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A REVIEW ON CHIMERIC ANTIGEN RECEPTORS AND THE CURRENT CLINICAL TRIALS ON BREAST CANCER THERAPY

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ABSTRACT The appearance of chimeric antigen receptors (CAR) T cells during the past few years has opened a new therapeutic approach for cancer, especially for hematopoietic malignancies, that has already proven to be effective and even considered to be established as the therapy of choice within the next few years. The effectiveness against solid tumors, however, still remains a challenge since many obstacles need to be surpassed, considering demarcation of tumor from normal tissue, homing, as well as tumor penetration and localized-to-tumor acting. The aim of this review is to make a brief report considering CAR T cells and to reveal the current stage-of-research considering the use of CAR T cells against breast cancer.

KEYWORDS : Chimeric Antigen Receptor; T cells; breast cancer; solid tumors

Introduction

The immune system is responsible for defending the human body against any threat with T lymphocytes being the key soldiers against infected or abnormal-for-the-human-body cells. However, some of the diseases presented to the human immune system have the ability of avoiding identification, thus remaining and acting free against the host. During the last decades, engineered T-cell therapy was presented as a novel therapy for cancer development proving to be very promising for treating the disease.

Chimeric antigen receptor (CAR) - also called chimeric immunoreceptors, chimeric T cell receptors or artificial T cell receptors - are engineered receptors grafted onto a T cell that target specific cancer cell types. CAR T cells combine the specificity of an antibody equipped with the cytotoxic functions of T cells. The aim of this review is to summarize the knowledge considering the use of CAR T cells against breast cancer.

The structure of CAR T Cells

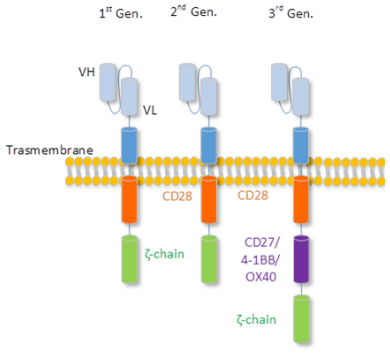
The innovative CAR/reprogrammed T cells are generated acquiring important significantly characteristics that make them capable of providing an ex-novo activation mechanism that bypass restrictions of the HLA-mediated antigen recognition avoiding therapeutic tolerance. These artificial T cell receptor molecules are capable of recognition and binding on specific tumor antigens expressed on the surface of cancer cells and they are able to activate, promote and propagate signaling of the lytic machinery.

Antigen specificity is due to the single-chain variable fragment (scFv) derived from the portion of a specific-to-target protein antibody, that is also expressed on the surface of the CAR T cell. In order for the scFv to be placed and expressed on the surface of the membrane, a spacer of varying flexibility connects the scFv part (extracellular) to the intracellular signaling domain through the transmembrane domain.

Eshhar et al., presented in 1993 the 1st generation of CAR T cells by combining the variable regions (Fv) of an antibody with the constant regions of γ or ζ T-cell receptor (TCR) chains (typically the cluster of differentiation -CD- 3ζ) resulting in T lymphocytes with antibody-type specificity (Eshhar, Waks, Gross, & Schindler, 1993). The last mentioned domain is responsible for stimulation and proliferation of the T cell, however, it is still unclear if it is responsible for sustained proliferation and activity in the absence of additional signal, even though, preclinical studies by Brentjens et al., and Stancovski et al., showed positive effects when directed against CD19 and HER2/Neu (R. J. Brentjens et al., 2003; Stancovski et al., 1993).

The development of 1st generation CAR T cells led the need of providing additional signals to ensure full activation of the reprogrammed cells (Figure 1). Thus, the 2nd generation of CAR T cells was equipped with a costimulatory domain (CD28) connected to the scFv via the transmembrane domain. The 3rd generation is supplied with an additional transducer domain (41-BB, CD27 or OX40) inserted between the costimulatory CD28 domain and the zeta chain while the 4th generation is equipped -instead of the 3rd generation transducer domain - with armored CARs, engineered to synthetize and deliver interleukins so as to increase persistence of the chimeric cells against tumor's microenvironment (R. Brentjens, 2015; Yeku, Purdon, Koneru, Spriggs, & Brentjens, 2017; Zhang, Liu, Zhong, & Zhang, 2017).

Figure 1. Structure of CAR T cells.



CARs are composed, as mentioned, of an extracellular domain derived from a tumor specific antibody linked to the T cells intracellular domains. For T cell gene modification, plasmid transfection and mRNA or viral vector transduction are used and tumor specific T cells are generated. These genetically modified and activated T cells are then inserted into the patient's blood to target specific surface proteins of the cancer cells.

Current Therapeutic Usage of CAR T Cells

Even though CAR T cells were widely used for solid and liquid tumors, the effectiveness of the therapy was proved by targeting B-cell hematologic tumors.

Patients affected by relapsed B cell acute lymphoblastic leukemia (B-ALL), chronic lymphocytic leukemia (CLL) and B-cell non-Hodgkin lymphoma revealed high antitumor efficacy when treated with anti-CD19 CAR T cells (complete remissions ranging from 70 to 94%) (Wang, Wu, Liu, & Han, 2017). Subsequently, the FDA approved -and are currently used as second line treatment- two anti-CD19 immunotherapy modified T cells, KYMRIAH [tisagenlecleucel (August 2017)] and YESCARTA [axicabtagene ciloleucel (October 2017)], used for patients with B-ALL up to 25 years of age, and for treatment of adults with B-cell lymphoma respectively.

A common problem occurred for treatment of B-cell malignancies with CAR-T cells is the entire B cells repertoire depletion partially solved with the administration of immunoglobulins. Nonetheless, depletion of other cell lineages could possibly restrict the use of CAR-T cell therapies to specific hematopoietic malignancies or even only on B-cell malignancies.

Similarly, patients treated with CAR T cells for large tumor masses presented acute cytokine release syndrome that required intensive care due to massive cytokines release from on-target activated cells (Fitzgerald et al., 2017; Xu & Tang, 2014).

CAR-T cells against solid tumors

Even though the therapeutic usage of CAR T cells against hematopoietic malignancies tends to be prevalent, use against solid tumors is still not widespread. Of the main reasons for this "delay" on manufacturing and using those artificial agents is the lack of specific targetable agents on the cell surface of the solid tumors, a characteristic that makes the use of such drugs ineffective, with poor specificity and poor efficacy against specific cancer types.

In order to overtake those problems, currently, there are several studies ongoing considering the use of artificial T cells against specifc antigens exclusively expressed on solid tumors and their results are still expected, however, there are already enough data to demonstrate the correct pattern for a successful development of CAR T cells against solid tumors. They key for a successful treatment is located on choosing the most suitable target, the proper dose and therapeutic frequency and on the efficient confrontation on avoiding not only tumor's tolerance, but also, fatal adverse outcomes. Some studies conducted so far reveal encouraging results for the systematic use of these therapies while other support that a lot more research is needed. Sampson et al., published a study on treating malignant glioma with CAR T cells targeting EGFRvIII mutation, while, Ahmed et al., pointed the positive results of treating HER2 positive sarcoma with anti –GD2 CAR T cells (Ahmed et al., 2015; Sampson, Archer, Mitchell, Heimberger, & Bigner, 2008). On the other hand Besser et al., and Goff et al., both supported that the adoptive cell therapy (ACT) in melanoma need infusion of more CAR T cells and the use of IL-2 support for the therapy, to retain a relative positive effect (Besser et al., 2009, 2010; Goff et al., 2016).

Obstacles against breast cancer and solid tumors

Even though CAR T cells have given a new and innovative therapeutic perception against hematopoietic malignancies, there are still major problems considering the use against breast cancer. Initially, a very important feature for the proper trafficking of T cells is $CD8^+$ T cells homing. T cells express different chemokines responsible for interaction with endothelial cells. For example, expression, of G protein-coupled receptors CXCR3 and CCR5 in breast cancer proves their key participation in regulating T-cell trafficking (Mikucki et al., 2015). However, tumor cells reveal an uncontrollable production of cytokines that dissuade homing of $CD8^+CXCR3^{high}$ T cells in tumor foci.

Of the main obstacles that T cells deal with while targeting solid tumors is tumor endothelium and tumor microenvironment characteristics, such as hypoxia and low nutrients, that makes the microenvironment hostile for T cells, preventing proliferation and chemokine production of the latter. Tumor endothelium overexpresses receptors and ligands that obstruct T cells. T lemphocytes degrade the main components of the sub-endothelial membrane and the extracellular matrix, including heparan sulphate proteoglycans (HSPGs). Thus, in order to be adequate, CAR T cells must release heparanase (HPSE), in order to be able to degrade HSPGs, and even though heparanase promotes antitumor activity of artificial T cell receptors, HPSE deficiency in in vitro-engineered and cultured tumor-specific T cells has proven to significantly limit their antitumor activity against solid tumors (Caruana et al., 2015).

Current clinical trials

There are currently only a few data considering the use of CAR T cells against breast cancer, especially due to the problems that still restrict the use of chimeric antigens in solid tumors. To date, there are still ongoing clinical trials testing the use of CAR T cells against breast cancer that aim on overcoming the obstacles of tumor microenvironment, limited antitumor activity and $CD8^+$ T cells homing (Table 1).

Table 1. Ongoing Clinical Trials on the use of CAR T cells against breast cancer.

No	Clinical Trial	Actual Study Completion Date	ClinicalTrials.gov Identifier
1	Chimeric Antigen Receptor-Modified T Cells for Breast Cancer	August 15, 2017 (Results not published yet)	NCT02547961
2	Phase I/II Study of Anti-Mucin1 (MUC1) CAR T Cells for Patients With MUC1+ Advanced Refractory Solid Tumor	October 2018	NCT02587689
3	Treatment of Relapsed and/or Chemotherapy Refractory Advanced Malignancies by CART133	October 2018	NCT02541370
4	Treatment of Relapsed and/or Chemotherapy Refractory Advanced Malignancies by CART-meso	November 2018	NCT02580747
5	EpCAM CAR-T for Treatment of Nasopharyngeal Carcinoma and Breast Cancer	July 2019	NCT02915445
6	A Clinical Research of CAR T Cells Targeting HER2 Positive Cancer	September 2019	NCT02713984
7	Genetically Modified T-Cell Therapy in Treating Patients With Advanced ROR1+ Malignancies	December 1, 2021	NCT02706392
8	A Clinical Research of CAR T Cells Targeting CEA Positive Cancer	December 2019	NCT02349724

DISCUSSION

Development of CAR T cells aims on having a more efficient antitumor efficacy than current therapies, combined to less adverse outcomes. The use against hematopoietic malignancies has already proven that the targeted treatment chimeric T cells offer is very promising and effective, with less side effects, nevertheless, there are still many challenges in dealing with solid tumors in general. Currently, there is little data considering the use against breast cancer, mainly derived from surveys on solid tumors in general. However, eight clinical ongoing trials are focused on overpassing the biological challenges set of breast cancer and CAR T cells, in order for the latter to be fully exploited and widely used.

Conflict of interest

The authors report no conflict of interest

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