



# Reprogramming Immune Cells for Enhanced Cancer Immunotherapy: Targets and Strategies

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Cancer is one of the leading causes of death and a major public health problem all over the world. Immunotherapy is becoming a revolutionary clinical management for various cancer types. Restoration of aberrant immune surveillance on cancers has achieved remarkable progress in the past years by either *in vivo* or *ex vivo* engineering of the immune cells. Here, we summarized the central roles of immune cells in tumor progression and regression, and the existing and emerging strategies for different immune cell-based immunotherapies. In addition, the current challenges and the potential solutions in translating the immunotherapies into the clinic are also discussed.

**Keywords:** cancer immune therapy, cell engineering, targets, strategies, immune cells

## INTRODUCTION

Cancer incidence and mortality have been increasing since 2010, making cancer the leading cause of death and a major public health problem all over the world (1, 2). The traditional cancer therapies, such as surgery, radiotherapy, and chemotherapy, have difficulty in completely eradicating cancer cells. The emerging immunotherapy is revolutionizing the clinical management of multiple tumors (3).

Tumor microenvironment (TME) is infiltrated by immune cells, which together with stromal cells, contribute to tumor escape from host immune surveillance and its progression (4, 5). Generally, the immune cells in TME can be divided into two types: tumor-antagonizing and tumor-promoting immune cells. The tumor-antagonizing immune cells consist of CD8<sup>+</sup> cytotoxic T cells, effector CD4<sup>+</sup> T cells, natural killer (NK) cells, dendritic cells (DCs), M1-polarized macrophages, and N1-polarized neutrophils. In contrast, the tumor-promoting immune cells mainly consist of regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs). MDSCs could be further divided into two subtypes: the polymorphonuclear MDSCs (PMN-MDSCs) and the monocytic MDSCs (M-MDSCs). The PMN-MDSCs are morphologically similar to N2-polarized neutrophils, whereas M-MDSCs are similar to M2-polarized macrophages. Notably, the role of B cells in TME is relatively unclear and controversial, with both tumor-antagonizing and protumorigenic roles being reported (6).

Aberrant innate and adaptive immune responses are closely related to immunosuppression and tumorigenesis (7). During the early stages of tumor progression, natural killer (NK) cells and CD8<sup>+</sup> T cells act as cytotoxic immune cells to recognize and kill tumor cells (8). However, there remains a subset of less immunogenic tumor cells that survived as the dominant cells afterward, which escaped immune surveillance (9, 10). As the tumor continuously grows, different kinds of immune cells adopt various ways to form the immune-suppressive microenvironment, which eventually weakens the tumoricidal effects (11).

The main mechanism of immunotherapy is to change the tumor microenvironment or the immune cells so that the immune system can achieve the purpose of killing tumors (5). Immunotherapies targeting accessory immune cells are considered as promising strategies against a variety of cancers (5, 11, 12). Here, we summarize the key roles of different immune cells in the tumor microenvironment and emerging immunotherapy strategies based on modulation of different immune cells. Future challenges and the possible solutions that translate the immunotherapies into clinical reality are also discussed.

## CHIMERIC ANTIGEN RECEPTOR T-CELL-BASED IMMUNOTHERAPY

Malignant progression stimulates adaptive immune responses and creates a subset of specific T cells that can precisely eliminate tumor cells (13, 14). However, with the development of tumors, tumor cells become less immunogenic and lose specific tumor antigens that activate adaptive response at the beginning of malignant progression (15, 16). Besides, low expression of class I MHC molecules on tumor cells also leads to the downregulation of CD8<sup>+</sup> cytotoxic T lymphocytes (CTLs) and thus immunosuppression (17). Immune checkpoints, CTLA-4 and PD-1/PD-L1 are also blamed to suppress T-cell activity when bound by the corresponding ligands on tumor cells (18–20).

Chimeric antigen receptor (CAR) T cell technology is an innovative therapy, which harnesses the inherent capacity of the immune system to fight cancer selectively in an MHC-independent way. CARs are synthetic antigen receptors that include both antigen recognition moieties and T-cell activation signaling domains (21). A CAR consists of three major domains: ectodomain, transmembrane domain, and endodomain (22, 23). The ectodomain is exposed to the extracellular space with signal peptide, antigen recognition region, and spacer. The antigen recognition region is usually a single chain variable fragment (scFv) formed by fusing the variable portions of heavy and light chains of a monoclonal antibody with a flexible linker (24, 25). The scFv presents the function of identifying and binding tumor antigens with high affinity. A spacer functions as a connection between the antigen-binding domain and the transmembrane domain (26). The transmembrane domain is derived from most of the membrane-proximal components of the endodomain and consists of a hydrophobic alpha helix spanning the membrane, which is related to the stability of the receptor. CD3 $\zeta$  serves as the most common component of the endodomain, which activates T cells after CAR binds the target antigen. Additionally, the CAR internal domain undergoes intergenerational changes, including one or more costimulatory domains, such as the commonly used CD28 and 41BB, to enhance the persistence and cytotoxicity of CAR-expressing cells (27, 28).

Based on the structure of the endodomain, CAR-T cells can be roughly divided into four generations (29). The first generation CARs mimicked the signals from endogenous T-cell receptor (TCR), which contains only one activating domain (usually a portion of the  $\zeta$  chain in the TCR complex). Additional activating

domains are then added in the next two generations, with more CAR-T-cell proliferation, stronger killing ability, and higher cytokine production. The fourth-generation CARs were typically characterized by the addition of IL-12 to the second generation of CAR-T (30). Currently, CAR-T have been tried to various types of cancer, including leukemia and solid tumors. Moreover, CAR-T could be even engineered to eradicate the cancer stem cells.

### CAR-T for Leukemia

CAR-T immunotherapy has produced a particularly successful clinical response in the treatment of hematologic malignancies. Up to now, three CAR-T cell products have been approved by the U.S. Food and Drug Administration, which all target CD19 antigen. CD19 is a cell-surface component of the B-cell receptor complex involved in B-cell activation, which expressed at high and stable levels on tumor tissue from most patients with B cell acute lymphoblastic leukemia (B-ALL), non-Hodgkin's lymphoma (NHL), and chronic lymphocytic leukemia (CLL). CAR-T cells targeting CD19 have emerged to present a marked efficacy to directly eradicate liquid tumors and induce sustained tumor regression of B lineage cell malignancies (31, 32). It is worth noting that cytokines released by CD19 CAR-T cells also demonstrated the ability to activate both innate and adaptive immune systems and enhance tumor rejection.

Besides CD19-targeted CAR-T, CD22 and CD123 targeted CAR-T were also developed to treat leukemia. CD22-targeted CAR-T cells have a potent antileukemic activity and modest off-target toxicity (33). Since CD123 is expressed on a range of hematological malignancies, CAR-T targeting CD123 has also a potential role in the prevention of tumor progression and with additional therapeutic effects on eradicating the central nervous system in hematological malignancies (34). In general, all these different targeting CAR-T therapies provide rapid, efficient, and uninhibited regression and destruction of B-cell cancers.

### Chimeric Antigen Receptor-T Cell for Solid Tumors

Besides leukemia, CAR-T-cell therapy has been demonstrated to be effective against several kinds of solid tumors recently, including melanoma, colon cancer, non-small cell lung cancer, ovarian cancer, mesothelioma, and neuroblastoma (35). Mesothelin, epidermal growth factor receptor (EGFR), and human epidermal growth factor receptor 2 (HER2) are the most commonly focused antigen targets in CAR-T therapy to solid malignancies (Table 1).

Mesothelin is a 40-kDa cell-surface glycoprotein. The precursor protein is proteolytically processed into two proteins, a 30-kDa soluble megakaryocyte potentiation factor and a 40-kDa GPI-anchored plasma membrane protein mesothelin (36). Mesothelin is limitedly expressed on mesothelial cells in different types of tissues including pleura, peritoneum, and pericardium but is highly expressed as a tumor-differentiation antigen in a broad spectrum of solid tumors, which make mesothelin as an attractive target for cancer immunotherapy (37). Mesothelin-specific CAR-T-cell therapy also has attracted widespread interest. Commonly, mesothelin-CAR-T-cells consist of anti-mesothelin scFv SS1 fused to TCRzeta signaling and

**TABLE 1** | Engineering strategies for effector chimeric antigen receptor-T (CAR)-T cell.

Specific antigens for engineered receptors	Treated cancer types	Function/effects
CD19	B-NHL (DLBCL, follicular lymphoma, mantle cell lymphoma), CLL, B-ALL	Leukemia-specific targeting
CD22	B-ALL	
CD123	AML B-ALL	
Mesothelin	Mesothelioma, TNBC, pancreatic cancer, lung cancer, gastric cancer, ovarian cancer, bile duct carcinoma	Cancer-specific targeting in solid tumor
EGFR/EGFRvIII	NSCLC, glioblastoma	
HER2	Ovarian cancer, breast cancer, osteosarcoma, HER2-positive sarcoma, glioblastoma	
CD133	Glioblastoma, lung cancer, breast cancer, liver cancer, gastric cancer, ovarian cancer, pancreatic cancer, colorectal cancer, prostate cancer	Cancer stem cell-specific targeting
CD90		
EpCAM		
ALDH		
PSCA	Prostate cancer	

ALDH, aldehyde dehydrogenases; AML, acute myeloid leukemia; B-ALL, acute B lymphoblastic leukemia; B-NHL, B cell non-Hodgkin's lymphoma; CLL, chronic lymphocytic leukemia; DLBCL, diffuse large B-cell lymphoma; EGFR, epidermal growth factor receptor; EGFRvIII, the epidermal growth factor receptor variant III; EpCAM, epithelial cell adhesion molecule; HER2, human epidermal growth factor receptor 2; NSCLC, non-small-cell lung carcinoma; PSCA, prostate stem cell antigen; TNBC, triple-negative breast cancer.

costimulatory domains. There are various clinical studies to investigate the safety and feasibility of mesothelin-specific CAR-T in mesothelioma, lung cancer, breast cancer with pleural metastases (38). All of these trials demonstrated that the application of mesothelin-specific CAR-T-cell therapy is a promising strategy for mesothelin-expressing malignancies.

EGFR is a 170-kDa transmembrane glycoprotein belonging to the ErbB oncogene family of tyrosine kinase receptors. Aberrant activation of EGFR leads to autophosphorylation of receptor tyrosine kinase that ultimately drives cell proliferation and metastasis in various types of tumors (39). Anti-EGFR CAR-T therapy is considered as an alternative way for EGFR-overexpressed solid malignancies (40).

Human epidermal growth factor 2 (HER2) is a membrane tyrosine of the EGFR family. HER2 promotes oncogenesis of several cancers and is found to be amplified in multiple tumors. An  $\alpha$ HER2/CD3 RNA-engineered CAR-T-like T cells have achieved success in treating HER2<sup>+</sup> malignancies (41). Furthermore, HER2-specific CAR-T cells present a prominent effect in targeting and killing HER2-positive cancers such as GBM, ovarian cancer, and breast cancer (42). HER2-specific CAR-T cells along with PD1 blockade was demonstrated to have a significant therapeutic potential for glioblastoma (43).

In addition to preclinical studies, clinical trials also revealed the safety, feasibility, and activity of CAR-T immunotherapy targeting HER2 in patients with advanced biliary tract cancers (BTCs) and pancreatic cancer (PCs) (44).

## Chimeric Antigen Receptor-T Cell Targeting Cancer Stem Cells

Cancer stem cells (CSCs) are a subpopulation of tumor cells that mimic self-renewal and multilineage differentiation capacity of normal tissues and are responsible for maintaining tumor heterogeneity, enhancing tumor growth, therapeutic resistance, immune evasion, invasion, and metastasis (45–47). Thus, specifically targeting CSCs is crucial for developing effective therapeutics. Numerous surface markers expressed on CSCs, namely, CD133, are applied to identify CSCs and thus provide potential targets for CAR-T cell therapy. A study demonstrated that anti-CD133 CAR-T cells efficiently eliminate glioblastoma stem cells *in vitro* and *in vivo* (48). Meanwhile, a case report indicated the safety and feasibility of the combination treatment of anti-EGFR CAR-T cells and anti-CD133 CAR-T cells in patients with cholangiocarcinoma (49). Besides CD133, prostate stem cell antigen (PSCA), CD90, EpCAM, and ALDH also can be considered as important target antigens for CAR-T-cell therapy in cancer treatment, which needs further study in both preclinical and clinical settings (50, 51).

## Challenges and Solutions of Chimeric Antigen Receptor-T Cell Therapy Adverse Effects

Adverse effects are accompanied by all cancer therapies and sometimes can be a big challenge. The toxicities of CAR-T cell therapy include cytokine release syndrome (CRS), immune effector cell-associated neurotoxicity syndrome (ICANS), cytopenias, and B-cell aplasia (related to CD19 targeting CART cells). CRS and ICANS have emerged as dominant CAR-T-cell-mediated toxicities. The onset of the CRS correlates with T-cell activation and high levels of cytokines, which have no target preference as it can be observed in both CD19 and other novel CARs. The choice of costimulation domain can be a major predictor of toxicity. For 4-1BB incorporated CARs, there were 58% patients who had CRS of any grade at a median of 5 days from infusion (52). Compared with 4-1BB costimulation domain, incorporation of CD28 leads to more rapid and higher peak expansion of CAR-T cells (53), leading to a 93% incidence of any grade CRS at a median of 2 days from CAR-T-cell infusion (54). The severity of the CRS is also associated with tumor burden at the time of treatment (55), as heavy tumor burden can provide more stimulation for CAR-T cell expansion. The cause of ICANS remains poorly understood. Potential causes include direct central nervous system toxicity by the CAR-T cells, diffusion of inflammatory cytokines through the blood-brain barrier (BBB), and the dysfunction of the BBB caused by CAR-T cells and/or cytokines.

The safety of CAR-T-cell therapies can be improved by early drug intervention such as using glucocorticoids and tocilizumab,

an anti-IL6 receptor antagonist, for CRS treatment, and optimal genetic engineering strategies to reduce CAR-T cell toxicity (56).

### Low Efficacy in Solid Tumors

Unlike hematological malignancies, solid tumors present several barriers that affect the safety and clinical outcomes of CAR-T-cell therapy. Target antigen specificity and heterogeneity, lymphocyte trafficking, and tumor-induced immunosuppression are three major factors that hindered the efficacy of CAR-T immunotherapy in solid tumors. Types of approaches are currently explored to address these challenges to enhance treatment efficacy (Table 1).

Selection of an optimal tumor-associated antigen (TAA) is considered as one of the most significant steps for CAR-T targeting. TAA should be highly expressed on all tumor cells but hopefully not expressed on the normal tissues. Although various tumor antigens including neoantigens, oncofetal antigens, and tumor-selective antigens are investigated for CAR-T-cell therapy, there remain no ideal ones meeting the criteria of specificity (57). Another major limitation to TAAs of solid tumors is antigen heterogeneity, which is a variable of the expression of antigen on the cells within a given tumor. To date, employing more targets, targeting multiple tumor antigens at once, and exploring new antigen-activated T-cell killing pathways are three approaches to address the problem of tumor antigen specificity and heterogeneity (58).

Insufficient migration and infiltration to tumor sites is the additional challenge of CAR-T-cell treatment to solid tumors. It is demonstrated that persistence and intratumoral accumulation of CAR-T is inevitably limited after adoptive transfer especially in the liver, lung, and spleen (59). It could be partially attributed to downregulation of cellular adhesion molecules, which inhibits T-cell transmigration. Besides, solid tumors have the capacity of modifying the structure of the adjacent tissue that hinders intratumoral lymphocyte accumulation. Plenty of strategies have been applied to increase lymphocyte migrating and infiltrating to tumor sites (60). Local administration can enhance the accumulation of engineered CAR-T cells at tumor sites and superior control of tumor growth compared with systemic administration, as shown by several preclinical solid tumor models (61, 62). Chemokine receptor–ligand interactions play an important role in mediating endogenous immune cell trafficking. CAR-T cells can be modified to express certain cytokine receptors to enhance trafficking into tumor tissue. For example, expression of a functional CCR2 receptor can enhance tumor localization and tumor eradication of the mesothelin-CAR-T cells (63).

Tumor immunosuppressive microenvironment hinders the efficacy of CAR-T therapy even if the T cell is successfully trafficking the tumor sites. The anatomical structure generated by tumor stroma and the associated high tissue pressure provide natural barriers for CAR-T therapy, while hypoxia and nutrient starvation are two factors of metabolic barriers (64). Limiting the numbers of tumor stroma cells, exposed to a hyperoxia environment, and manipulating key cellular regulators of nutrients, have shown the attractive outcomes to augment antitumor immunity and repress tumor growth

(65, 66). Additionally, tumor-derived cytokines, namely, TGF $\beta$ , might reduce the antitumor response of CAR-T therapy (67). Theoretically, engineering novel CAR-T cells expressing negative TGF $\beta$  receptor might be beneficial. Furthermore, inhibitory leukocytes like regulatory T cells, tumor-associated macrophages (TAMs), and myeloid-derived suppressor cells (MDSCs) present as potent barriers of CAR-T therapy (68, 69). Reprogramming of the immunosuppressive nature of the TME by genetically engineering CAR-T cells with immune-modulating cytokines is the most commonly used strategy to address this problem, which should be explored in future studies.

### Chimeric Antigen Receptor-T-Cell Therapy Resistance

CAR-T therapy resistance is also a challenge in the field. Various CD19 mutations and alternative splicing have been the dominant cause of CAR-T-cell resistance. In this setting, multivalent targeting CARs or serial manipulation with multiple different CAR-T cells may prevent single-agent resistance. The combination of CD19 and BCMA targeted CAR-T cells, either combined infusion of both anti-CD19 and anti-BCMA CAR-T cells or a tan-CAR with both a scFv-CD19 and scFv-BCMA in tandem orientation, may help to reduce the rate of relapse in the treatment with single scFv-CAR-T cells (70, 71). The same strategy can be explored for solid tumors.

Altogether, CAR-T cell therapy has proven to be an inspiring strategy for cancer treatment. Various studies have highlighted that CAR-T cells have achieved encouraging outcomes in various malignancies, while several barriers including the selection of TAA, lymphocyte trafficking, and tumor immunosuppression in solid tumors restrict the effects. There remains much for us to explore to enhance the therapeutic effects of CAR-T cells in cancer treatment (72).

## THERAPEUTIC STRATEGIES TARGETING BONE MARROW-DERIVED SUPPRESSOR CELLS

Bone marrow-derived suppressor cells (MDSCs) consist of a cluster of highly heterogeneous cells generated from myeloid progenitors, which protect a tumor from the immune system and restrain the efficacy of immunotherapy (73, 74). The immunosuppressive cytokines caused by tumor-related chronic inflammation induce normal myeloid cell precursors to proliferate and differentiate into MDSCs, which suppress the antitumor effect and promote tumor progression (75). Therefore, targeted numerous enzymes, growth factors, and cytokines regulating the lifecycle of MDSCs may serve as efficient ways to eliminate cancer (76). For instance, neutralizing antibody to KIT significantly reduced MDSC expansion and unleash anti-tumor efficacy of T cells in colon carcinoma (77). Antagonists of CXCR2 (S-265610) and CXCR4 (AMD3100) altered the recruitment of immature myeloid cells (iMCs) to the tumor and thus reverted the environment that favors tumor progression (78). Besides, anti-IL-6R mAb could eliminate the accumulation of MDSCs, subsequently upregulating IFN $\gamma$  and enhancing

antitumor T-cell response (79). In summary, eliminating MDSCs is a promising way to unleash immunosuppression in a tumor microenvironment and kill cancer.

## THERAPEUTIC STRATEGIES TARGETING TUMOR-ASSOCIATED MACROPHAGES

Tumor-associated macrophages (TAMs) are the most abundant MDSCs in the TME. They secrete various cytokines, growth factors, chemokines as well as inflammatory mediators that promote key processes in tumor progression (80–82). TAMs function in the processes of angiogenesis, invasion, and metastasis. TAM-induced immunosuppression is mediated by the expression of inhibitory checkpoints, including PD-L1, PD-L2, and the non-classical major histocompatibility complex (MHC) class I (MHC-I) molecules (83). Meanwhile, TAMs secrete several cytokines including IL-10, TGF $\beta$ , and CCL5, maintaining a strong immunosuppressive microenvironment by inducing regulatory T (T<sub>reg</sub>) cell expansion. TAMs also release arginase I to deplete L-arginine, which directly inhibits T-cell cytotoxicity (84).

TAMs exhibit roles of promoting tumor or inhibiting tumor upon different stimuli, which depend on the status of the polarization of macrophage (85). M1-like TAMs accumulating at very early phases of oncogenesis stimulate antitumor immunity and hold the capacity of tumoricidal effect. However, the persistence of M1-like TAMs can induce chronic inflammation, hence, enhancing genomic instability in tumor cells and acts as a driver of oncogenesis in the early oncogenesis (86). Various studies have demonstrated that M2-like TAMs present as the dominant subtype through the progress of “re-education” by contexture changes of immune cells and metabolic factors in TME (87, 88). With the characterization of plasticity and heterogeneity, reprogramming macrophage from M2- to M1-like may provide a viable strategy to eliminate tumor cells (89, 90). Accumulating researches are devoted to reversing the pro-tumor effect of TAMs (Table 2).

Currently, depletion, recruitment inhibition, and reprogramming are three commonly used strategies of TAM targeting under clinical trial investigation (91). First, the most advanced approaches of TAM depletion depends on inhibition of colony-stimulating factor 1 and its receptor (CSF1/CSF1R) signaling. CSF1 binds with CSF1R, a class III receptor tyrosine kinase, regulating differentiation, migration, and survival of macrophage and its precursors (92). Various small molecules inhibiting CSF1R tyrosine kinase have been investigated in several researches. Preclinical researches revealed that PLX3397 inhibited tumor-associated microglia and enhances sensitivity to chemotherapy of glioma, c-kit-mutated melanoma, prostate cancer, and classical Hodgkin lymphoma (cHL) (93–95). In addition, CSF1R targeting small molecules, including ARRY-382, PLX7486, BLZ945, and JNJ-40346527, which target the intracellular tyrosine kinase of CSF1R, are in completed or ongoing studies in solid tumors and classical Hodgkin lymphoma (96). Antibodies also play an essential role in targeting CSF1/CSF1R. A study showed that a monoclonal

antibody RG7155 strongly reduces TAMs with an increase in T-cell ratio in diffuse-type giant cell tumor patients (97). Furthermore, the compounds MCS110 and PD-0360324 targeting the ligand CSF1 are also found to have the capacity to effectively clear TAMs (95). Compared with the inhibitors of CSF1/CSF1R, bisphosphonates in liposomes seems to be a more directed approach of TAM depletion. Evidence was shown in lung cancer, melanoma, hepatocellular carcinoma, and lung metastasis from breast cancer that bisphosphonates significantly reduced TAM infiltration (98–100). However, there are plenty of side effects remaining in the strategy of TAM depletion. Anti-CSF1R antibodies also non-selectively target non-tumor macrophages with many safety concerns especially accompanied by complications. The increased expression of IFN $\gamma$  and IFN $\alpha$  after CSF1R inhibition directly leads to upregulation of immune checkpoint molecules, such as PD-1 and CTLA-4, possibly restraining its therapeutic effects (101).

Second, blockade of monocyte recruitment to tumors serves as an alternative approach to hinder TAMs, namely, the application of CCL2/CCR2 inhibitors. CCR2, which is highly expressed in monocytes/TAMs, is the only known receptor for chemokine CCL2 (102). It is reported that employing CCR2 antagonist inhibits monocyte/TAM recruitment and M2 polarization in hepatocellular carcinoma (HCC) (102). Carlumab is the most representative CCL2-targeted antibody, which successfully represses macrophage infiltration and thus reduces tumor growth (103). Recently, PF-04136309, a small molecule targeting CCR2, was investigated in a clinical trial of pancreatic cancer, which could enhance sensitivity to chemotherapy (104). However, anti-CCL2/CCR2 therapy might have a notable side effect that the antitumor immune cells might be unable to target the tumors (105). Generally, both depletion and recruitment inhibition present inevitable toxicity and side effects.

Functional reprogramming of TAMs is an attractive way for cancer therapy and holds the capacity of providing an opportunity to rebalance the microenvironment immune infiltrate therapeutically from a pro-tumoral one to an antitumoral one (92, 106, 107). Anti-CD47 antibodies block the binding of CD47 to SIRP $\alpha$ , and thus increase phagocytosis of cancer cells, representing an efficient strategy of TAM reprogramming (83, 108). Inhibition of IL-10, a TAM-derived cytokine with the ability to block IL-12 and suppress T-cell tumoricidal function, was identified to improve the efficacy of chemotherapy (109). As Toll-like receptors (TLRs) act a critical role in innate immune response and polarize macrophages into a pro-inflammatory subtype, studies investigate different ligands to change the subtype of TAMs to an antitumoral one (110). The results indicated that agonists of TLR7, TLR8, and TLR9 induced macrophage repolarization and increased tumoricidal activity in several cancers (111–113). Additionally, CD40, a receptor commonly expressed in antigen-presenting cells, interacts with CD40L expressed by T cells to increase pro-inflammatory cytokines. Agonists of CD40/CD40L were identified to affect the protumoral effects in several cancers. Furthermore, strategies targeting crucial processes especially epigenetic regulation in gene expression also obtain effective outcomes to reprogramming TAMs (114). Studies revealed that

**TABLE 2** | *In vivo* or *in vitro* engineering strategies for macrophage-based immune therapy.

Aims	Targets	Delivery strategy	Treated cancer type	Effects/function
Recognition	CD47/SIRP $\alpha$	<i>Ex vivo</i> and <i>in vivo</i> engineering	AML, pediatric brain cancer, B cell lymphoma, lung cancer, ovarian cancer	Enhancing phagocytosis
	CD40/CD40L	Activation <i>via</i> drug delivery (antibody, adenovirus vector)	Melanoma, mesothelioma, pancreatic ductal adenocarcinoma,	Activating antigen-presenting; substituting function of CD4+ T cells; apoptosis induction
Inhibition	CSF1/CSF1R	Drug delivery (antibody and small molecules)	Glioblastoma, tenosynovial giant cell tumors, Hodgkin lymphoma, colorectal cancer, fibrosarcoma, breast cancer	TAM depletion
	Phagocytosis	Delivery of bisphosphonates	Teratocarcinoma, rhabdomyosarcoma, breast cancer, lung cancer, melanoma, liver cancer, prostate cancer	
	CCL2/CCR2	Drug delivery (antibody and small molecules)	Ovarian cancer, lung cancer, melanoma, prostate cancer, liver cancer, breast cancer, pancreatic cancer	Blocking TAM recruitment
	IL-10	<i>In vivo</i> delivery of inhibitors	Lung cancer, ovarian cancer	Unleashing expression of IL-12
	DICER	NA	Lung cancer, colorectal cancer	Enhancing expression of IFN $\gamma$ -STAT1 and repolarizing TAMs <i>via</i> miRNA biosynthesis inhibition
	HDACs	Delivery of TMP195	Breast cancer	Repolarizing TAMs, activating CCL1 and CCL2 expression <i>via</i> epigenetic remodeling
	LDHA	NA	Lung cancer	Downregulation of VEGF and PD-L1; reducing glycolysis and reversing TAM-driven immunosuppression
	PIK3 $\gamma$	<i>In vivo</i> drug delivery (IPI-549)	Lung cancer, head and neck cancer	Reducing glycolysis and reversing TAM-driven immunosuppression; upregulation of MHC-II and IL-12; recruitment of antitumor immune cells
	Activation	TLRs	Delivery of small molecules	Melanoma, breast cancer, ovarian cancer, lung cancer, head and neck cancer, renal cancer, endometrial cancer, cervical cancer, and types of leukemia

AML, acute myeloid leukemia; CCL1, chemokine (C-C motif) ligand 1; CCL2, chemokine (C-C motif) ligand 2; CCR2, C-C chemokine receptor type 2; CSF1, colony-stimulating factor 1; CSF1R, colony-stimulating factor 1 receptor; HDACs, histone deacetylases; IFN $\gamma$ , interferon gamma; IL-10, interleukin 10; IL-12, interleukin 12; LDHA, lactate dehydrogenase A; MHC-II, major histocompatibility complex class II; PD-L1, programmed death-ligand 1; PIK3 $\gamma$ , phosphoinositide 3-kinases gamma; SIRP $\alpha$ , signal regulatory protein  $\alpha$ ; STAT1, signal transducer and activator of transcription 1; TAM, tumor-associated macrophage; TLRs, Toll-like receptors; VEGF, vascular endothelial growth factor.

inhibition of DICER, a key enzyme for microRNA synthesis in macrophages switches the subtype accompanied by tumor regression and infiltration of effective immune cells (115). Inhibitors of histone deacetylase (HDACs) can repolarize the phenotype of TAMs and alter CCL1 expression in monocytes (116). Besides, metabolic reprogramming also plays a significant role in functional modifying of TAMs. Deletion of LDHA and inhibition of phosphatidylinositol-4, 5-bisphosphate 3-kinase catalytic subunit gamma (PIK3 $\gamma$ ) aimed to reduce glycolysis and hence relieved TAM-driven immunosuppression (117, 118). What is more, lactic acid produced by aerobic or anaerobic glycolysis has an essential function in inducing M2-like polarization of TAMs, suggesting the possibilities to reprogram TAMs by suppressing the production of lactic acid (119). However, TAM reprogramming *via* depleting M2-like TAMs and/or favoring their repolarization toward an M1-like phenotype is limited by innate and acquired resistance, compensation by alternative immunosuppressive cells, and relapse during treatment discontinuation. Besides, side effects including anemia and autoimmunity disease are hard to overcome.

Similar to CAR-T-cell therapy, a recent study engineered the macrophage to express CARs (CAR-Ms) to have antigen-specific phagocytosis capacity, which induced a pro-inflammatory tumor environment, enhanced antitumor T-cell activity, and alleviated tumor burden (120). Another study generated induced pluripotent stem cell (iPSC)-derived, CAR-expressing macrophage cells (CAR-iMac) with antigen-dependent macrophage function and antitumor effects both *in vitro* and *in vivo* (121). In general, these studies provided potent strategies for reprogramming tumor microenvironment and set good examples reverting immunosuppression for cancer immunotherapy, though the persistence and efficacy of the CAR macrophage may be further modified.

## THERAPEUTIC STRATEGIES TARGETING REGULATORY T CELLS

Regulatory T (Treg) cells suppress abnormal/excessive immune responses to self- and non-self-antigens to prevent chronic inflammatory, allergic, and autoimmune diseases and maintain

immune homeostasis (122, 123). Infiltration of Treg cells into the TME occurs in various murine and human tumors (124). Treg-induced immune homeostasis can significantly limit the efficacy of antitumor effect as many tumor antigens are either overexpressed or mutated self-antigens. Notably, increasing expression of Tregs regulator forkhead box protein 3 (FOXP3) is identified in many tumors such as breast cancer, melanoma, and pancreatic cancer with a complicated function and cell type-related manner (125). It is reported that FOXP3 plays two key roles: (1) the tumor suppressor in prostate, ovarian, and breast cancers *via* activating tumor-suppressor genes and inhibiting several oncogenes; (2) a biomarker related with poor prognosis in melanoma, non-small cell lung cancer, urinary bladder cancer, and esophageal cancer (126). Besides, FOXP3 is also involved in immune functions of Tregs *via* inhibition of APC function mediated by CTLA-4, an increase in immunosuppressive cytokines and metabolites, IL-2 exhaustion (127). There are mainly three strategies to reprogram Treg function based on the immune-suppressive mechanisms of Treg cells (Table 3).

High expression of several cell surface receptors makes them attractive targets to selectively deplete Treg cells. Interruption of costimulatory molecule CD28 in Tregs impairs their differentiation and function selectively within tumors, reducing their capacity to suppress antitumor immune responses and promoting tumor control (128). Similarly, targeting surface receptor CD25 successfully represses Treg cells with antitumor immune response (129–131). Moreover, since cytotoxic T-lymphocyte antigen-4 (CTLA4) is expressed by Treg cells and increased after activation of effective T cells, mAbs targeting CTLA4 was applied to antagonize inhibitory signal and activate effective T cells to regain tumoricidal activity (132). Other antibodies against GITR, OX40, and molecules predominantly expressed by Treg cells have long been used to selectively deplete Tregs and inhibit their suppressive capacity (133, 134). Additionally, blocking chemokine and chemokine receptors (CCL22-CCR4, CCL28-CCR10, and CCL1-CCR8) associated with Treg chemotaxis into TME could reduce the number of Tregs and increase antitumor immune responses (135–137). In murine cancer models, deletion of neuropilin 1 (Nrp-1) specifically in Tregs leads to enhanced immunity to many transplantable tumors (138).

Reprogramming metabolic profiles including glycolysis and lipid oxidation has been considered as another strategy to suppress Treg cells and change the fate of immunotherapy. Recent studies suggest that metabolic regulations are actively involved in Treg differentiation, Foxp3 expression, and Treg stability. Studies showed that inhibiting Akt–mTOR may regulate metabolic programs to facilitate the suppression of Treg cells (139). Besides, low oxygen tension combined with TCR activation, can stabilize hypoxia-inducible factor 1 $\alpha$  (HIF1 $\alpha$ ) and promote Foxp3 expression (140). TLR1 and TLR2 signaling activation in Treg cells enhances Treg glycolysis and proliferation and unleash the immunosuppressive capacity (141). TLR8 signaling selectively inhibits glucose uptake and glycolysis in human Treg cells, resulting in reversal of Treg suppression in melanoma (142).

Modulation of critical factors and chromatin regulators associated with transcription can also transform the function of Treg cells. Foxp3 serves as the most significant transcription factor in Tregs, which is involved in the differentiation and stability of Tregs. Loss of Foxp3 results in autoimmunity in the normal situation, while deficiency of Foxp3 unleashes immunosuppressive capacities and, hence, improves tumoricidal activities (143, 144). In addition, epigenetical inhibition of Foxp3 *via* interruption of histone acetylation [histone acetyltransferase (HAT) EP300] and DNA methylation [ten-eleven translocation (TET)] reduces the immunosuppressive function of Tregs and leaves effective T cells to regain their antitumor function (145, 146). Foxp3 also regulates Tregs by interacting with other transcription factors; disruption of these factors provides an alternative way to reprogram Tregs with antitumor ability. It is reported that the disruption of the CARMA1–BCL10–MALT1 (CBM) signalosome complex and induction of IFN $\gamma$  secretion suppress Tregs, activate the adaptive immune response, hinder tumor growth, and improve the efficacy of immune checkpoint therapy (147). Moreover, genetic or pharmacologic disruption of transcription factors Eos, Helios, Foxo1/Foxo3, and EZH2 reprograms Tregs to enhance cancer immunity and improve tumoricidal activity (148–151). However, while there is some success through the treatment of targeted Tregs, there are still some obstacles that need to be addressed. First, specific targets for reprogramming Tregs are limited, especially for tumor-infiltrating Tregs. Second, while an immune-related adverse effect resulting from systemic depletion of Treg cells becomes a risk for patients, strategies specifically tuning Treg cell function in TME are needed.

## THERAPEUTIC STRATEGIES TARGETING NATURAL KILLER CELLS

Natural killer (NK) cells, which present in the peripheral blood, lymph nodes, spleen, and bone marrow, are innate immune cells involved in cytotoxicity and cytokine production (152). Tumor necrosis factor alpha (TNF- $\alpha$ ), granulocyte–macrophage colony-stimulating factor (GM-CSF), and IFN- $\gamma$  are the main cytokines activated by NK cells (153). In addition, a complicated network of activating and inhibitory receptors regulates the function of NK cells. C-type lectin receptors (CD94/NKG2C, NKG2D), killer cell C-type lectin-like receptor (NKp65, NKp80), natural cytotoxicity receptors (NKp30, NKp44, and NKp46), SLAM family receptors (2B4, SLAMF6, and SLAMF7, function in the recognition of hematopoietic cells), Fc receptor Fc $\gamma$ R (function in antibody-dependent cell cytotoxicity), killer cell immunoglobulin-like receptors (KIR) (KIR-2DS and KIR-3DS), DNAM-1, and CD137 (41BB) serve as activating receptors, while KIR-2DL and C-type lectin receptors CD94/NKG2A/B serve as inhibitory receptors of NK cells (154, 155). NK cells play an important role in initiating and promoting cancer with effective capacity at the first-line defense for tumor elimination. The major functions of NK include cytotoxicity and cytokine production, which help in killing tumor cells. Higher infiltration of NK cells usually associates with a good prognosis in various cancers. However,

**TABLE 3** | Therapeutic strategies targeting regulatory T cell (Treg) for cancer therapy.

Targets	Treated cancer type	Effects/function
CD28*	NA	Inhibiting stability and function of Treg
CD25*	Breast cancer	Depleting Treg
CTLA4*	Melanoma, colorectal cancer, fibrosarcoma	Depleting CTLA4 expressing Treg through ADCC
GITR <sup>#</sup>	Bladder cancer, sarcoma, melanoma, lung cancer	Inhibiting the suppressive activity of Tregs; activating effector T cells
OX40 <sup>#</sup>	Glioma, breast cancer, colon carcinoma, prostate cancer, sarcoma, melanoma, lung cancer	
CCL22/CCR4*	Lung cancer, esophageal cancer	Attenuating Treg accumulation
CCL28/CCR10*	Ovarian cancer	
CCL1/CCR8*	Breast cancer	
Nrp1*	Melanoma, CLL, cervical cancer	Preventing Treg recruitment; downregulating VEGF, and producing IFN $\gamma$
Akt-mTOR	Melanoma, ovarian cancer	Increasing glucose uptake and glycolysis; destabilizing Treg
TLR1 <sup>#</sup>	AML, metastatic colorectal cancer, mantle cell lymphoma	Enhancing Treg glycolysis and proliferation
TLR2 <sup>#</sup>	Melanoma, AML, metastatic colorectal cancer, mantle cell lymphoma	
TLR8 <sup>#</sup>	Melanoma	Inhibiting glucose uptake and glycolysis
HIF1 $\alpha$ *	Metastatic melanoma in lungs	Impairing Treg stability and driving Foxp3 degradation
HAT*	Breast cancer, prostate cancer, pancreatic cancer, ovarian cancer	Epigenetical inhibition of Foxp3
TET*		
Foxp3*		Inhibiting function of Tregs
CBM complex*	Melanoma, colorectal cancer	Enhancing IFN $\gamma$ and suppressing tumor growth
Eos*	Lung cancer	Reprogramming Treg to gain immune-stimulating capacity; decreasing expression of Foxp3
Helios*	Melanoma, colorectal cancer	Decreasing expression of Foxp3
Foxo1/Foxo3*		
EZH2*		

\*Inhibition via drug delivery, decoys, siRNA, and others.

<sup>#</sup>Activation via mimics or ligands.

ADCC, antibody-dependent cellular cytotoxicity; Akt, protein kinase B; AML, acute myeloid leukemia; CBM complex, CARMA1–BCL10–MALT1 signalosome complex; CCL, chemokine (C-C motif) ligand; CCR, C-C chemokine receptor; CLL, chronic lymphocytic leukemia; CTLA4, cytotoxic T-lymphocyte-associated protein 4; EZH2, enhancer of zeste homolog 2; Foxo1, forkhead box O1; Foxo3, forkhead box O3; Foxp3, forkhead box P3; GITR, glucocorticoid-induced tumor necrosis factor receptor; HAT, histone acetyltransferase; HIF1 $\alpha$ , hypoxia-inducible factor 1-alpha; IFN $\gamma$ , interferon gamma; mTOR, mammalian target of rapamycin; Nrp1, neuropilin 1; TET, Ten-eleven translocation methylcytosine dioxygenase; TLRs, toll-like receptors; VEGF, vascular endothelial growth factor.

due to the limited ability of homing and immunosuppressive tumor microenvironment, solid cancers commonly present a poor NK cell infiltration with increasing inhibitory signals. Therefore, targeting with an inhibition signal may serve as a meaningful approach to restore cytotoxic function of NK cells against cancer cells (154, 156, 157).

To date, NK-cell-based immunotherapy is roughly divided into two types: directly targeting cytokines and receptors involved in NK cell proliferation and function; and chimeric antigen receptor (CAR)-engineered NK cells (Table 4). IL-2 and IL-15 are two of the most commonly employed cytokines in targeting NK cells. IL-2 was applied to produce lymphokine-activated killer (LAK) cells with unsatisfactory outcomes, which is probably attributed to the expansion of Treg cells at the same time. Compared with IL-2, IL-15 met a great success in targeting NK cells for tumor treatment. Expansion of NK cells and CD8 effector memory T cells after IL-15 therapy was identified in both mouse model and clinical studies (158, 159). Other cytokines, including IL-18 and IL-21, have also been shown to promote NK cell functions (158, 160). What is more, it is worth concerning

that a combination therapy of cytokine and other traditional therapy can elevate NK cell proliferation, cytotoxicity, and memory, which is more effective than single cytokine treatment. Additionally, antibodies targeting activating receptors involved in antibody-dependent cell-mediated cytotoxicity (ADCC) can also improve cytotoxicity of NK cells to tumor cells. Similarly, blocking of inhibitory receptors like KIR reverse the suppressive state of NK cells (161).

CAR-NK cell therapy has largely been investigated. CAR-NK cell therapy exhibits enhanced tumoricidal capacity with advantages that are not responsible for GVHD and do not induce cytokine storms (162). In addition, the sources of CAR-NK cells can be generated from cord blood (CB), peripheral blood (PB), adult hematopoietic stem cells (HSCs), embryonic stem cells (ESCs), and induced pluripotent stem cells (iPSCs) (163, 164). Similar as in CAR-T cells, an intracellular signaling domain-like CD3 $\zeta$  and costimulatory signaling domain (CD28, 4-1BB) are basic structures for a CAR. Other molecules like DNAX-activation protein 12 (DAP12), DAP10, and NKG2D can also be selected as intracellular or ectodomain (162). For



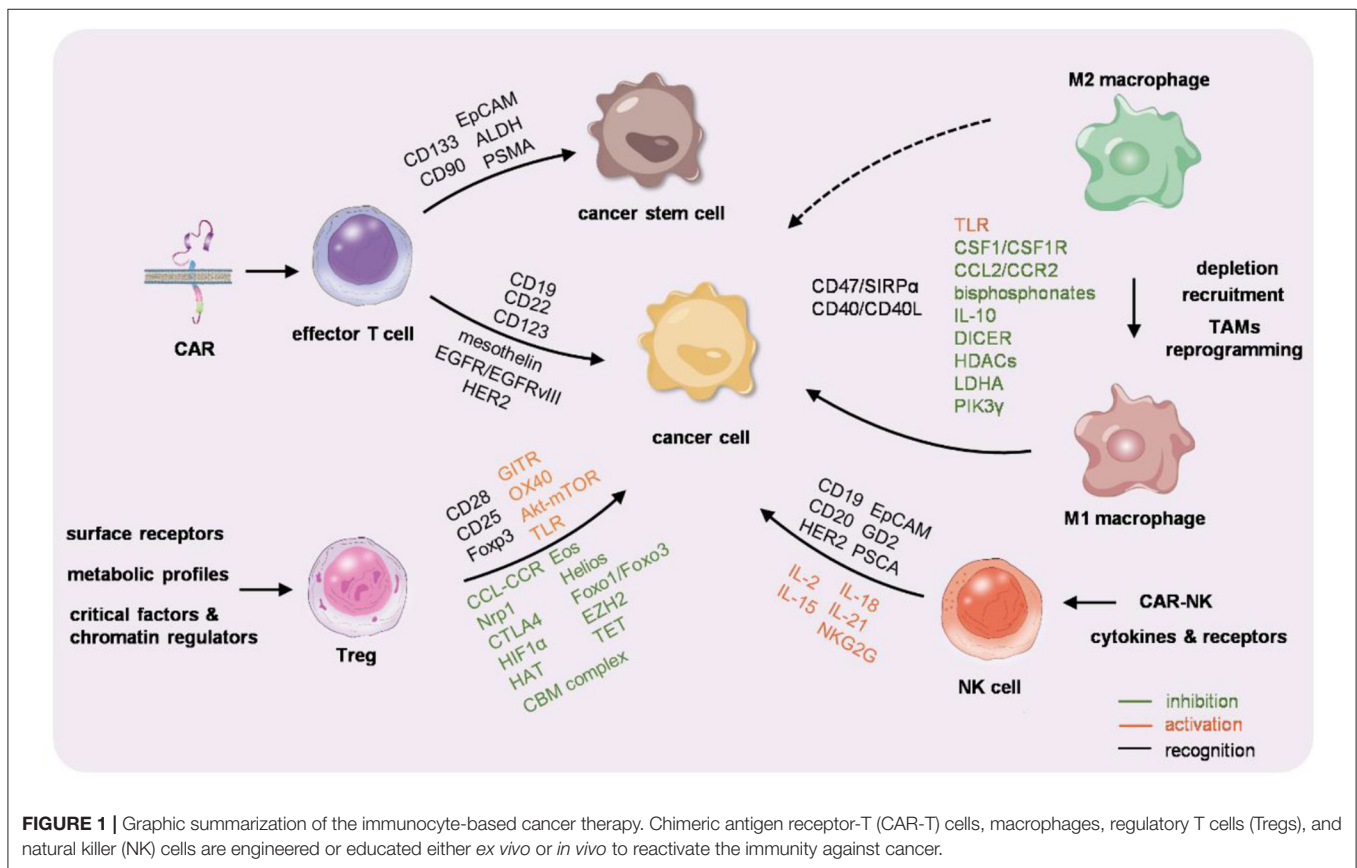
**TABLE 4 |** Natural killer (NK) cell-based immune therapy.

Targeted genes	Delivery strategy	Treated cancer type	Effects/function
IL-2*	Delivery of superkine or fusion protein	AML	Promoting NK cell proliferation and activating NK cells
IL-15*	Delivery of fusion protein	Ovarian cancer, myeloid leukemia	Enhancing cytotoxicity of NK cells
IL-18*	Delivery of cIAP2 and TRAF1	Triple-negative breast cancer, lung cancer, melanoma	Sustaining NK cell survival
IL-21*	Delivery of rIL-21	Pancreatic cancer, mantle cell lymphoma, melanoma	
NKG2D*	Inhibition via antibody	Lung cancer, colon cancer, prostate cancer, ovarian cancer, CLL	Triggering cytokine production and NK cell cytotoxicity
CD19# CD20# HER2#	Ex vivo engineering NK	AML, ALL, multiple myeloma	Specific targeting via the CAR engineered
EpCAM# GD2# PSCA#		Neuroblastoma, ovarian cancer, colon cancer, renal cell cancer, osteosarcoma	
		Prostate cancer	

\*Inhibition via drug delivery, decoys, siRNA, and others.

#Recognition targets for ex vivo engineering.

NKG2D, natural-killer group 2, member D. ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CARs, chimeric antigen receptors; CLL, chronic lymphoblastic leukemia; EpCAM, epithelial cell adhesion molecule; HER2, human epidermal growth factor receptor 2; IL, interleukin; PSCA, prostate stem cell antigen.



**FIGURE 1 |** Graphic summarization of the immunocyte-based cancer therapy. Chimeric antigen receptor-T (CAR-T) cells, macrophages, regulatory T cells (Tregs), and natural killer (NK) cells are engineered or educated either *ex vivo* or *in vivo* to reactivate the immunity against cancer.

antigen selection, most CAR-NK cells target CD19 and CD20 in hematological malignancies, and HER2, EpCAM, GD2, and PSCA in solid cancers. Recent studies suggested that the most

effective responses of CAR-NK cells were observed in ALL, prostate carcinoma, and osteosarcoma, while the effects in other cancers tested were not that satisfactory (165–167).

To sum up, the development of NK cell-based cancer immunotherapy is a fast-evolving field. Unleashing NK cell antitumor responses by harnessing surface receptors and involved cytokines depict potentially successful immunotherapeutic strategies for cancer. The foremost challenge of CAR-NK cell therapy is expansion of primary NK cells *ex vivo*. Additionally, limited transfection efficacy in NK cells to express CARs is also notable. Selection of a suitable method, such as viral infection, electroporation, and nanoparticles, is a prerequisite for successful CAR-NK therapy. Although there remain pressing obstacles of CAR-NK cells, the striking outcomes in several cancers make it a promising new strategy for cancer immunotherapy.

## CONCLUSIONS AND PERSPECTIVES

With the advances in the knowledge in the cross-talk between different immune cells and tumor cells, the techniques in cell engineering and drug delivery, and immunotherapies targeting accessory immune cells either *ex vivo* or *in vivo* have been intensively studied (Figure 1). Some of them have been widely

used in clinics; some have been already under phase 2/3 clinical trial. Generally, immunotherapy is emerging as a promising strategy against a variety of cancers and might be the final therapeutic tool. Translational researches using different strategies for various types of cancers are intensive studies worldwide. Future challenges rely on improvement of the safety, efficacy, and convenience in personalization and customization.

## AUTHOR CONTRIBUTIONS

YD, GY, and LL designed the paper. YD, ZW, XG, GY, and LL analyzed the references and edited the paper. YD and GY wrote the paper. All authors contributed to the article and approved the submitted version.

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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