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Chimeric Antigen Receptor T-Cell Therapy (Car T-Cells) in Solid Tumors, Resistance and Success

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Mini Review

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Abstract

CARs are chimeric synthetic antigen receptors that can be introduced into an immune cell to retarget its cytotoxicity toward a specific tumor antigen. CAR T-cells immunotherapy demonstrated significant success in the management of hematologic malignancies. Nevertheless, limited studies are present regarding its efficacy in solid and refractory tumors. It is well known that the major concerns regarding this technique include the risk of relapse and the resistance of tumor cells, in addition to high expenses and limited affordability. Several factors play a crucial role in improving the efficacy of immunotherapy, including tumor mutation burden (TMB), microsatellite instability (MSI), loss of heterozygosity (LOH), the APOBEC Protein Family, tumor microenvironment (TMI), and epigenetics. In this minireview, we address the current and future applications of CAR T-Cells against solid tumors and their measure for factors of resistance and success.

Keywords: Chimeric; Resistance; Receptor; T-Cell Therapy

Abbreviations: GVHD: Graft-Versus-Host Disease; MSI: Micro Satellite Instability; LOH: Loss of Heterozygosity; TMI: Tumor Microenvironment; TMB: Tumor Mutation Burden; TCR: T-Cell Receptors; TILs: Tumor-Infiltrating Lymphocytes.

Introduction

Revolutionary discoveries have been made regarding cancer treatment modalities. Chimeric antigen receptor (CAR) T-cell therapy is a challenging method that uses re-engineered lymphocytes expressing specific modular synthetic surface antibodies. The CARs are encoded by specific genes and inserted into the genome of T-cells, which are either autologous or derived from allogeneic donors.

The outcome of obtaining effective autologous T cells from the patient is suboptimal due to the quality and quantity of the harvested cells in addition to being time-consuming and unprofitable.

On the other hand, using allogeneic T cells presents several challenges due to the presence of endogenous Major Histocompatibility Class I (Human Leukocyte Antigen HLA barriers) and T-cell receptors (TCR) on donor's T lymphocytes,

which lead to alloreactivity and graft-versus-host disease (GVHD). Therefore, to overcome the aforementioned HLA barriers, advanced technology was applied to produce modified CAR T-cells by knocking out TCRs and HLA-I in allogeneic T cells. The CARs are separately generated on a case-by-case basis to achieve high personalized effectiveness against tumors [1-3].

Indicators of Improved Response to CAR T-Cell and Immunotherapy

- The presence of a high extent of tumor-infiltrating lymphocytes (TILs).
- In breast cancer, the quantity and composition of TILs play a major role in determining the response to therapy [4].
- Expression of immune microenvironment markers that affect prognosis and treatment response in several cancer subtypes. For example, the expression of HER2, KI67, PIK3CA, P53, PD1, PDL1, and CTLA4 in breast cancer indicate promising targets for adjuvant therapies [5,6].
- Detection of other circulating biomarkers including lactate dehydrogenase (LDH), which is associated with poor prognosis in solid neoplasms, particularly in breast, melanoma, prostate, and renal cell carcinomas [7].
- Elevated neutrophil-to-lymphocyte (N/L) ratio or high expression of tumor-associated neutrophils (TAN) could be related to worse prognosis in multiple solid tumors including prostate, bladder, and nasopharyngeal carcinomas. Nevertheless, results are still limited regarding other types of neoplasms [8].
- High tumor mutational burden (TMB) due to an increased level of nonsynonymous somatic mutations, which revealed an improved response to CAR T-cell therapy combined with depletion of tumor cells by chemo or radiation therapy. Therefore, a low tumor mutation burden might worsen the response to immunotherapy despite the expression of immune checkpoint genes [9,10].
- Homologous recombination repair (HR) deficiency increases genomic instability in tumors and subsequently improves tumors immunogenicity and response to CAR T-cell therapy [11].

Genome Engineering Editing Technologies That Produce CAR T-Cells

Zinc-finger nucleases (ZFNs): ZFNs have been used to modify endogenous genes in a variety of cell types. Genomic alterations including insertions, duplications, and point mutations could be introduced based on this method. ZFNs are used to obstruct gene expression of TCR β and α chain with an increased level of highly-specific cell surface exogenous TCR [12].

Transcription activator-like effector nucleases (TALENs): TALENs are defined as a non-specific DNA-cleaving nuclease connected to a DNA-binding domain. This technique enables modifying any sequence of interest in living cells or organisms. Recently, TALENs have rapidly emerged as an alternative method to ZFNs in introducing targeted double-strand breaks (DSBs) and genome editing [13].

Lentiviral vectors: Another possible technique involves transducing CARs into T cells via genetically engineered lentiviruses that represent viral vectors with their ability to infect non-dividing cells [14].

Clustered regulatory interspaced short palindromic repeats/ CAS9: (CRISPR) is a novel genome editing technique that exhibits the insertion of CARs at precise target regions of the genome with relative ease. This technique revealed a robust antitumor activity upon knocking out beta-2-microglobulin (β2M), in addition to PDCD1 and TRAC, which aid in preventing GVHD in the leukemia mice model [12,15,16].

Cas-cLOVER: Cas-CLOVER is a novel gene-editing technology alternative to CRISPR/Cas9, demonstrating high fidelity with no detectable off-targets while maintaining robust editing efficiency. It is functionally similar to CRISPR/Cas9 technologies but uses a different nuclease protein called Clo51 fused to a nuclease-inactivated Cas9 protein.

Cas-CLOVER may represent a combination of the CRISPR–Cas9 and TALEN as applied in deletion of αβTCR and β2M that has been successfully achieved.

Cas-CLOVER revealed greater specificity through the utilization of two guide RNAs as well as a nuclease activity that requires dimerization of subunits associated with each guide RNA [17-19].

Resistance to CAR T-Cell Therapy

Major challenge for CAR T-Cell application is the resistance and the side effects to therapy Figure 1

- Constant modification of surface proteins of tumor cells facilitates their ability to escape from the immune system [20].
- Abnormal expression of tumor-specific antigens on the surface of tumor cells. These antigens are presented by MHC class I or II molecules on the surface of tumor cells. They are called tumor-specific antigens (TSAs) and typically result from a tumor-specific mutation [20].
- Alterations in the signaling of antigen-presenting pathways, including mutations interfering with the

proteasome, and modifications in the structure of MHC. These changes inhibit the correct activation of T cells and encourage escaping immunosurveillance by tumor cells. In addition, evolving of new mutations in tumor cells themselves represent an additional factor in increasing resistance to therapy [21].

- Tumor microenvironment, including cancer-associated fibroblasts CAFs, fibroblast, regulatory T cells, extracellular collagen matrix, and blood vascular network. These factors facilitate tumor growth, invasion, and relapse after therapy. Also, alteration of metabolism in tumor cells as a response to the environmental metabolic changes represent an additional factor [21].
- Immature vascularization in tumors promotes the expression of hypoxia-inducible factor-1 (HIF-1), and subsequently induces the expression of vascular endothelial growth factor (VEGF), epidermal growth factor, fibroblast growth factor (FGF), hepatocyte growth factor (HGF), platelet-derived growth factor (PDGF), and other angiogenic proteins. This cascade stimulates hypervascularization that contributes to resistance to immunotherapy [22].
- Exhaustion of T cells due to continuous expression of PDL1 by tumor cells and the severity of exhaustion correlated with the level of antigen stimulation. Interestingly, epigenomic alteration is most likely result in exhaustion phenotype in T cells [23].
- Secretion of immunosuppressive molecules including TGF-β, IL-10, in addition to the increase in mTORC1 activity mediated by IL15 which participates in resistance to therapy [9,24].

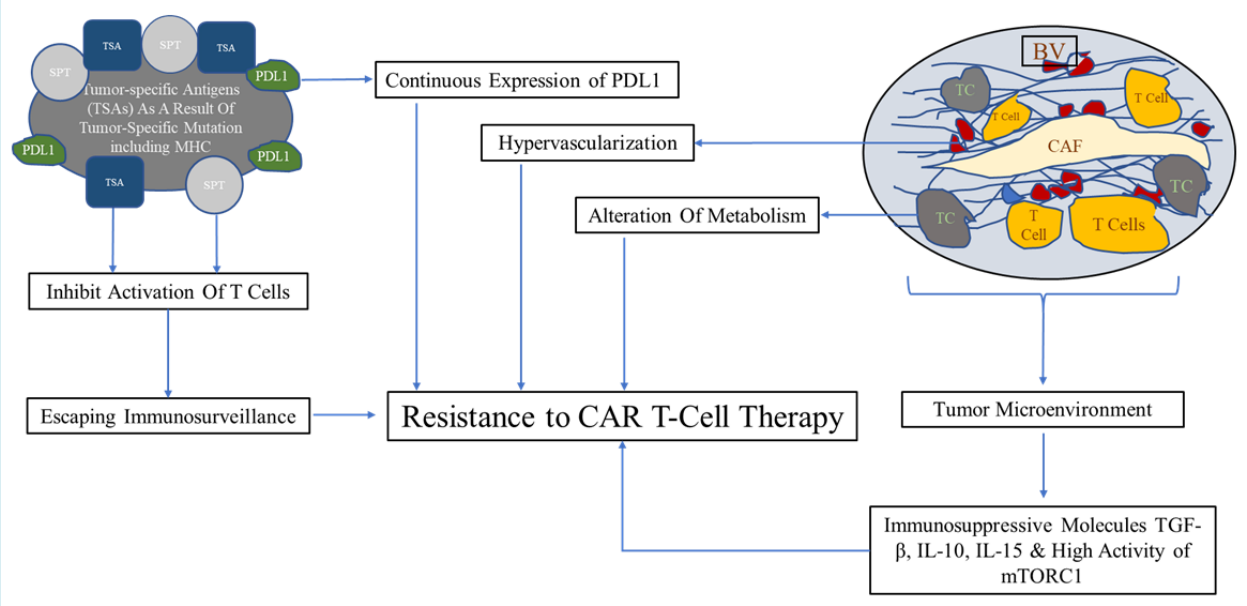

Figure 1: CAR T-Cell Therapy Resistance Scheme.
Surface Proteins of Tumor Cells (SPT), Tumor-Specific Antigens (TSP), Tumor Cell (TC), Blood Vessels (BV), Cancer-Associated Fibroblasts (CAF).

CAR T-Cells and Loss of Heterozygosity (LOH)

The link between CAR T Cells therapy and LOH was not studied long enough in solid tumors, however, irreversible loss of heterozygosity in CD19 on Chromosome 16q could be used as a biomarker for an outcome prediction after the CAR T cells therapy for B cell acute lymphoblastic leukemia (B-ALL) [25].

CAR T-Cells and Microsatellite Instability (MSI)

Studies on patients with gastric carcinoma who expressed high MSI were found to have higher expression levels of MET gene than normal tissue, and this result provided strong support for the selection of c-Met as the target of CAR-T.

However, the presence of the immunosuppressive microenvironment will reduce the efficacy of CAR-T in solid tumors. The PD1/CD28 chimeric-switch receptor (CSR) improves immunosuppression by fusing the extracellular domain of PD-1 with the transmembrane and intracellular domains of CD28, thus transforming the inhibitory signal of PD-1 into the activation signal of CD28. Therefore, CAR-Ts with PD1/CD28 CSR had a better anti-tumor effect than CAR-T combined with PD-1 antibody alone [26].

CAR-T cells and APOBAEC

The APOBEC (Apolipoprotein B mRNA Editing Catalytic Polypeptide-like) is a family of proteins that are characterized by their ability to bind to RNA and single-stranded DNA. Several factors regulate their activity, including genetic alterations, changes in transcription, and interactions with intracellular macromolecules. Loss of cellular control of APOBEC activities is associated with cancer progression and treatment resistance [27,28].

CAR-T cells and Immune Checkpoint Inhibitors

Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed death1(PD-1) are defined as checkpoint receptors that are expressed by activated T cells to negatively control their cytotoxic activities. Overexpression of these receptors leads to excessive activation and exhaustion of T-cells. In contrast, blocking and inhibition of these products improve the effect of CAR T cells [29].

CAR T Cells and Bispecific Antibody

Because of several mechanisms of Cart T Cells resistance, recently, a new approach has been proposed and advanced to overcome such resistance by producing Bispecific Antibody Armed T cells (BATs), which revealed encouraging clinical results in breast, prostate, and pancreatic cancer [30].

The bispecific monoclonal antibody (BsMAb, BsAb) is an artificial protein that can simultaneously bind to two different antigens or two different epitopes on the same antigen [31].

bispecific antibodies can be engineered by nonbinding two epitopes of different antibodies such as, anti-HER2 and CD3, anti-HER2/HER3, anti-CD19/CD3, EpCAM/CD3TF2, CD28/PDL1, and Her2/PDL1 (Figure 2) [32].

In one study, BsMAb targets CD28 homolog (CD28H) (a newly identified B7 family receptor), which is constitutively expressed on T and natural killer (NK) cells with a PD-L1 receptors on tumor cells to potentiate tumor-specific immune responses.

The best CD28H–PD-L1 bispecific antibody which retained equivalent binding of both antigens to their parental IgG, (anti-CD28H) as the Fab and (anti–PD-L1) as an scFv appended to the C-terminus of the human IgG4P Fc domain.

On the other hand, CD28H–PD-L1 bispecific antibody perform more functions than the two separate antibodies (anti PDL1 and anti CD28H) because binding in one arm to the PDL-1 receptor results in T-cell costimulation, inducing NK-cell cytotoxicity of PD-L1–expressing tumor cells and activated tissue-resident memory CD8+ T cells that may lead to induction of durable, therapeutic antitumor responses.

Mechanistically, the CD28H agonistic arm of the bispecific antibody reduced PD-L1/PD-1–induced SHP2 phosphorylation while simultaneously augmenting T-cell receptor signaling by activating the MAPK, AKT and MTOR pathways [33].

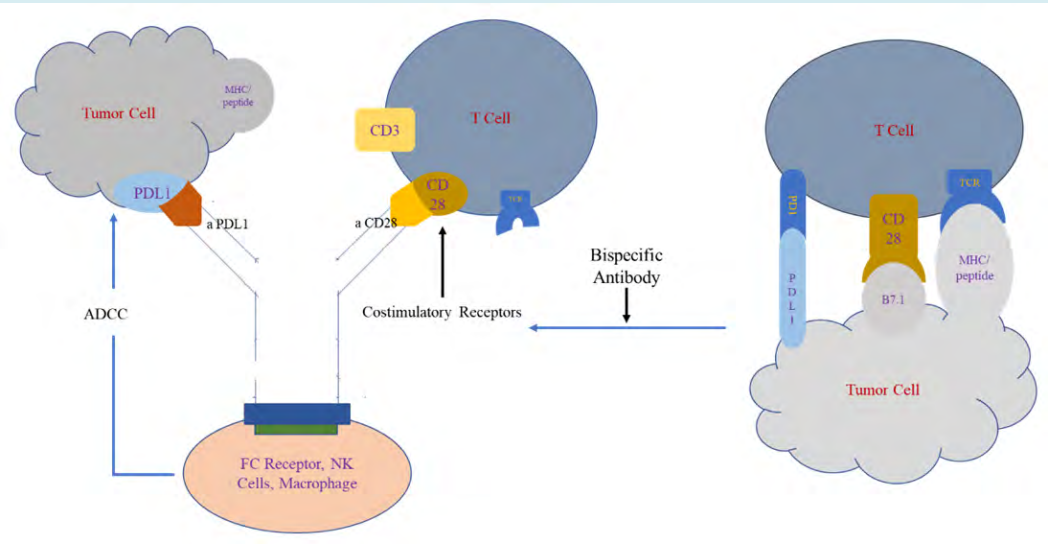

Figure 2: Binding sites of the bispecific antibody. TCR (T cell receptor), PD1(program death 1), PDL1(program death ligand 1), aCD28 (agonist CD28 epitope), aPDL1(agonist PDL1 epitope), ADCC (antibody dependent cytotoxicity), MHC (major histocompatibility molecule).

CAR T-cells and Epigenetic Factor

Despite the high preservation of DNA methylation patterns, several studies highlighted a significant association between epigenetic factors and various cancer subtypes, which present epigenetics as a major determiner in fetal cell development and differentiation.

The chromatin might represent a signaling platform to integrate signals to control gene expression. These changes are demonstrated by different chromatin appearances characterized by histone post-translational *modifications* and DNA modifications, which affect the gene expression pattern in T cells. Subsequently, epigenetic modifications could ultimately improve the efficacy of CAR-T cell therapy [34].

However, the epigenetic regulation (DNA methylation and histone modifications) plays an important role in the differentiation of T cells.

It has been reported that acquired Cancer Cell Resistance to T cell bispecific antibodies and car T targeting her2 in Vivo was conducted through Jak Down-Modulation and Due to Epigenetic Modification as well [35].

Conclusion

CAR-T cell therapy is a challenging method that represents a promising tool in assessing better outcomes in cancer patients. This technique faces several challenges in solid tumors, including tumor mutational burden and tumor microenvironment. In our manuscript, we aimed to present challenges, innovations, and improvements to this revolutionary treatment method.

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