ma S, Li X, Wang X, et al. Current progress in CAR-T cell therapy for solid tumors[J]. *Int J Biol Sci*, 2019, 15(12): 2548–2560.
- [36] Slaney CY, Kershaw MH, Darcy PK. Trafficking of T cells into tumors[J]. *Cancer Res*, 2014, 74(24): 7168–7174.
- [37] Toole BP. Emmprin (CD147), a cell surface regulator of matrix metalloproteinase production and function[J]. *Curr Top Dev Biol*, 2003, 54(1): 371–389.
- [38] Zhang J, Wang Z, Zhang X, et al. Large-scale single-cell and bulk sequencing analyses reveal the prognostic value and immune aspects of CD147 in pan-cancer[J]. *Front Immunol*, 2022, 13: 810471.
- [39] Zhang L, Tian L, Dai X, et al. Pluripotent stem cell-derived CAR-macrophage cells with antigen-dependent anti-cancer cell functions[J]. *J Hematol Oncol*, 2020, 13(1): 153.
- [40] Yang M, Tang X, Zhang Z, et al. Tandem CAR-T cells targeting CD70 and B7-H3 exhibit potent preclinical activity against multiple solid tumors[J]. *Theranostics*, 2020, 10(17): 7622–7634.
- [41] Han X, Wang Y, Wei J, et al. Multi-antigen-targeted chimeric antigen receptor T cells for cancer therapy[J]. *J Hematol Oncol*, 2019, 12(1): 128.
- [42] Lee S, Wong WW. The most logical approach to improve CAR T cell therapy[J]. *Cell Syst*, 2020, 11(5): 421–423.
- [43] Urak R, Walter M, Lim L, et al. *Ex vivo* Akt inhibition promotes the generation of potent CD19CAR T cells for adoptive immunotherapy[J]. *J Immunother Cancer*, 2017, 5(1): 26.
- [44] Jiang W, Li T, Guo J, et al. Bispecific c-Met/PD-L1 CAR-T cells have enhanced therapeutic effects on hepatocellular carcinoma[J]. *Front Oncol*, 2021, 11: 546586.
- [45] Feng Z. The research progress and tendency of CAR-T cell technology[J]. *J Nanjing Med Univ (in Chinese)*, 2020, 40(7): 937–939.
- [46] Zhao W, Jia L, Zhang M, et al. The killing effect of novel bi-specific Trop2/PD-L1 CAR-T cell targeted gastric cancer[J]. *Am J Cancer Res*, 2019, 9(8): 1846–1856.
- [47] Bartok E, Hartmann G. Immune sensing mechanisms that discriminate self from altered self and foreign nucleic acids[J]. *Immunity*, 2020, 53(1): 54–77.
- [48] Bobadilla S, Sunseri N, Landau NR. Landau. Efficient transduction of myeloid cells by an HIV-1-derived lentiviral vector that packages the Vpx accessory protein[J]. *Gene Ther*, 2013, 20(5): 514–520.
- [49] Laguet N, Sobhian B, Casartelli N, et al. SAMHD1 is the dendritic- and myeloid-cell-specific HIV-1 restriction factor counteracted by Vpx[J]. *Nature*, 2011, 474(7353): 654–657.
- [50] Nilsson M, Ljungberg J, Richter J, et al. Development of an adenoviral vector system with adenovirus serotype 35 tropism; efficient transient gene transfer into primary malignant hematopoietic cells[J]. *J Gene Med*, 2004, 6(6): 631–641.
- [51] Lam E, Stein S, Falck-Pedersen E. Adenovirus detection by the cGAS/STING/TBK1 DNA sensing cascade[J]. *J Virol*, 2014, 88(2): 974–981.
- [52] Cha EB, Shin KK, Seo J, et al. Antibody-secreting macrophages generated using CpG-free plasmid eliminate tumor cells through antibody-dependent cellular phagocytosis[J]. *BMB Rep*, 2020, 53(8): 442–447.
- [53] Moradian H, Roch T, Lendlein A, et al. mRNA transfection-induced activation of primary human monocytes and macrophages: dependence on carrier system and nucleotide modification[J]. *Sci Rep*, 2020, 10(1): 4181.
- [54] Wang X, Wang G, Wang N, et al. A simple and efficient method for the generation of a porcine alveolar macrophage cell line for high-efficiency Porcine reproductive and respiratory syndrome virus 2 infection[J]. *J Virol Methods*, 2019, 274: 113727.
- [55] van der Heide D, Weiskirchen R, Bansal R. Therapeutic targeting of hepatic macrophages for the treatment of liver diseases[J]. *Front Immunol*, 2019, 10: 2852.
- [56] Kim J, Bae JS. Tumor-associated macrophages and neutrophils in tumor microenvironment[J]. *Mediators Inflamm*, 2016, 2016: 6058147.
- [57] Weiskopf K, Weissman IL. Macrophages are critical effectors of antibody therapies for cancer[J]. *MAbs*, 2015, 7(2): 303–310.
- [58] Gordon SR, Maute RL, Dulken BW, et al. PD-1 expression by tumour-associated macrophages inhibits phagocytosis and tumour immunity[J]. *Nature*, 2017, 545(7655): 495–499.

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