

The Chimeric Antigen Receptor Macrophages (CAR-M): An Immunotherapy Against Solid Tumors



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Introduction

Cancer still tribulates death in different population sectors worldwide. Around 10 million deaths occurred due to cancer, as per the World Health Organization (WHO) 2020 statistics (1). The solid tumor treatment encompasses major solicitude than the liquid tumor. Existing chemotherapeutic strategies include novel drugs and nanoparticles targeting checkpoint proteins and receptors causing downregulation of immune reactions, immunotherapy, and monoclonal antibodies. However, existing treatment is associated with drawbacks including a rise in immunosuppressive cells including tumor-associated macrophage (TAM), myeloid deriver monocyte (MDM), and regulatory T-cells along with death receptor suppression onto cancer cells, low chemokine expression, inefficient drug concentration due to phagocytosis, p-glycoprotein efflux, hostile tumor microenvironment, tumor vasculature barrier, and T-cell exhaustion (2,3). This indeed paves a way for the scientific community worldwide towards the development of novel translational anti-cancer therapy.

Novel cell-based therapy includes a strategy to educate and instigate the body's defense cells against cancer. Chimeric antigen receptor-T cells (CAR-T) were widely developed in this regard. The USFDA approved three CAR-T products in 2020, including Tecartus®, Breyanzi®, and Carvykti® for the treatment of liquid tumors. However, the therapy failed to penetrate and survive within a solid tumor. The development of chimeric receptor-based other immune cell therapy may serve as a suitable alternative.

Although macrophages are inconspicuous but can be lucrative cell-based strategies against cancer. The macrophages encompass the ability to penetrate the stromal tumor tissues and proliferate within solid tumors. The functional plasticity of macrophages may lead to various anti-tumor and pro-tumor activities under different conditions. The macrophage exists in varying phenotypes and expresses various receptors including but not limited to the C-C chemokine ligand-2 (CCL-2), colony-stimulating factor (CSF-R), and a cluster of differentiation 206 which are engaged in tumor proliferation (4). The expression of the phenotypes and receptors could be modulated by the delivery of chimeric antigen receptor-expressing macrophages (CAR-M) (Figure 1). The succeeding section includes the significance of macrophages in cancer, various efforts directed toward the *ex-vivo* genetic integration of a few anti-tumor genes within macrophages, its advantages, and its translational potential.

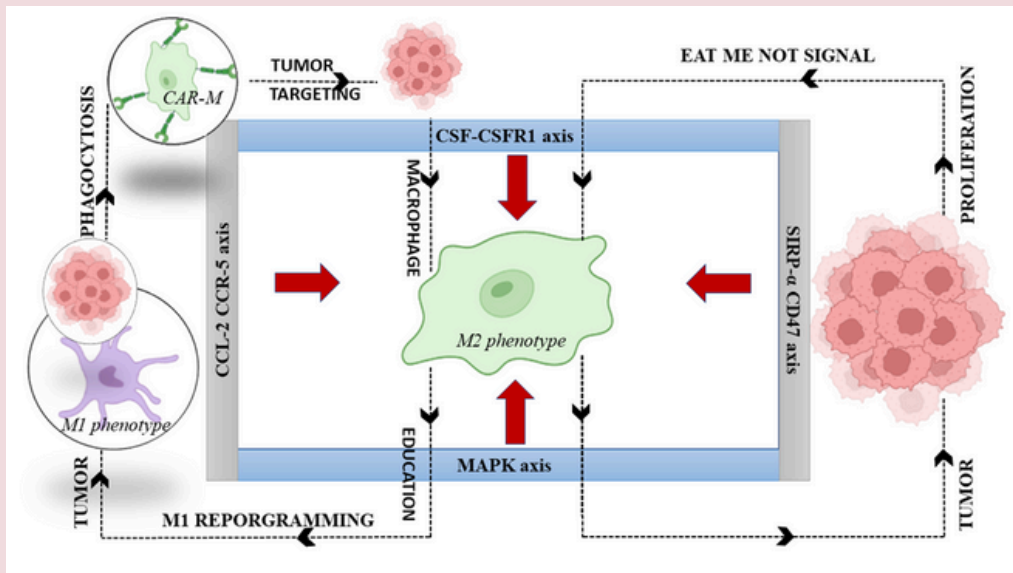


Figure 1. Pathways inhibiting macrophage repolarization and CAR-M therapy for macrophage education in solid tumor

Macrophages in solid tumors

Due to the secretion of cytokines (IL-6), tumor growth factor- β (TGF- β), and the low oxygen environment of tumor tissues, the macrophage mistakenly recognizes the tumor cells as damaged tissues and initiates its repair. Moreover, macrophage exists naturally in two phenotypic forms which function as an anti-inflammatory (M1 type) and pro-inflammatory (M2 phenotype). The M1 phenotype is activated by the Interferon- γ (IFN- γ), CSF, and lipopolysaccharide (LPS). While the M2 phenotype can be activated by Interleukin-4 (IL-4), bacterial LPS, and glucocorticoid. The M1/M2 ratio varies based on the disease condition (4). The M2 polarized state is responsible for the 'eat me not' condition in the case of solid tumors. The tumor cells secrete CSF which binds to the MCSF-receptors (MCSF-R) on myeloid cells and causes the downregulation of signals including the mitogen-activated protein kinase pathway (MAPK)(5,6), polarizing the M2 and generating the TAM through a variety of soluble and mechanical factors. Moreover, the cancer cells express the CD47 proteins which are generally recognized by the signal regulatory proteins (SIRP- α) expressed by myeloid cells and activate the Src homology region 2(SH2) phosphatase activating the 'eat me not' signal (7). The expression of antigen presentation, activation of the inflammatory response, and CD4+ and CD8+ activation are widely reduced in TAM. The CCL2 expressed by TAM can target C-C receptor 2(CCR-2) and instigate tumor invasion and metastasis (8) (Figure 1).

Therefore, the inhibition of the CSF-CSFR axis, SIRP- α -CD47 axis, MAPK axis, and CCL2-CCR2 axis would repolarize the M2 into M1 and diminish the M2 cells. Moreover, the CD40 cells expressed on monocytes, macrophages, and B-cells and the TLR activation might enhance the antigen presentation and pro-inflammatory signals, thereby repolarizing M2 to M1. Additionally, the TAM is infiltrated into the tumor through CCR5 and CCL5 receptors. While the trafficking of myeloid cells to the tumor is caused by IL-8 via the CXCL-8 receptors. Therefore, blocking the CCL5, CCR5, and CXCL8 may enhance the anti-tumor efficacy (9). Various small molecules and antibodies have been developed targeting single or combination pathways mentioned (Table 1). However, the therapy suffered from drawbacks including tumor relapse, subject variability, and inconsistency of M1 or M2 state in various solid tumors which coax to

limited application.

The CAR for macrophages-treatment against solid tumors

Genetically engineered educated macrophages would be advantageous compared to existing chemotherapy against the solid tumor. The synthesis of CAR-M involves a week-long process. Subcutaneous administration of G-M-CSF leads to the mobilization of the monocytes. Later, leukapheresis is done and appropriate CD14+ monocyte cells are selected and separated. The monocytes are differentiated into macrophages ex-vivo. Lastly, the CAR-M is synthesized by mRNA or viral transfection using lentivirus or adenovirus to express antigen-specific against cancer cells or train the pluripotent stem cells by stimulation into M1 macrophages which enhances the anti-tumor effect.

Breast cancer relapse is majorly associated with overexpression of anti-human epidermal growth factor receptor-2 (HER2) expression. Targeting the HER-2 using CAR-M could be an effective therapy to eradicate breast cancer (10). To this end, the differentiated human CD14+ macrophages from human monocytes were transduced using an adenovirus vector (Ad5f35) to elicit the M1 activity of the macrophages specifically in HER2+ expressing solid tumors. The CD14+ CAR-M were active against GM-CSF and M-CSF-expressing tumors and were devoid of M2 phenotype and immune suppression. Moreover, the developed CAR-M did not cause phagocytosis of normal human tissues. In vivo studies revealed persistent CAR expression in NSG and NSG-S mouse models and were effective in the treatment of HER+ solid tumors (11). Moreover, the anti-HER-2 CAR-M (CT-0508) induced MHC-II and TNF- α through pro-inflammatory signals in human TME. It induced the activation of markers on immature dendritic cells and activated T-cells against the HER-2+ solid tumors (12). Due to its enormous potential observed during the pre-clinical trial, the CT-0508 has been translated to the first in a human trial in patients with over-expressed HER-2 solid tumors (13).

Table 1: Recent clinical trials of small molecules and monoclonal antibodies against solid tumors*

Sr no	Molecule/Monoclonal antibody	Clinical stage	Target
1	BI 765063+ BI 754091	Phase I	SIRP- α PD-1 receptor antagonist
2	ALX148+Doxorubicin liposome+ Pembrolizumab	Phase II	CD47 inhibitor
3	IB1322	Phase I	
4	TTI-621	Phase Ia/Ib	SIRP- α -CD47 axis
5	TTI-622+Doxorubicin	Phase I/II	inhibition
6	JSI-1187	Phase I	MAPK inhibitor
	Trametinib	Phase II	
8	Carlumab	Phase II	CCL-2 inhibitor

*All the clinical trial information has been adapted from ClinicalTrials.gov

Klichinsky et. al. transduced THP-1 cells with CD3ζ to develop CAR-M. The developed CD3 ζ CAR-M elicited polyphagocytosis of antigen-positive target cells. Additionally, they developed anti-HER-2 CAR-M against HER2+ metastasized lung cancer. In-vivo studies in NSGS mice revealed consistent tumor growth in the control group as compared with the HER2+ CAR-M treated mice where a significant increase was observed in the survival rate of the mice with decreased tumor progression. Moreover, the treatment was nontoxic with no change in the body weight of the mice. The CAR-M-activated inflammasome, and induced M1 phenotype, antigen-presenting ability, MHC class I/II expressions, and interferons due to the use of an adenovirus vector. Moreover, the M1 phenotype was maintained upto 40 days after transduction. The expressed phenotype was consistent despite M2 stimulations induced using IL4 and IL13 (14). In yet another study, CAR-M (CT-1119) is under pre-clinical development targeting the overexpressed mesothelin receptors onto solid tumors. The CAR comprised human scFv transduced using adenovirus onto human monocytes. The CT-1119 demonstrated robust killing of A549 tumor cell lines expressing MES-OV mesothelin, M1 polarization, dose-dependent pro-inflammatory TNF-α release, and tumor reduction in the murine xenograft model in lung cancer(15).

The CAR-M would emerge as a personalized therapy with the transduction of CAR onto the specific human monocyte based on the overexpressed receptors onto tumor macrophages. The immune response may not be triggered against the CAR-M but, a specific tumor. Tumor relapse shall be a rare occurrence due to prolonged expression of the CAR. Unlike the T-cells, the macrophages have low graft versus host disease and can be prepared in advance for administration. Nevertheless, an uncontrollably triggered immune response due to insertion or mutation of the gene through viral transduction and the safety and efficacy in humans still needs to be thoroughly evaluated for the clinical success of CAR-M.

Conclusion

Despite of tremendous cost of chemotherapy, the treatment regimen was prolonged due to limited efficacy until their in-vivo administration. The surface-modified nanoparticles actively targeting cancer cells could target only specific pathways which led to incomplete eradication of cancer cells. The lack of wholesome treatment and rising deaths due to cancer requires consolidated treatment measures without off-target side effects and selective instigation of the immune response against the solid tumor. Education of macrophages against specific inhibition of cancer cells would be a boon to the current stumbling block. Moreover, CAR-modified other adaptive immune cells including T-cells, natural killer cells, and lymphocytes were ineffective due to the hostile tumor environment and failed to penetrate within the solid tumor. On the contrary, the genetic modification of host monocytes sustains the hostile conditions, penetrate the solid tumors, and antigen presentation potential stimulating the cytotoxic T cell response. However, the extent of proliferation of the CAR-M post-injection, their efficacy against the target tumor in humans, migration of CAR-M to the target site compared with their residence site in the liver, and optimal expression of the target receptors onto cancer cells to elicit appropriate CAR-M efficacy in humans remains elusive.

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