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CAR T-Cell Therapy for Solid Tumors: How Far Are We from Reality?

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ABSTRACT

Chimeric antigen receptor (CAR) T-cell therapy is an emerging cancer treatment option throughout the world. This approach to cancer treatment uses a patient's own immune cells and engineers them to directly target and kill cancer that has been evading their immune system. There has been much success when using CAR T-cell therapy in hematological malignancies due to the almost universal expression of the CD19 antigen. Though this success has not translated into success with solid tumors, there has been extensive research to improve the results. Solid tumors pose as a more difficult target due to their variable expression of target antigens, difficulty to penetrate, and hostile microenvironment. These challenges have been investigated and tested to improve CAR T-cell therapy success in the future.

INTRODUCTION

Cancer, second only to heart attack, is a leading cause of death throughout the world. Although modern technology has improved therapies such as surgery, radiation, and chemotherapy, the general cancer survival rate has not risen significantly. Immunotherapy is one area of cancer treatment research that has shown promising results. Adoptive T-cell therapy (ACT) is one approach where a patient's own lymphocytes are removed and engineered to recognize tumor antigens through T-cell receptors (TCRs) or chimeric antigen receptors (CARs). CAR-T cell therapy has proved successful and has multiple benefits over other treatments such as the aforementioned TCR-modified T-cells. These benefits include recognizing antigens independently of the major histocompatibility complex (MHC), ability to target proteins, carbohydrates, and glycolipids expressed by tumor cells, and the ability to modify the CARs with co-stimulatory molecules to augment cytotoxicity and prolong lifespan *in vivo* (Muhammad, Niaz, et al., 2017).

CAR T-CELL ANATOMY

The basic anatomy of a CAR includes three sections: an extracellular domain, transmembrane domain, and an intracellular signaling domain. The extracellular domain is an antigen-binding domain composed of a single-chain variable fragment (scFv) of a tumor antigen-reactive antibody. This scFv, consisting of the variable portions of heavy and light chains from the antibody, are joined to each other by a peptide linker and fused to the transmembrane domain by a hinge domain (Nair, Ranjit, and Sattva S Neelapu, 2018). The transmembrane domain is comprised of a hydrophobic alpha helix traversing the cellular

membrane to be linked to the intracellular signaling domain within the cytosol of the cell. This intracellular signaling domain is the functional end of the CAR. In the first generation of CARs, the intracellular signaling domain was a single CD3ζ chain. Based off the amount of signaling molecules on the intracellular signaling domain, there are currently four generations of CARs (Zhang, Cheng, et al., 2017).

The first generation of CARs contained the single CD3ζ chain for the intracellular signaling domain. This assembly did not perform well *in vivo* due to the inability to produce sufficient interleukin-2 (IL-2) and did not yield results in studies due to poor proliferation, short lifespan *in vivo*, and insufficient cytokine products (Zhang, Cheng, et al., 2017). It was concluded that CARs with only the CD3ζ chain could not adequately activate the CAR T-cells (Zhang, Cheng, et al., 2017).

The second generation of CARs were produced by complementing the CD3ζ chain with co-stimulatory molecules such as CD28 or CD137 (4-1BB and CD134 (OX40)). This addition enhanced the proliferation, cytotoxicity, and extended the lifespan of the CAR T-cells *in vivo*.

The third generation of CARs were produced by fusing multiple signaling domains (Figure 1). These two co-stimulatory molecules derived from p56 Ick + CD28, OX40 + CD28, or 4-1BB+CD28 in addition to the CD3ζ chain. This assembly showed to increase the potency with higher levels of cytokine production (Zhang, Cheng, et al., 2017).

The fourth generation CAR was produced by combining the second generation CARs with a cytokine expression cassette (Figure 1). These are known as T-cell redirected for universal cytokine-mediated killing (TRUCKs). These CARs have the ability to increase the

activation of T-cells and attract immune cells to eliminate antigen-negative tumor cells by releasing anti-tumor cytokines (Yan Ll, and Liu Bn., 2018).

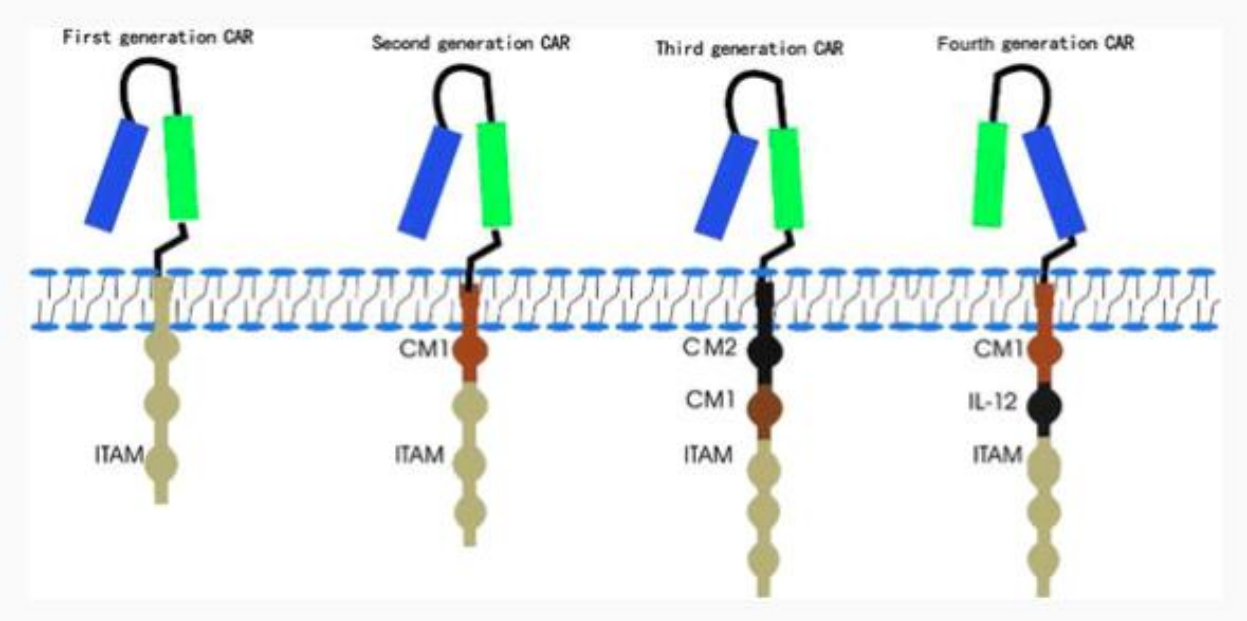

Figure 1. The evolution of chimeric antigen receptors (CARs). This shows the progression from the first generation to the fourth. The CD3ζ chain is conserved through all generations (Zhang, Cheng, et al., 2017).

CURRENT SUCCESS

Thus far, Car T-cell therapy has shown great success in treating hematological malignancies. This is due to the expression of CD19 by almost all B-cell malignancies such as chronic lymphocytic leukemia (CLL), B-cell acute lymphoblastic leukemia (B-ALL), and Non-Hodgkin Lymphomas (NHLs) (Muhammad, Niaz, et al., 2017). The first successful treatment of CLL by CAR T-cell therapy used CAR T-cells that targeted the B-cell antigen, CD19 (Kalos, Michael, et al., 2011). Although CAR T-cell therapy has been very successful in treating hematological malignancies, the same success has not been provided in the treatment of solid tumors. There may be numerous unknown obstacles accounting for the poor success, but the

known challenges of solid tumors are finding, infiltrating, and surviving within the solid tumor (Marina Martinez, and Edmund Kyung Moon., 2019).

FINDING ANTIGENS

The main difference with solid tumors versus hematological malignancies is that solid tumors do not universally express a targetable antigen such as the CD19 antigen in hematological malignancies. This makes it difficult for CAR T-cells to differentiate between normal tissues and tumor cells. The ideal target for solid tumors should be overexpressed on tumor cells and show none or very little expression on normal, healthy tissues in the cancer patient. Some tumor associated antigens (TAAs) that are being researched are mesothelin (MSLN), HER2, EGFR/EGFRvIII, GD2, CEA, IL13Rα2, MUC1, FAP, PSMA, and PSCA (Li, Jian, et al., 2018)

Unlike hematological malignancies, solid tumors do not universally express a TAA that is not also expressed on normal tissues. Therefore, a major concern with CAR T-cell therapy for solid tumors is off-target cytotoxicity. An example of this is in a CAR T-cell therapy for neuroblastoma using a high affinity anti-GD2 CAR. This construct caused lethal central nervous system toxicity through excessive CAR T-cell infiltration and proliferation and resulted in lethal encephalitis (Richman, Sarah A, et al., 2018). This shows that selecting the correct antigen is essential because off-target cytotoxicity on normal tissues may have unfavorable consequences. Regulating the binding affinity of CAR T-cells to antigens may be a way to avoid toxicity in normal tissues. In a study of tuning the affinity of CAR T-cells to ICAM-1 in thyroid tumors, the safety and efficacy was compared between CAR T-cells with a one million-fold

difference in binding-affinity. This difference was from low nanomolar to high micromolar affinity to the ICAM-1 antigen. The results of this study concluded that the micromolar binding-affinity performed better in safety and efficacy when compared to the nanomolar. At the high micromolar affinity, CAR T-cells produced death of host by on/off-target toxicity as well as high levels of cytokine release (Spencer Park, et al., 2017). These results show that a higher binding affinity is not always better and may cause unwanted toxicity to the host. Another solution to the problem of off-target toxicity is to insert a safety switch that can turn CAR T-cells on or off. One such switch is an inducible Capsase 9 (iCasp9) gene that can induce apoptosis of CAR T-cells that may cause toxicity to normal tissue. Administration of a small molecule dimerizer drug, AP1903, causes mass apoptosis of the activated cells expressing the transgene (Tessa E. gargett, and Michael E. brown, 2014). Opposite to the inducible iCasp9 off switch, there is an on switch that is capable of introducing the CAR T-cells in a gradual fashion. The design of the on switch requires both binding of antigen and a small molecule drug for dimerization and activation in a split-receptor approach. This assembly allows for exogenous control over CAR T-cell antitumor processes such as proliferation, cytokine production, and cytotoxicity (Wu, Chia-Yung, et al., 2015). Another inducible on switch for CAR T-cells has been found to be an ultrasound-based mechanogenetics system. This system has the ability to noninvasively control the genetics of cells with high frequency ultrasound. For this to work, the mechanically sensitive Piezo1 ion channel linked to transcriptional activities was engineered into Jurkat T-cells. Application of ultrasound to the T-cells induced the expression of CARs. This method allows for precise activation of CAR T-cells to protects against off-target toxicity (Pan, Yijia, et al., 2018).

INFILTRATION

Even if a good solid tumor antigen is found, the next obstacle a CAR T-cell encounters is infiltrating the solid tumor from the bloodstream. This homing from the bloodstream to tissues requires adhesive interactions between the CAR T-cells and the wall of the blood vessel. The stages of these interactions can be grouped into rolling, activating-induced arrest, and movement into the tissue (Ager, Ann, et al., 2016). The ability of CAR T-cells to home to a solid tumor is controlled by their expression of chemokine receptors matching the chemokines within the solid tumor. Chemokines expressed by the tumor cells may not match the chemokine receptor of the CAR T-cells. This limits recruitment into the solid tumor (Harlin, Helena, et al., 2009).

Chemokines provide a chemotactic gradient for immune cells to follow to produce an immune response. There has been a study that used CXCR3 ligands (CXCL9, CXCL10, and CXCL11) and transfected mice with them using a recombinant adenovirus-based vaccination. It was found that the strongest effect on CD8+ T cells was produced when CXCL11 was administered with the vaccine. These results were confirmed with a therapeutic tumor mouse model. This showed that delivery of CXCL11 into a tumor may provide increased CAR T cell infiltration and toxicity to solid tumors (Namkoong, Hong, et al., 2014).

There may be a way to evade the barriers of CAR T-cells infiltrating into solid tumors. One study of HER2-CAR T-cells against $HER2^{+}$ metastases of the brain showed that injecting the CAR T-cells intratumorally produced complete tumor regression. This study was conducted using a xenograft mouse model. When treated with intravenously injected HER2-CAR T-cells at 10-fold higher doses, the model only showed partial antitumor responses (Priceman, Saul J, et

al., 2018). The results of this study show that intratumoral delivery of CAR T-cells against solid tumors may be a way to increase efficacy, gain complete tumor regression, and avoid off-target toxicities.

SURVIVAL IN THE TUMOR MICROENVIRONMENT

If a CAR T-cell is able to cross a solid tumor's barriers and find itself inside the tumor, there are more challenges to overcome. The tumor microenvironment (TME) is significantly suppressive to immune cells. The TME is composed of non-neoplastic cells that provide support for the neoplasm of the tumor. Within the TME, there are multiple cells and secretory factors that suppress the function of CAR T-cell. These include T-regulatory Lymphocytes (Tregs), myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs), tumor associated neutrophils, and stromal fibroblasts. Tregs have the ability to uptake IL-2 to blunt the effect of CAR T-cells that are present. Both Tregs and TAMs secrete IL-10, a suppressive cytokine that aids in the proliferation of tumor cells (Gowrishankar, Kavitha, et al., 2018). MDSCs are able to suppress anti-tumor functions by stimulating Tregs to produce reactive oxygen species to suppress the response of T-cells by oxidative stress. In the inflammatory environment of a solid tumor, it upregulates the expression of the programmed cell death ligand ½ (PD-L1/2). This pathway is used by the tumor to induce exhaustion of CAR T-cells. This exhaustion results in decreased proliferation and production of cytokines like IL-2, TNF-α, and IFN-γ. When a T-cell is exhausted, it expresses elevated levels of receptors that can bind PD-L1, which results in suppression of their anti-tumor activities to allow survival of the tumor Morgan, Michael A., and Schambach, Axel., 2018).

The stromal compartment of the solid tumor provides protection and serves as a physical barrier to inhibit infiltration by CAR T-cells. This compartment is comprised of non-malignant fibroblasts and mesenchymal cells surrounded by blood vessels, immune cells, matrix, and inflammatory mediators. The fibroblasts have the ability to convert to cancer-associated fibroblasts (CAFs) and express fibroblast activating protein (FAP). The expression of FAP by CAFs can bring in endothelial cells for increased vasculature, cause collagen crosslinking, and matrix degradation. These, together, promote the development of the tumor. The collagen crosslinking introduces complexity to the outside of the tumor and makes it more difficult for CAR T-cells to infiltrate (Gowrishankar, Kavitha, et al., 2018). There has been a study conducted on the results of targeting FAP by FAP-targeted CAR T-cells. The results showed that FAP^+ cancer-associated stromal cells are necessary for the progression of a solid tumor. Using the FAP-CAR T-cells decreased vasculature and inhibited growth of tumors (Lo, Albert, et al., 2015). These results are promising for the future therapeutic advantages of controlling tumor growth in an immune-dependent and -independent fashion.

Solid tumors have another blockade for infiltrating CAR T-cells. The low oxygen (hypoxic) environment within the TME is due to abated blood flow, abnormal vascularization, and the production of hydrogen peroxide. Reactive oxygen species (ROS), such as hydrogen peroxide, impair the anti-tumor activities and put stress on CAR T-cells. One way to combat oxidative stress from ROS is to use CARs that are able to express the enzyme Catalase (Gowrishankar, Kavitha, et al., 2018). Catalase metabolizes hydrogen peroxide into water and oxygen. A way to protect CAR T-cells from oxidative stress within the TME was conducted by engineering T-cells with a bicistronic vector to express catalase with the CAR co-expressing catalase (CAR-CAT).

These CAR-CAR T-cells proved to reduce the amount of oxidative stress and even exhibited a bystander effect. This bystander effect protected NK cells from oxidative stress and allowed them to exert anti-tumor functions (Ligtenberg, Maarten A, et al., 2016).

FUTURE DIRECTIONS

CAR T-cell therapy for solid tumors has shown promising results over the last few years. But there is still much room for improvement in the areas of efficacy and safety. The gene-editing power of CRISPR/Cas9 could be the future for allowing CAR T-cells to find, enter, and survive within solid tumors. One application for CRISPR/Cas9 is the production of off-the-shelf CAR T-cells. Currently, CAR T-cell formation requires the use of a patient's own lymphocytes. This involves knocking out the TRAC and B2M genes through CRISPR/Cas9 to produce universal CAR T-cells made from allogenic cells. Also, CRISPR/Cas9 has been utilized to knock-out the PD-1 gene in T-cells. This allows CAR T-cells to pass immune checkpoints on solid tumors and improves cytotoxicity (Qianqian Gao, et al., 2019). There is an example of this approach being used in a phase I clinical trial investigating CD19-specific CAR T-cells with PD-1 knockout (NCT03298828).

CONCLUSION

In conclusion, CAR T-cell therapy has come a long way but still has much potential to keep progressing into the cancer therapy of the future. The success seen in hematological malignancies has not yet transferred to solid tumors. There are still current efficacy and safety issues that present as major hurdles to overcome before CAR T-cell therapy becomes the

mainstream treatment option for solid tumors. There have been many advances in the areas of target antigens, controlling off-target toxicity, improving infiltration, and surviving within solid tumors. With the problems presented, CRISPR/Cas9 may be the solution to providing cheap, safe, and universally available CAR T-cells to those in need.

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